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Contribution of *BRCA1/2* Mutation Testing to Risk Assessment for Susceptibility to Breast and Ovarian Cancer

Agence d'évaluation des technologies
et des modes d'intervention en santé

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Québec



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Contribution of *BRCA1/2* Mutation Testing to Risk Assessment for Susceptibility to Breast and Ovarian Cancer

Monograph prepared for AETMIS
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Foreword

This health technology assessment was undertaken following a request from the Quebec Ministry of Health and Social Services (MSSS, *Ministère de la santé et des services sociaux*). Questions had been brought to the attention of MSSS by the Quebec health insurance agency (RAMQ, *Régie de l'assurance maladie du Québec*) regarding whether sending samples to Myriad Genetics Inc. for the sequencing of *BRCA1* and *BRCA2* genes was justified, and which indications for testing should be followed. Additional concerns were raised by the Quebec Association of Medical Geneticists (AMGQ, *Association des médecins généticiens du Québec*) regarding the organisation of cancer genetics services in the province. The MSSS request asked AETMIS to consider a broad range of issues, including 1) *BRCA1/2* molecular test validity, 2) testing indications, 3) psychosocial and ethical implications, 4) impact on clinical management, 5) cost-effectiveness, and 6) organisational aspects of cancer genetics service delivery.

Since the Canadian Coordinating Office of Health Technology Assessment (CCOHTA) had also received a mandate to review *BRCA1/2* testing, a collaboration between CCOHTA and AETMIS was established to avoid duplication of work. The present monograph is the result of the analysis undertaken at AETMIS on 1) prevalence and penetrance of *BRCA1/2* mutations, 2) risk assessment models and testing indications, 3) clinical validity of molecular tests, and 4) the impact of molecular testing on risk assessment and genetic counselling. The forthcoming CCOHTA report will address the analytical validity of molecular tests, the potential benefits of molecular testing for clinical management, and the psychosocial and ethical issues. The complementary nature of the work undertaken by the AETMIS and CCOHTA researchers is clearly an asset, but overall conclusions in this area will need to take the content of both reports into account. The scope of the present work does not therefore allow recommendations to be made regarding the complex range of questions related to the clinical use of *BRCA1* and *BRCA2* molecular tests.

A follow-up AETMIS report is also in preparation, which will build upon the present work, the CCOHTA report, and other systematic reviews on selected topics, as well as the research undertaken at AETMIS with respect to organisational and economic issues related to cancer genetics services. This broader-ranging review will allow conclusions to be drawn with regard to the policy questions raised by the use of the genetic testing technology. The present monograph provides an understanding of the nature of the scientific evidence that needs to underpin these decisions and of the unresolved questions and uncertainties that complicate the decision-making process.

The purpose of health technology assessment is to examine the use of a specific technology for a given target condition and population. For diagnostic tests, the central question is that of the contribution of the technology to the diagnosis and management of the target condition (be it a disease or a risk factor for disease). Defining the target condition is somewhat problematic in the case of the present assessment. Current clinical practice does not support the widespread use of *BRCA1* and *BRCA2* molecular testing for all cases of breast cancer and their families. Instead, families with a high prior probability of carrying a genetic susceptibility to breast and ovarian cancer, on the basis of family history and ethnic origin, are targeted. With a view to coherence with the above perspective and given that clinical management was not covered in this review, the present monograph focuses on the contribution of *BRCA1* and *BRCA2* testing to risk assessment for susceptibility to breast and ovarian cancer.

Dr. Luc Deschênes

Président-directeur général

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DISCLOSURE OF CONFLICT OF INTEREST

None declared.

Executive summary

INTRODUCTION

For both breast and ovarian cancer the strongest known risk factor, after adjusting for age, is family history. The figure most often cited is that approximately $\frac{1}{3}$ of breast cancer is *familial*, where family history is defined by the presence of at least two cases of breast cancer, at any age, in first- or second-degree relatives.

Approximately 5% of all breast cancer cases are currently thought to be hereditary, that is, related to the transmission of mutations in a single gene. Clustering of cancer cases in families does not imply that genetic susceptibility is always present, since environmental factors and chance can also play a role. Conversely, the presence of a genetic susceptibility will not always translate into the occurrence of multiple cancer cases within one family. However, persons considered to be at highest risk of carrying a mutation in a breast or ovarian cancer susceptibility gene are those displaying the characteristics of a hereditary breast and ovarian cancer syndrome, HBOC. The HBOC syndrome is characterized by a pattern of occurrence of cancers in a family, involving multiple relatives affected by breast and/or ovarian cancer (and possibly other cancer such as prostate or male breast), and by early age at diagnosis. However, there is currently no consensus as to the minimal set of criteria defining an HBOC family.

Two genes conferring susceptibility to breast and ovarian cancer have been associated with HBOC: Breast CAncer gene 1 (*BRCA1*) on chromosome 17 and Breast CAncer gene 2 (*BRCA2*) on chromosome 13. The mutations in the *BRCA1* or *BRCA2* genes are transmitted via an autosomal dominant mode of inheritance. Both *BRCA1* and *BRCA2* are very large genes that span more than 100 kb of DNA, and since cloning, more than a thousand mutations have been identified in these genes. Many of the *BRCA1/2* mutations occur in only one or a few families, except in certain ethnic groups where a *founder effect* appears to exist (that is, higher frequencies of particular *BRCA1/2* mutations in

descendants of common ancestors, due to geographic or ethnic isolation). Slight homology exists between the *BRCA1* and *BRCA2* proteins, and both have a function in DNA repair. *BRCA1/2* genes are considered to be tumour suppressor genes. In general, tumour development related to this class of genes occurs when two copies of mutated alleles are present. This implies that carrying an inherited mutated allele is not a sufficient condition for abnormal cell growth and that inactivation of the second, normal allele is a necessary step for carcinogenesis.

BRCA1-related breast tumours appear to have pathological features that are distinct from those in non-hereditary tumours, whereas differences with non-hereditary forms are less clear for *BRCA2*-related breast tumours and ovarian tumours associated with *BRCA1* or 2. Molecular profiling of tumours is an active field of research which is likely to improve the characterisation of a distinctive pathological *phenotype* (that is, the expression of the genetic makeup) for *BRCA1/2*-related cancers.

According to population-based studies, *BRCA1/2* mutations explain 16-17% of the clustering of breast cancer in families (*familial* cancer defined here as at least two family members being affected in first degree relatives), compared to 55-60% of the clustering in ovarian cancer. However, the contribution of *BRCA1/2* to familial breast or ovarian cancer may be greater in certain ethnic groups with a founder effect (e.g., Ashkenazi Jewish, Icelandic). At the moment, a substantial proportion of familial breast cancer cases has not been linked to any known breast or ovarian cancer susceptibility genes and clustering of cancer cases in these families is most likely related to low penetrance genes, environmental factors or a combination of both. However, the isolation of genetic variants associated with a lower risk of developing cancer (i.e., genes with lower *penetrance*) poses an important challenge as these rarely produce a familial pattern that is striking enough for

traditional linkage analysis, even though their prevalence could be substantially greater than that of high penetrance genes. Some of these low penetrance genes may be implicated in the modulation of cancer risk in *BRCA1/2* carriers.

Since the cloning of *BRCA1* and *BRCA2*, in 1994 and 1995 respectively, considerable progress has been made in the understanding of the role of the *BRCA1/2* mutations in carcinogenesis, in their characterization in a variety of populations, and in the assessment of the risk of developing cancer for *BRCA1/2* mutation carriers. Molecular tests to detect mutations have been developed, but the transition from research to clinical practice has occurred under a variety of conditions and at a different pace from one center to the next. Even today, substantial heterogeneity can be observed in terms of the precise testing indications, the analytical techniques used, the organization of services accompanying testing, and the professionals involved in these services. This heterogeneity, in turn, means that combining and making sense of the evidence in this complex field is particularly challenging.

In order to reflect both current clinical practice and the emphasis in the scientific literature, our analysis of the contribution of *BRCA1/2* testing to risk assessment focuses on individuals with a high prior probability (that is, at high risk) of carrying a genetic susceptibility to breast and ovarian cancer. This comprises families thought to fit the definition(s) of a hereditary cancer syndrome, as well as individuals with other risk-related characteristics, such as early age at onset of cancer.

The present monograph is part of a broader project. As a first step, a systematic literature review was conducted with respect to:

1. the prevalence of *BRCA1/2* mutations in HBOC families, breast or ovarian cancer cases, and certain founder populations,
2. the penetrance associated with *BRCA1/2* mutations,
3. the available risk assessment models,

4. existing guidelines as to testing indications, and
5. the clinical validity of available molecular tests.

The evidence retrieved from the systematic review was integrated to provide an understanding of the contribution of molecular tests to risk assessment and genetic counselling of individuals and families with HBOC, understood in a broad sense, and to situate these findings within the wider context of decision and policy making issues related to the clinical use of *BRCA1/2* mutation testing.

Important considerations, such as the analytical validity or technical performance of available tests, the psychosocial consequences of testing, the impact of testing on clinical management, and the ethical and legal issues raised by the use of these tests in clinical practice, have either been dealt with in recent systematic reviews or are the object of ongoing projects elsewhere. Such topics will not be covered here, but the conclusions of these reviews will be taken into account in a follow-up document to the present monograph,¹ which will cover issues of particular concern to policy makers. This companion report will include a review of organisational modalities of cancer genetics services in different jurisdictions, a review of the impact of models of care delivery on psychosocial consequences of testing, and a comparative cost analysis for different organisational modalities.

MUTATION PREVALENCE

BRCA1/2 mutation prevalence data are reviewed separately for 1) individuals with a family history of breast or ovarian cancer, 2) individuals with these cancers unselected for family history, 3) the general population and 4) several founder populations, since mutation prevalence varies according to population. Prevalence estimates also vary substantially from one study to the next as a result of differing mutation detection techniques and study sample

1. AETMIS. Cancer Genetic Services: Economic and organisational issues (in preparation).

selection criteria, in particular with respect to family history and age at diagnosis.

Estimated frequency of *BRCA1/2* mutations in families ascertained because of multiple cases of cancer is high (i.e., up to 84% of families are linked to these genes when at least 4 breast cancer cases are diagnosed prior to 60 years of age, with or without ovarian cancer). In individuals referred to cancer clinics due to family history, frequencies of *BRCA1/2* mutations are, in general, lower than in highly selected families, at least on the basis of point estimates of prevalence, and estimates vary from 3.5 to 45% (no CIs provided) for *BRCA1* and from 1.2 to 22.5% (no CIs provided) for *BRCA2*.

In breast cancer cases unselected for family history and age at onset, *BRCA1* mutation prevalence estimates vary from 1.1 (95% CI: 0.4-2.2) to 2.6 (95% CI: 0-5.5), and prevalence is estimated in one study as 1.1 for *BRCA2* (95% CI: 0.4-2.2). In ovarian cancer series, prevalence estimates vary from 1.9% (no CI provided) to 9.6% (95% CI: 6.7-13.5) for *BRCA1* mutations and from 0.9 to 4.1% (no CIs provided) for *BRCA2* mutations. In breast cancer cases unselected for family history only, prevalence estimates vary for *BRCA1* mutations from 0.7% (95% CI: 0.3-1.3) to 6% (95% CI: 3.8-8.8) and, for *BRCA2* mutations, from 1.3% (95% CI: 0.8-2.1) to 3.9% (95% CI: 2.2-6.3). Up to 36% of women with breast cancer who are mutation carriers report no family history of breast or ovarian cancer. The figures presented above for breast and ovarian cancer cases arise from heterogeneous populations. In the Ashkenazi Jewish population, between 1.9 and 2.7% of individuals are estimated to be carriers of one of three common mutations, which is approximately ten times the overall carrier prevalence estimated in heterogeneous populations. *BRCA1/2* mutation frequencies in heterogeneous general populations have been extrapolated from breast cancer series.

Stratified analyses show that mutation prevalence is associated with various risk factors: breast cancer diagnosed at an early age, specific ethnic origin (e.g., Ashkenazi Jewish), ovarian cancer and male breast cancer. Cut-off

values for defining ‘early’ age at diagnosis of breast cancer vary widely in the literature, and there is no general agreement as to the value that justifies offering tests on the basis of age as the only risk factor. The discriminating power of the number of breast cancer cases and the presence of bilateral cancer as risk factors appears to depend on the presence of ovarian cancer in the family history and age of onset of cancer, respectively.

The fact that recurrent mutations have been discovered in certain populations makes the molecular analysis of the *BRCA1/2* genes easier for these groups, since one can screen for common mutations first rather than proceeding directly to screening all exons. However, the testing strategy should take the importance of the founder effect into account. The three most striking examples of founder mutations are those in the Ashkenazi Jewish (AJ), Icelandic and Polish populations. When testing is performed, it is common to screen for selected founder mutations in some ethnic groups (e.g., AJ), while optimal approaches have not yet been established in other founder populations, such as French Canadians.

The identification of a fairly large proportion of *BRCA1/2* variants of unknown clinical significance raises the problem of classifying and interpreting these test results, deciding whether or not to include them in prevalence calculations, and choosing appropriate clinical follow-up strategies.

PENETRANCE

BRCA1/2 mutations are associated with a high risk of breast and ovarian cancer, clearly elevated over that of the general population. Absolute risks of cancers other than breast or ovarian appear to be relatively small. In studies on families with at least four affected individuals, estimated risk of breast cancer at age 70 is similar for *BRCA1* mutations (85-87%; 95% CI: 72-95 for the latter estimate) and *BRCA2* mutations (84%; 95% CI: 43-95), but risk of ovarian cancer appears to be higher for *BRCA1* (44% [95% CI: 28-56] to 63% [no CI provided]) than for *BRCA2* (27% [95% CI: 0-

47]). According to the only meta-analysis published in which data from 22 studies on heterogeneous and founder populations unselected for family history were pooled, risk of breast cancer at age 70 is lower based on point estimates (i.e., 65% [95% CI: 51-75] for *BRCA1* mutations and 45% [95% CI: 33-54] for *BRCA2* mutations). In this meta-analysis, ovarian cancer risk was significantly higher for mutations in *BRCA1* (39% [95% CI: 22-51]) than in *BRCA2* (11% [95% CI: 4.1-18]). Breast cancer risk point estimates for individuals referred to specialized clinics due to family history fall in between the values reported above (i.e., 73% [95% CI: 68-78] at 70 years for *BRCA1* mutations).

Penetrance of *BRCA1/2* mutations has been estimated in one study of French Canadian high risk families and is in line with point estimates of penetrance in high risk families among other ethnic groups, but confidence intervals are particularly large. For the Ashkenazi Jewish population, breast cancer risk estimates at 70 to 75 years vary from 36% (no CI provided) to 69% (95% CI: 59-79) for the *BRCA1* 185delAG and the 5382insC mutations, and from 26% (95% CI: 14-50) to 74% (95% CI: 58-90) for the *BRCA2* 6174delT mutation. For ovarian cancer, risk estimates vary from 10.1 to 51.2% (no CIs provided) for *BRCA1* 185delAG, from 16 (95% CI: 6-28) to 46% (no CI provided) for *BRCA1* 5382insC, and from 12% (95% CI: 0-26) to 26.6% (no CI provided) for the *BRCA2* 6174delT mutation.

Penetrance estimates with respect to breast and ovarian cancer vary widely from study to study. In general, confidence intervals are wide, rendering most differences between point estimates statistically non-significant. Analyses are underway to examine whether the observed differences are attributable to the effects of disparate designs and analytical methodologies, or to real differences between high risk and moderate risk populations. With the exception of certain populations, where penetrance has been determined for specific founder *BRCA1/2* mutations (e.g., AJ, Icelandic, Norwegian), estimates to date are mean values for a broad range of *BRCA1/2* mutations. Given the number

of different factors that influence the phenotypic expression of hereditary breast cancer (type of *BRCA1/2* mutation, and reproductive, environmental and other genetic factors) a more precise personal cancer risk assessment is likely to be unrealistic, especially in heterogeneous populations. Contradictions exist between studies on cancer risk modifiers and these cannot be used presently at the clinical level. At the moment, the information on penetrance is conveyed to patients and families as a range of values derived from empirical data on groups of families with similar risk factors. No biological markers are currently available to predict which *BRCA1/2* mutation carriers will develop cancer. In practice, family pedigree information and ethnicity are used to modulate risk assessment.

RISK ASSESSMENT

Given the complexity of the data on prevalence and penetrance of *BRCA* mutations, statistical modelling has been seen as a means of simplifying risk assessment and of identifying those families that are most likely to carry *BRCA* mutations. The earliest statistical models were designed to predict risk of breast cancer and did not integrate results of molecular testing. Subsequently, several models have been developed to estimate the probability of a *BRCA1/2* mutation explaining the familial aggregation of cancer. These models are either based on published data on population mutation prevalence, age-specific and cumulative penetrance and cancer incidence, or are derived from the analysis of samples of high risk families fulfilling particular eligibility criteria and for whom molecular testing was performed. Some models are limited to *BRCA1* only, whereas others consider *BRCA1* and *BRCA2*. The range of risk factors integrated in these models is variable (AJ ancestry or male breast cancer may or may not be considered, for example) and such factors are not defined in a consistent way (for early onset of cancer, for instance). The complete family structure is not systematically considered. Most models, therefore, do not apply to all possible familial constellations of risk factors.

Some common predictors of *BRCA* mutation status have been identified through multivariate analysis. Among these, the presence of ovarian cancer and the proband's age at diagnosis are prominent. Although similar patterns emerge in the various models, specific results are still dependent upon the particular study samples, the testing methods and the precise definitions of variables used.

A number of authors have compared the carrier probabilities generated by one or several models with *BRCA* mutation testing results. Others have compared the predictions of several models to each other. On the whole, these models are reported to have fairly high sensitivity and negative predictive value, but disappointing specificity and positive predictive value. The approaches used to compare the performance of these models are varied and sometimes questionable. In addition, most comparative studies have been performed on samples of exclusively high risk families, so that the ability to discriminate between carrier and non-carrier families among borderline low to moderate risk families may not have been assessed accurately. More recent risk models strive to account for all facets of the familial aggregation of breast cancer and these may become important tools for risk assessment in the low to moderate risk range in the future. Efforts are also underway to develop more user-friendly tools for use in risk assessment in tertiary care and as referral guidelines for primary care.

The lack of proper validation and the numerous classification schemes of risk factors may contribute to the fact that none of these models has been unanimously adopted in clinical practice. Indeed, these statistical models are currently used as supplements to the genetic counsellor or clinical geneticist's own judgement of risk.

GUIDELINES FOR TESTING INDICATIONS

Clinical guidelines have been produced by a variety of bodies on indications for performing molecular testing for *BRCA1/2* mutations or on risk classification schemes, with a view to referring families to the appropriate level of

care. Most guidelines are based on expert opinion or are extrapolated from research study evidence. However, the links between the recommendations and the supporting evidence, i.e., the source and the specific levels of mutation penetrance and prevalence associated with familial and personal risk factors, are not stated.

Genetic testing is currently recommended for high risk families only, and there is general concordance between the various guidelines as to the most important risk factors used to define high risk families. The identified risk factors are consistent with findings from the literature on penetrance and prevalence and with the common results of the risk assessment statistical models discussed earlier. It should be noted that this concordance applies to broadly defined risk factors, such as early onset of breast cancer, male breast cancer, bilateral breast cancer, ovarian cancer, multiple affected family members and Ashkenazi Jewish ancestry. In contrast, there is little consensus as to the precise criteria that should guide testing within each of these broad risk factors, or the combination thereof. This is not a trivial issue, particularly if some risk factors are used in isolation. If, for instance, early age at diagnosis is used as a sufficient criterion for testing (without requirements with respect to number of cancer cases in relatives for instance), the choice of age cut-off greatly influences the number of tests being requested and the ability of laboratories and the health care system to assume these activities. A similar situation arises if male breast cancer or ovarian cancer are used without additional criteria related to age at onset or family history. If such guidelines were to be considered in Quebec, the probability of detecting mutations under these conditions would have to be appropriately documented and the implications estimated, in terms of impact on services and health care organisation.

The most recent guidelines have placed more emphasis on criteria for referral to tertiary genetic services or for access to genetic counselling than on precise testing indications. Likewise, the 2003 ASCO statement has backed away from the use of a numerical threshold as a

criterion to recommend *BRCA1/2* mutation testing, placing greater emphasis on the role of the evaluation made by health care professionals experienced in cancer genetics in determining appropriateness of testing. Such a position may be more coherent with current practices and more realistic at the present time, given the limitations of available data and the lack of consensus around precise guidelines and statistical models. In terms of planning of clinical and laboratory services, flexible testing indications make it more difficult to predict volume of testing. Cost implications could be significant, if testing were to extend beyond the environment of specialized centres. In addition, flexible testing indications will defer standardisation of practices and will thus have implications on future data collection, unless clear selection criteria are agreed upon for research and monitoring purposes.

CLINICAL VALIDITY

The length of the *BRCA1* and *BRCA2* genes, the distribution of mutations throughout these genes, and the diversity of types of mutations found markedly complicate molecular analyses. No single technique is appropriate to detect all mutation types. The sequencing of all exons and exon-intron junctions has traditionally been considered as the gold standard technique to detect point mutations and small insertions and deletions in these parts of the genes. Sequencing is both costly and labour-intensive and is not an appropriate technique to detect large rearrangements, such as large deletions or insertions. The proportion of *BRCA1/2* mutations that cannot be detected by sequencing has been the object of research only in the past few years, and it has become clear that the proportion of large rearrangements, for *BRCA1* in particular, cannot be neglected.

Molecular tests are assessed for both their technical and clinical performance. Analytical validity refers to the technical performance of the test and reflects the comparison of test results with the genotype, or the DNA sequence. This literature has not been reviewed for the purposes of this document. Clinical validity refers to the comparison of test results with the

phenotype, i.e., the clinical expression of disease. Clinical validity is a function of the target population, depending both on the ethnic group and on the familial risk factors used to select study samples. Data were extracted from the literature to estimate clinical sensitivity of:

1. exon and splice site screening techniques (EX/SP screening, involving a variety of methods) for heterogeneous (non-founder) populations, by examining the proportion of HBOC families carrying point mutations and small rearrangements in *BRCA1*, as opposed to large genomic rearrangements and non-coding region mutations,
2. testing for common mutations in different populations with founder effects (Ashkenazi Jewish, Polish, Dutch, Finnish, Hungarian and French Canadian), using the distribution of common and non-common *BRCA1/2* mutations, and
3. the protein truncation test (PTT)

Several methodological challenges were encountered in reviewing this literature. The approach we developed for deriving clinical sensitivity estimates accounts for the conceptual problems underlying these difficulties and the empirical constraints resulting from the quality and nature of available data. A wide variety of different molecular techniques are used both for the point mutation/small rearrangement search and for the detection of large rearrangements. Therefore, resulting data are implicitly dependent on the various levels of analytical sensitivity associated with these methods, and it is thus problematic to combine values across investigations. To account for the limited analytical sensitivity of most techniques used and the possibility of misclassification of families in which all genetic tests were negative (likely a mixture of false negative and true negative results), our clinical sensitivity estimates of EX/SP screening are expressed as a range of values. The minimum value places all the tested families in the denominator, including those who tested negative (with the assumption that they actually carry mutations); the maximum value excludes all negative families from the denominator (by assuming they are truly negative). At the time of our analysis, the

literature on large *BRCA2* rearrangements was limited; our computations of clinical sensitivity of EX/SP screening methods in heterogeneous populations therefore apply only to *BRCA1* mutations. Finally, the studies we identified involved testing only affected cases from HBOC families, which precludes evaluation of clinical specificity.

Among eligible studies, a single, small-scale study used sequencing for all families and the estimated clinical sensitivity varies from 65 to 76.5%. A greater range of estimates is obtained from the studies which used other EX/SP screening methods. If point mutation/small rearrangement and large rearrangement/non-coding region screening were systematically combined, a higher level of clinical sensitivity would be reached; however, exhaustive testing for large rearrangements and non-coding region mutations is not routine in the clinical setting. A number of laboratories have recently switched to combining the DHPLC technique (to detect point mutations and small rearrangements) with the MLPA analysis (to detect large rearrangements), but there were not enough data in the literature we reviewed to examine a joint clinical sensitivity for this approach.

Large variations in estimates of clinical sensitivity of techniques designed to detect point mutations and small insertions/deletions in exons and exon-intron junctions are observed in the literature. These variations are in part related to founder effects for large deletions, described in the Dutch population, for example, in which large genomic rearrangements could contribute up to 38% of all clinically important *BRCA1* mutations in high risk families; they are also the result of differences in study selection criteria and in methods for estimating clinical sensitivity. Underlying these discrepancies is the lack of an unequivocal definition of clinical validity and of the reference phenotype, as well as the progressive and technique-dependant accrual of knowledge. The result is an as yet incomplete portrait of the distribution of *BRCA1/2* mutations in most populations.

In populations with strong founder effects, such as the Ashkenazi Jewish, Icelandic and Polish

populations, the testing protocol can be restricted to the search for the common founder mutations. Such testing strategies achieve comparable or even higher clinical sensitivity than the screening of all coding regions in heterogeneous populations. In Finland and Quebec, in contrast, several common founder mutations have been identified, but their prevalence is lower and their distribution could vary by region.

IMPACT OF TESTING ON RISK ASSESSMENT AND GENETIC COUNSELLING

The utility of testing is determined, in part, by the extent to which test results contribute to a more definite and precise risk assessment, both in terms of personal and familial risk. With respect to the establishment of an increased familial risk of breast and ovarian cancer, the discovery of a *BRCA1/2* mutation provides confirmation of an increased risk, information which may be useful for families with an *a priori* moderate risk on the basis of the pedigree. In families with an *a priori* high risk, molecular testing may not substantially modify breast or ovarian cancer risk assessment, even if the test result is inconclusive (negative), because of the limited clinical sensitivity of most techniques. The detection (or not) of a mutation may also affect the assessment of risk for other types of cancer (e.g., prostate, male breast cancer).

The impact of testing on the personal risk assessment within moderate to high risk families is a different issue, and depends on whether a mutation has previously been identified in the family or not. If a mutation has been previously documented in a given family, a negative test result for that particular mutation brings the test-negative individual's risk down to that of the general population, assuming that the test's analytical validity is high and that only one mutation runs in the family. This finding has clear implications in terms of clinical management in the sense of a 'demedicalisation' for the individual and her/his offspring. A positive test result, on the other hand, indicates a risk level clearly above that of the general population, but does not necessarily yield a

precise estimate of the age-specific or cumulative risk of developing breast and/or ovarian cancer. Precise individual estimates would require detailed knowledge about the joint impact of genetic and environmental risk factors on disease probability, which is unlikely to become available. For current counselling purposes, a wide range is usually provided for the cumulative risk of disease expression. In some founder populations (e.g., Ashkenazi Jewish), risk figures that are specific to particular mutations can be provided. In terms of consequences for family members, first-degree relatives can be informed of their 50% probability of having inherited the same mutation. This information may modify their risk perception and their interest in counselling and testing.

When a mutation has not been previously detected in the family, three outcomes are possible. If a mutation is detected in the index case and is known to be deleterious, the interpretation of the test result is not particularly problematic, since it should be considered as a true positive. In contrast, when a variant of unknown clinical significance (VUC) is detected, the *a priori* risk estimate remains applicable as long as the clinical significance of the sequence variation has not been clarified. This result should not be used to guide management decisions and tests are usually not offered to other family members. To clarify whether the sequence variation has any impact on cancer risk, different types of investigations—both laboratory-based and epidemiological—may be required (e.g., analysis of proteins, association studies). For the genetic counsellor, this situation is particularly complex, and for the family such an uninformative result is unlikely to relieve anxiety. The third possible outcome—when no DNA alteration is found—should be presented to the family as an ‘inconclusive’ result. Indeed, a *BRCA1/2* mutation may have been missed by the standard molecular techniques or the family may carry a mutation in another, yet undiscovered breast cancer susceptibility gene. The interpretation here is also complex because, depending on the family history, the post-test probability will either be reduced (but not to the

general population level) or will remain unchanged. In the latter case, decisions regarding clinical management would likely be based on the familial risk assessment.

The interpretation of a test result and its implications in terms of familial risk and personal management options are not straightforward, in view of the complexity and degree of uncertainty associated with some of these results. Interpretation of test results and estimation of post-test probabilities need to consider the pedigree information, the analytical and clinical validity of the technique used, and the penetrance and nature of the detected mutation. Services need to be in place to support patients and families adequately. Qualified personnel and ample time have to be dedicated to providing this information and to allow an opportunity for questions and clarifications.

CONCLUSION

In the process of reviewing the literature for this assessment important limitations in the evidence have been found. Major problems are the lack of a consensual definition of HBOC and the quality of the study designs and reporting of data, which are not up to epidemiological standards for molecular test evaluation studies. The variability in the study population selection criteria and in the testing protocols for molecular testing complicate the synthesis of the evidence. This variability is particularly striking in the literature on prevalence, penetrance and clinical validity. Current knowledge in areas such as the distribution of *BRCA1/2* mutations is dependent on the evolution of molecular techniques and testing criteria. Both prevalence and penetrance have been studied more thoroughly in high risk families and in some founder populations than in families at moderate or low risk. Residual uncertainties and gaps in current knowledge have implications regarding decision-making for individuals and families, for health care providers and for policy makers.

Among the conditions put forward by the American Society of Clinical Oncology for molecular testing in cancer genetics in general, the evidence was reviewed with respect to the

assessment of prior (pre-test) risk based on family history and on the informativeness of test results for the post-test updating of the risk assessment. As far as current practices are concerned, the majority of families considered eligible for testing in cancer genetics clinics (typically families with two or three affected individuals) are not found to carry a *BRCA1/2* mutation. Such a result is considered inconclusive and the residual risk of cancer remains higher than that of the general population. Providing precise post-test probabilities to these families is, however, difficult because good data on clinical negative predictive value are lacking. Another inconclusive test result which represents a challenging task for geneticists and genetic counsellors, and does not substantially alleviate a family's anxiety, is the discovery of a variant of unknown clinical significance. This situation is relatively frequent, possibly almost as frequent as a positive test finding, but may be resolved over time as new knowledge about these variants accrues. In practice, for the very high risk families, the pre-test (prior) risk will not be substantially modified by an inconclusive test result.

Testing primarily benefits families in which a *BRCA1/2* mutation has been discovered. For unaffected relatives that undergo testing and are found not to carry the mutation present in the family, breast cancer risk drops from a high prior probability to a post-test risk comparable to that in the general population. Unaffected

relatives in whom a mutation is identified, on the other hand, have a substantially higher cancer risk than that of the general population. In addition, individuals with breast or ovarian cancer in whom a *BRCA1/2* mutation is identified are at increased risk of developing a second cancer. For families in which a *BRCA1/2* mutation is identified, both positive and negative test results have implications for clinical management, either in the sense of a 'demedicalisation' or of increased requirements for surveillance, prevention or prophylactic measures. A review of the effectiveness of these interventions did not fall within the purview of this assessment.

Consequently, a balanced view of benefits and risks, and formal recommendations with respect to the use of *BRCA1/2* mutation testing, cannot be presented at this time. It is, however, already apparent that the balance of benefits and risks will be heavily dependent on the prior risk assessment and that the knowledge base to derive decisions is stronger for high risk families than for populations at lower risk. In the subsequent AETMIS report, the forthcoming evidence from other systematic reviews on issues such as the analytical validity of the molecular tests, psychosocial consequences of testing and the effectiveness of available interventions, will be integrated with our further analysis of the economic and organisational issues surrounding cancer genetic services.

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Glossary²

Alleles

Alternate forms of a given gene that differ in their nucleotide sequence.

Amino acids

Aminocarboxylic acids that form the building blocks of protein and peptides, for which DNA carries the genetic code.

Analytical sensitivity of molecular genetic tests

The proportion of individuals with the genotype of interest (the mutations detectable by the test) in whom the test will be positive. Analytical sensitivity refers to the ability of the test to detect the mutations it was designed to detect.

Analytical specificity of molecular genetic tests

The proportion of individuals who do not have the genotype of interest (the mutations detectable by the test) in whom the test will be negative. Analytical specificity refers to the ability of the test to detect only the mutations it was designed to detect.

Autosome

Any chromosome other than a sex chromosome (in humans there are normally 22 pairs of autosomes).

Base

One of the building block molecules that form DNA and RNA. In DNA, two bases (adenine and thymine or guanine and cytosine) held together by weak bonds are called *base pairs*. The two strands of DNA are held together in the shape of a double helix by the bonds between base pairs.

cDNA

Complementary DNA or copy DNA; synthetic DNA transcribed from a specific RNA through the action of the reverse transcriptase enzyme.

Chromosome

The genetic material contained in the cell nucleus and consisting of DNA. In humans, the genetic material is normally divided into 46 chromosomes, 22 pairs of autosomes and two sex chromosomes.

Clinical sensitivity of molecular genetic tests

The proportion of individuals with the phenotype of the disease (or who will develop this phenotype) in whom the test will be positive. A more theoretical interpretation of this definition is sometimes used by considering only the proportion of individuals with the phenotype of the disease (or who will develop this phenotype) in whom the mutations targeted by the test are present. This definition represents the upper limit of clinical sensitivity when the analytical sensitivity is 100%.

2. Sources: Blancquaert and Caron [2002]; Thompson *et al.*, [1991]; website for the genome programs of the US Department of Energy Office of Science: http://www.ornl.gov/sci/techresources/Human_Genome/glossary/ (Human Genome Project Information glossary accessed August 2 and 8, 2005).

Clinical specificity of molecular genetic tests

The proportion of individuals who do not have the phenotype of the disease (or who will not develop this phenotype) in whom the test will be negative. Clinical specificity is the probability that the molecular test will be negative in individuals that do not express the phenotype of interest.

Codon

A triplet of three nucleotides in a DNA or RNA molecule, specifying a single amino acid.

Deletion

The loss of one or more consecutive base pairs without a break in the continuity of the DNA molecule.

DNA (deoxyribonucleic acid)

The genetic material contained in the chromosomes within the cell nucleus and mitochondria. DNA consists of two chains made up of substances called *nucleotides* and coiled into a double helix. There are four types of nucleotides, and each can pair only with one other, the sequences on both DNA strands being complementary.

DNA polymerase

An enzyme that catalyzes the synthesis of DNA using preexisting templates, assembling DNA from nucleotides.

Dominant

An allele is dominant when it is expressed even in the heterozygous state, i.e., when present on only one of the two homologous chromosomes. The carrier of a dominant disorder has inherited the mutation from one of his or her parents, unless there was a new mutation. Each child of a carrier has a probability of 50% of inheriting the mutated gene.

Exon

A gene sequence whose transcript persists in the final messenger RNA and which can therefore be translated into a polypeptide chain. Each gene contains several noncontiguous exons, which are separated by introns.

First degree relative

An individual who is expected to share 50% of her/his genes with a given case (parent, sibling or child).

Founder effect

The occurrence of a higher frequency of one or more specific mutations (called *founder mutations*) in the descendants of a group of common ancestors, as a result of geographic and/or ethnic isolation.

Gene

A physical and functional unit of heredity, being a sequence of nucleotides situated at a specific locus on a given chromosome and coding for specific functions.

Genetic linkage

The co-segregation of two or more alleles over the generations because of the physical proximity of their loci on the genome.

Genetic marker

Any variation in the DNA sequence creating different alleles at a given locus and used to identify this locus.

Genetic test

A test for detecting a mutation, a defective gene, an abnormal protein, a chromosomal abnormality or the presence of a genetic marker near or in a gene.

Genetics

The study of the structure, regulation, expression, transmission and frequency of genes, and of the pathologies associated with structural defects in genes.

Genome

All the genetic material in the chromosomes of a particular organism; its size is generally given as its total number of base pairs.

Genotype

An individual's genetic makeup, as contrasted with his or her phenotype.

Germ cell

Sperm and egg cells and their precursors. Germ cells normally have only one set of chromosomes (23 in all), while all other cells have two copies (46 in all).

Haplotype

A group of alleles from closely linked loci, usually inherited as a unit.

Heterozygosity

A genotypic situation in which two homologous loci in a given chromosome pair each carry a different allele.

Homozygosity

The presence of the same allele on both chromosomes in a given chromosome pair. By extension, is also said of the genotype of individuals who have inherited a double dose of an abnormal allele, whether the mutated version is the same or different on each chromosome.

Index case

An affected family member who first draws attention to a pedigree.

Insertion

The presence of one or more additional consecutive base pairs without a break in the continuity of the DNA molecule.

Intron

A DNA sequence transcribed and subsequently eliminated by splicing during RNA processing.

Kilobase (kb)

In the case of DNA, 1,000 base pairs (bp); in the case of RNA, 1,000 bases.

Locus

The position of a DNA segment on a chromosome. A locus is defined by its information content (gene) or its sequence, whether the latter is polymorphic or not.

Lod score

Result from a statistical method that tests genetic marker data in families to determine whether two loci are linked. The lod score is the logarithm to base 10 of the odds in favour of linkage. By convention a lod score of 3 (odds of 1000:1 in favour) is taken as statistical evidence of linkage.

Mendelian trait or disease

A characteristic or disease due to a single gene transmitted by a simple pattern of inheritance (e.g., autosomal dominant, autosomal recessive, X-linked).

Messenger RNA

RNA that serves as a template for protein synthesis.

Mutation

Any change occurring in the DNA sequence that can result in pathological manifestations. If the change involves only one base, one speaks of a *point* mutation. If a mutation occurs in a germ cell, it can be passed onto subsequent generations. A gene that has undergone mutation is called a *mutant*.

Negative predictive value

The probability that individuals with negative test results will not develop the disease.

Nucleotides

Basic units of DNA and RNA structure. Nucleotides consist of a phosphorylated sugar (ribose or deoxyribose) bound to a base. Two purine bases, adenine (A) and guanine (G), and two pyrimidine bases, cytosine (C) and thymine (T), are the constituents of DNA. In RNA, thymine is replaced by uracil (U). Each strand of DNA consists of a sequence of nucleotides, whose bases pair two by two (adenosine with thymine and guanine with cytosine) to form the DNA double helix.

PCR (polymerase chain reaction)

The selective amplification of a sequence of double-stranded DNA carried out *in vitro* by the iterative extension of two primers, one on either side of the region of interest, using a DNA polymerase.

Amplification is carried out by repeated cycles of denaturation, annealing and extension, which result in the logarithmic replication of each strand.

Pedigree

A family tree diagram that shows which family members have been diagnosed with a disease and how a particular genetic trait or disease has been inherited.

Penetrance

The percentage of individuals that have a specific genotype and in whom the phenotype associated with this genotype is expressed. The cumulative risk of disease expression at a specific age given a carrier genotype.

Phenotype

The outward manifestation of the makeup of the genome in the form of a morphological trait, clinical syndrome or physiological characteristics, such as a qualitative or quantitative variation in the final product expressed by a gene (i.e., protein or metabolites).

Polymorphism

The co-occurrence in a population of two or more alternative alleles which lead to indistinguishable phenotypes, each with appreciable frequency.

Polypeptide

A protein or part of a protein made of several chains of amino acids.

Prevalence

The ratio of the number of individuals with a disease over the number of individuals in a given population at a given point in time.

Primer

A DNA sequence of approximately 15 to 30 nucleotides (an *oligonucleotide*) serving as an anchor and starting point for the replication, by DNA polymerase, of a specific DNA sequence during PCR.

Proband

The family member through whom a family is ascertained. If affected, may be called the *index case*.

Point mutation

A single nucleotide base pair change in DNA.

Positive predictive value

The probability that people with positive test results will develop the disease.

Probe

A nucleic acid sequence, usually at least 15 nucleotides in length, homologous to a DNA or an RNA sequence, to which it anneals in a stable and specific manner by re-association between complementary nucleotides.

Restriction enzymes

Bacterial endonucleases that specifically cleave the two DNA strands at a particular DNA sequence (4 to 8 nucleotides in length), referred to as a *restriction site*.

Restriction site

The double-stranded DNA sequence specifically recognized and cleaved by a given restriction enzyme.

RNA (ribonucleic acid)

Nucleic acid formed upon a DNA template containing ribose sugar instead of deoxyribose sugar.

Second degree relative

An individual who is expected to share 25% of her/his genes with a given case (grandparent, grandchild, aunt/uncle, niece/nephew, or half-sibling).

Sensitivity

A measure of a diagnostic or screening test's ability to correctly detect people with a given target condition (be it a disease or a risk factor). The proportion of all persons with the target condition for whom the test is positive.

Specificity

A measure of a diagnostic or screening test's ability to correctly identify people without a given target condition (be it a disease or a risk factor). The proportion of all persons free of the target condition for whom the test is negative.

Splice site

Location in the DNA sequence, at the interface of exons and introns, where non-coding sequences (introns) are removed to form the mature messenger RNA for translation into a protein (this action is called *splicing*).

Transcript

The first product resulting from the synthesis of an RNA copy of a DNA sequence. This action is called *transcription*.

5' untranslated region

Region that contains the molecular “start” signals for the synthesis of mRNA transcribed from a gene.

3' untranslated region

Region that contains the molecular “stop” signals for the synthesis of mRNA transcribed from a gene.

Abbreviations

AJ	Ashkenazi Jewish
ASCO	American Society of Clinical Oncology
ASO	Allele-specific oligonucleotide
BCLC	Breast Cancer Linkage Consortium
Br	Breast
<i>BRCA1</i>	Breast cancer gene 1
<i>BRCA2</i>	Breast cancer gene 2
BrOv	Breast and ovarian
ca	Cancer
CDGE	Constant denaturant gel electrophoresis
cDNA	Complementary DNA
CI	Confidence interval
CGSC	Cancer Genetics Studies Consortium
CSGE	Conformational sensitive gel electrophoresis
DDF	Dideoxy fingerprinting
<i>del</i>	Deletion
DGGE	Denaturing gradient gel electrophoresis
DHPLC	Denaturing high performance liquid chromatography
DNA	Deoxyribonucleic acid
DSA	Direct sequence analysis
DSDI	Detection of small deletion and insertion
dx	Diagnosis
EMD	Enzymatic mutation detection
EX/SP	Exon and splice site junction
FC	French Canadian
F-CSGE	Fluorescent conformation sensitive gel electrophoresis
FDR	first degree relative
FLA	Fragment length analysis
F-MD	Fluorescent mutation detection
FHAT	Family History Assessment Tool
FS	Frame shift
HDA	Heteroduplex analysis
HBC	Hereditary breast cancer

HBOC	Hereditary breast and ovarian cancer
hx	History
<i>ins</i>	Insertion
IR	Inferred regulatory
LHR	Loss of heterozygosity at the RNA level
LOH	Loss of heterozygosity
LR	Large rearrangement
MLPA	Multiplex ligation-dependent probe amplification
mRNA	Messenger ribonucleic acid
MS	Missense
MS-PCR	Multiplex mutagenically separated PCR
NPV	Negative predictive value
NC	Non-coding mutation screening
nr	Not reported
NS	Nonsense
OCCR	Ovarian cancer cluster region
OR	Odds ratio
Ov	Ovarian
PCR	Polymerase chain reaction
PPV	Positive predictive value
PTT	Protein truncation test
R	Regulatory
RNA	Ribonucleic acid
RR	Relative risk
SA	Sequence analysis
SD	Standard deviation
SEQ	Sequencing
SSCA	Single-strand conformation analysis
SSCP	Single-strand conformation polymorphism
SSCP/HA	Single-strand conformation polymorphism combined with heteroduplex analysis
SP	Splice site
TDGS	Two-dimensional gene scanning
UTR	Untranslated region
VUC	Variant of unknown clinical significance
+ve	positive result
-ve	negative result

1. Introduction

1.1 BREAST AND OVARIAN CANCER

Breast cancer is the second leading cause of death in Canadian women after lung cancer, with 5,400 women expected to die from the disease in 2003 [Bryant, 2004]. Of invasive cancers, breast cancer remains the most often diagnosed, with more than 20,000 new diagnoses yearly. As of 1998, Canadian women had a lifetime risk of about 11.4% or 1 in 9 to develop the disease [Bryant, 2004]. The mortality rate has fallen by about 15% in the past 30 years.³ The known risk factors for breast cancer include older age, family history of breast cancer, country of birth, reproductive and hormonal factors (early menarche, late menopause and late age at first full-term pregnancy), lifestyle and environmental factors (e.g., alcohol consumption, obesity, hormone replacement therapy), and the presence of benign breast lesions (e.g., atypical hyperplasia) [Bryant, 2004; Singletary, 2003].

Ovarian cancer has a lower incidence than breast cancer but a higher case-fatality rate. Lifetime risk of ovarian cancer in North America is estimated as 1.4% [Chappuis and Foulkes, 2002], but most cases are diagnosed when the disease is at an advanced stage, less amenable to treatment. This is due to the insidious and non-specific nature of the symptoms, and to the difficulty of physically examining the ovaries [Gladstone, 1994]. In Canada, 2,600 women were diagnosed with ovarian cancer in 1999 and 1,500 died of the disease [Elit, 2001]. Important reproductive risk factors for ovarian cancer are infertility, nulliparity, early menarche and late menopause.

For both breast and ovarian cancer the strongest risk factor, after adjusting for age, is family history [Chappuis and Foulkes, 2002]. Breast cancer risk estimates have been generated by a

re-analysis of data from 52 cohort and case-control studies with data on family history of breast cancer in first-degree relatives⁴ (FDR) [CGHFBC, 2001]. The cumulative incidence of breast cancer by age 80 for women without affected FDR is estimated as 7.8%. In women with one and two affected FDRs, the risk increases to 13.3% and 21.1%, respectively [CGHFBC, 2001]. In an earlier meta-analysis, the relative risks for breast cancer were estimated as 3.6 in women with an affected mother and sister (5 studies) and 2.1 for at least one affected FDR (38 studies) [Pharoah *et al.*, 1997]. For ovarian cancer, the lifetime incidence is estimated as 5%, or 3.6 times the general population risk, when only one FDR is affected with the disease. The ovarian risk may be as high as 30-40% in women who have two or more affected FDRs [Elit, 2001; Langston and Ostrander, 1997].

1.2 CLASSIFICATION OF HEREDITARY BREAST AND OVARIAN CANCER SYNDROMES

In 1866, the French surgeon Paul Broca was the first to present a detailed pedigree showing a pattern of familial breast cancer [Lynch *et al.*, 2004]. Several reports in the early 1970s suggested transmission of a single, dominant gene in families with many cases of breast cancer over multiple generations [Iau *et al.*, 2001]. The first documentation of a possible genetic association between breast and ovarian cancer that was consistent with an autosomal dominant mode of inheritance seems to be the account by Lynch and Krush in 1971 [Lynch *et al.*, 2004]. Subsequently, an association between breast cancer and other cancers has been described in certain families. Associated cancers and inheritance mode have been used to classify families with cancer cases according to different clinical syndromes thought to be related to genetic susceptibility.

3. This reduction appears to be due to better survival in affected women but it is unclear whether screening or improved treatment explains this trend [Bryant, 2004].

4. A female first degree relative could be the mother, a sister, or a daughter.

Tables 1 and 2 present classifications of hereditary breast cancer syndromes and hereditary ovarian cancer syndromes, respectively, according to the associated clinical manifestations and gene(s). Hereditary breast cancer syndromes can be divided into three main groups (Table 1): i) site-specific breast cancer or hereditary breast cancer (HBC); ii) breast and ovarian cancer or hereditary breast and ovarian cancer (HBOC); and iii) other rare syndromes (Li-Fraumeni, Peutz-Jeghers, Cowden, Ataxia telangiectasia). Hereditary ovarian cancer syndromes⁵ can also be divided into three main groups (Table 2): i) site-specific ovarian cancer or hereditary ovarian cancer (HOC); ii) breast and ovarian cancer or HBOC; and iii) in association with colorectal cancer (HNPCC).

With the development of molecular genetics, intense efforts have been devoted to the discovery of the genes associated with the above clinical syndromes. The two main genes that confer susceptibility to breast and ovarian cancer are the BREast CAncer gene 1 (*BRCA1*) and the BREast CAncer gene 2 (*BRCA2*). The mutations in these genes are associated with both the Hereditary Breast and Ovarian Cancer (HBOC) and the Hereditary Breast Cancer (HBC) syndromes, and can also be involved in cases of cancer that appear to have arisen outside of such syndromes, as in a single case of cancer in a family (as will be discussed further below). These syndromes are essentially defined by the pattern of occurrence of cancers in the family and their age at onset. A restrictive definition of HBOC was proposed early on by one of the first collaborative projects in this field.⁶ More relaxed criteria have been used since and no widespread consensus on this issue has been adopted in the scientific community. Furthermore, most studies have included both HBOC and HBC families. The present assessment will focus on a systematic review of the literature concerning *BRCA1/2* mutation testing in HBOC and HBC, understood in a

broad sense, without selecting studies on the basis of the definition of HBOC or HBC. For ease of reading, we will use the term HBOC to designate both HBOC and HBC unless otherwise stated (when discussing details about family history, for instance). In order to reflect both current clinical practice and the emphasis in the scientific literature, we have also systematically reviewed the available literature on other groups thought to be at high risk for a genetic susceptibility, notably for certain populations in which a founder effect is suspected.

The germline mutations in the *BRCA1* or *BRCA2* genes are transmitted via an autosomal dominant mode of inheritance. Individuals who have a germline mutation in the *BRCA1* or *BRCA2* genes have a 50% chance of transmitting the mutant allele to their children.

1.3 CONTRIBUTION OF HEREDITARY FACTORS TO BREAST AND OVARIAN CANCER

It is generally agreed that the majority of breast and ovarian cancer cases arise in persons with no relatives with cancer (that is, no family history). We refer to such cases as *sporadic* in this monograph. The proportion of cases that are *familial* (that is, arising in persons with a family history of cancer), and the proportion of cases that are *hereditary* (that is, with a pedigree thought to be related to the transmission of mutations in a single gene) are still the object of some debate. The figures most often cited are that approximately 1/3 of breast cancer is familial and that hereditary cases account for 5% of all cancer cases. In this context, familial cases are defined by the presence of at least two cases of breast cancer,⁷ at any age, in first-or second-degree relatives⁷ [Nathanson *et al.*, 2001].

5. There are other hereditary syndromes that include ovarian cancer but these confer a lower risk of the disease and are very rare [Chappuis and Foulkes, 2001].

6. The Breast Cancer Linkage Consortium (BCLC) has proposed a working definition of a hereditary breast and ovarian cancer (HBOC) family: a family containing at least 4 or more cases of early-onset breast cancer (diagnosed at age <60 years) or ovarian cancer (diagnosed at any age), and with at least 1 case of ovarian cancer [Iau *et al.*, 2001].

7. A first degree relative is an individual who is expected to share 50% of her/his genes with a given case (parent, sibling or child). A second degree relative is an individual whom is expected to share 25% of her/his genes with a given case (grandparent, grandchild, aunt/uncle, niece/nephew, or half-sibling).

Table 1.
Hereditary breast cancer syndromes* (adapted from Chang-Claude [2001]; Robson *et al.* [2001])

SYNDROME	CLINICAL MANIFESTATIONS	GENE(S)
Hereditary breast cancer (HBC)	Breast cancer	<i>BRCA1/2</i>
Hereditary breast and ovarian cancer (HBOC)	Breast, ovarian cancer	<i>BRCA1/2</i>
Li-Fraumeni	Sarcoma; cancer of the brain, thyroid, breast; adrenocortical tumours; acute leukemia	<i>TP53</i>
Peutz-Jeghers	Melanocytic macules of the lips; polyps; cancer of the breast, ovaries, intestinal tract etc.	<i>STK11/LKB1</i>
Cowden	Multiple hamartomatous lesions of the skin and mucous membrane; breast and thyroid cancer	<i>PTEN</i>
Ataxia telangiectasia	Progressive cerebral ataxia, hypersensitivity to radiation, increased risk of cancer	<i>ATM</i>

Table 2.
Hereditary ovarian cancer syndromes* [Chappuis and Foulkes, 2001]

SYNDROME	CLINICAL MANIFESTATIONS	GENE(S)
Hereditary ovarian cancer (HOC)	Ovarian cancer	<i>BRCA1</i>
Hereditary breast and ovarian cancer (HBOC)	Breast, ovarian cancer	<i>BRCA1/2</i>
Hereditary nonpolyposis colorectal cancer (HNPCC)	Colorectal, endometrial, stomach, ovarian, urinary tract, small bowel cancer	<i>Mismatch repair genes (MLH1, MSH2, MSH6, PMS2)</i>

Markedly different approaches have been used to determine the contribution of genetic factors to breast cancer. Early on, most studies initiated within the clinical and research genetics community focussed on HBOC families with multiple cancer cases. In parallel, some epidemiological studies on breast cancer cases collected information on cancer occurrence among close relatives. More recently, with the development of genetic epidemiology, pedigree information was collected more thoroughly and systematically in ‘population-based families’ [Hopper, 2001]. The latter approach, as well as extensive statistical modelling, reflect the efforts being made to develop a coherent understanding of the familial clustering of breast cancer, since bridging the information obtained from the other sources did not turn out to be a straight-forward undertaking.

Early segregation analyses, carried out before the identification of the *BRCA1/2* genes. In order to examine which patterns of inheritance best fit to the clustering of breast cancer cases within families, these studies suggested that the overall contribution of highly penetrant, autosomal dominant genes is limited, accounting for not more than between 4 and 7% of all breast cancer in the population [Iau *et al.*, 2001; Robson *et al.*, 2001]. Studies estimating the proportion of breast and ovarian cancer cases in the general population carrying *BRCA1/2* mutations yield a wider range of values, as outlined in section 5.1. To date, *BRCA1/2* genes remain the major highly penetrant genes known to be involved in hereditary breast cancer; the total contribution of all the other known genes with high penetrance listed in Tables 1 and 2 is estimated as less than 1% [Chang-Claude, 2001].

The re-analysis of data from 52 association studies (cohort and case-control designs) on breast cancer yielded estimates of the proportion of breast cancer cases with affected first-degree relatives (FDRs). The resulting estimate was that 12.9% of women with breast cancer have one or more FDRs with breast cancer [CGHFBC, 2001]. The collaborative study counts only first degree, and not second degree, relatives and family history of cancer in FDRs may not take the history of cancer on the paternal side into account.⁸

Clustering of cancer cases in families does not imply that genetic susceptibility is always present, since environmental factors and chance can also play a role. Conversely, the presence of a genetic susceptibility will not always translate into the occurrence of multiple cancer cases within one family. Population-based family studies have shown that cancer cases arising in *BRCA1/2* mutation-carrying families only rarely present themselves as hereditary cases. In fact, most will actually fall in the category of sporadic cases, arising in individuals without a family history of cancer [Hopper, 2001].

It remains difficult to present a clear picture of the relative contribution of each known susceptibility gene to familial breast and ovarian cancer, but the model that has gained the widest acceptance is that the minority of cases are related to a few highly penetrant susceptibility genes—the most frequent being *BRCA1/2*—and that the majority are explained by other causes, such as low penetrance susceptibility genes and/or environmental factors [Hodgson *et al.*, 2004; Houlston and Peto, 2004; Narod and Foulkes, 2004; Pharoah *et al.*, 2004; Wooster and Weber, 2003; Pharoah *et al.*, 2002; Hopper, 2001; Nathanson *et al.*, 2001]. Assuming that a large number of yet undiscovered low risk genes also confer cancer susceptibility, it has been estimated using segregation analysis that 50% of all breast cancer cases arise from the minority of the general population at highest risk (that is, the 12% of the population with a lifetime risk of 10% or greater by age 70) [Pharoah *et al.*, 2002].

8. J. Simard, personal communication, May 30, 2005.

However, such an estimate relies heavily on various statistical assumptions related to the segregation analysis and distribution of breast cancer risk in the population.

1.4 OVERVIEW OF CURRENT CHALLENGES

The discovery of the *BRCA1/2* genes and the identification of mutations in families at high risk of developing breast and ovarian cancer have raised enormous hopes with regard to the understanding of carcinogenic mechanisms, the ability to refine risk assessment and to tailor preventive and prophylactic management to needs, and the development of new therapeutic measures.

Research efforts have involved numerous teams from several countries, in a very competitive dynamic. Alliances have been established across centres,⁹ but no globally concerted effort has been organised to coordinate research endeavours. Despite the complexity of this field, spectacular progress has been made in certain domains (such as the understanding of carcinogenesis), while other areas of research have lagged behind. The transition between research and clinical practice has proceeded in a rather disorderly manner and, until recently, few initiatives had been mounted to standardise clinical and laboratory practices. The patents granted on these genes and the monopolistic licensing practices may have had negative consequences on both research and services, as initially anticipated by American and European professional societies. [Clinical Molecular Genetics Society (CMGS), 1999; American College of Medical Genetics (ACMG), 1999a]

The gaps in the current state of knowledge with respect to *BRCA1/2* have implications not only for patients and families, but also for service delivery and organisation. Uncertainties persist in areas such as the full spectrum of mutations,

9. The Breast Cancer Linkage Consortium (BCLC), the Breast Cancer Information Core (BIC), the Interdisciplinary Health International Research Team on Breast Cancer Susceptibility (INHERIT BRCA), financed by the Canadian Institutes of Health Research since 2001), and the Breast Cancer Family Registry (financed by the National Cancer Institute in USA since 1995) are examples of some of these initiatives.

the penetrance estimates, the precise contribution of hereditary factors to the burden of breast and ovarian cancer, the analytical validity of tests, the interactions between environmental and genetic risk factors, and the impact of surveillance and preventive measures for *BRCA1/2* mutation carriers. As a result, the development of a consensus around testing indications, the choice of optimal testing protocols, the definition of molecular strategies that are coherent with the regional distribution of mutations, the optimization of service delivery to minimise risks to patients and families—while taking into consideration the available resources—are all challenges that will have to be faced in the near future.

1.5 OBJECTIVES OF THIS ASSESSMENT

The present monograph is part of a broader project that strives to analyse the knowledge base needed to address some of the challenges outlined above. As a first step, this assessment focuses on the initial questions that individuals, families and health care providers are faced with when envisioning the use of molecular tests for hereditary breast and ovarian cancer:

1. For whom should testing be considered? and
2. What information do the test results provide?

The first question refers to testing indications, which are in part based on who is most likely to carry a *BRCA1/2* mutation, or, in other words, in which types of families or individuals the highest prevalence of mutations has been found. The second question relates to the added-on value of the test result in terms of risk assessment for the individual and family. The difference between the prior and post-test risk assessment depends on the test's clinical validity, which is partly driven by the mutations' penetrance, that is, the cumulative risk of developing disease expression when a mutation is present. These questions are not only relevant to personal and clinical decision-making, but also to policy-making, since the breadth of testing indications will largely determine future use, costs, and resource requirements to organise clinical services in this field.

To provide insight into these questions, a systematic literature review was conducted with respect to:

1. the prevalence of *BRCA1/2* mutations in HBOC families, cancer cases, and founder populations,
2. the penetrance associated with *BRCA1/2* mutations,
3. the available risk assessment models,
4. existing guidelines as to testing indications, and
5. the clinical validity of the available molecular tests.

Background information on the evolution of knowledge about the genetics of breast and ovarian cancer will be provided first, as well as an overview of the molecular methods in current use. The concepts involved in the assessment of molecular tests, such as analytical and clinical validity, are introduced, and the methodology underlying the systematic review is presented. The findings from the systematic review of the five topics listed above are summarised. Finally, the evidence retrieved from the systematic review is integrated to provide an understanding of the contribution of molecular tests to risk assessment and genetic counselling of individuals and families with HBOC or other risk factors for genetic susceptibility, and to situate these findings in the broader picture of decision- and policy-making issues related to the clinical use of *BRCA1/2* mutation testing.

Of note, this assessment will not systematically review the evidence on the analytical validity or technical performance of available tests, the psychosocial consequences of testing, the impact of testing on clinical management, the ethical and legal issues raised by the introduction of these tests in clinical practice, and the organisational and economic implications of their integration in the health care system. A few recent systematic reviews are available on selected issues [Kmet *et al.*, 2004; Lostumbo *et al.*, 2004] and ongoing projects are expected to

help provide a more comprehensive view of the evidence for these other topics (CCOHTA, FBR, USPSTF).¹⁰ Conclusions of these reviews will be taken into account in a follow-up to the present assessment, which is in preparation and will cover issues of particular concern to policy makers in the province of Quebec. This companion report¹¹ will include a review of organisational modalities of cancer genetic services in different jurisdictions, a review of the impact of models of care delivery on psychosocial consequences of testing, and a comparative cost analysis for different organisational modalities.

10. Foundation for Blood Research (FBR). Family history and *BRCA1/2* testing for identifying women at risk for inherited breast/ovarian cancer (preliminary report) (www.cdc.gov/genomics/gtesting/ACCE/FBR.htm); CCOHTA (Canadian Coordinating Office for Health Technology Assessment). Systematic review of *BRCA1* and *BRCA2* genetic testing for breast and ovarian cancer susceptibility (in preparation); US Preventive Services Task Force/Agency for Healthcare Research and Quality (USPSTF/AHRQ). Genetic risk assessment and *BRCA* mutation testing for breast and ovarian cancer susceptibility. Evidence Synthesis no. 37, 2005 (www.ahrq.gov/clinic/upstf/uspbngen.htm).

11. AETMIS. Cancer genetic services: Economic and organisational issues (in preparation).

2. Genetics of hereditary breast and ovarian cancer

2.1 *BRCA1* AND *BRCA2* GENES

2.1.1 Identification of the *BRCA1/2* genes

The localisation and identification of the *BRCA1/2* genes are the result of years of collaborative efforts from many laboratories around the world and the participation of numerous families with multiple cases of breast cancer. Many of these studies were carried out by an international network of scientists, known as the Breast Cancer Linkage Consortium (BCLC). Associations between breast and ovarian cancer and the existence of male breast cancer were key elements in the localisation of the *BRCA1* and *BRCA2* gene, respectively [Narod and Foulkes, 2004]. Genetic linkage analysis was used to localise *BRCA1* on chromosome 17q in 1990 and *BRCA2* on chromosome 13q in 1994 [Wooster and Weber 2003; Hall *et al.*, 1990]. The *BRCA1* gene was identified using labour-intensive strategies almost four years after its localisation [Miki *et al.*, 1994]. In comparison, information on the human genome sequence from the Human Genome Project greatly reduced the time needed to isolate *BRCA2* by Wooster and colleagues [1995] and Tavtigian and colleagues [1996].

Between December 1997 and September 2000, the U.S. Patent and Trademark Office awarded several patents on the *BRCA1* and *BRCA2* genes to Myriad Genetic Laboratories. These patents cover all possible uses derived from the knowledge of the gene sequence, including any methods to detect mutations in these genes. In Canada, patents were granted to Myriad Genetic Laboratories in October 2000 on *BRCA1* and in April 2001 on *BRCA2*, together with a second patent on *BRCA1* relative to a method to detect the existence of a predisposition for breast and ovarian cancer.

In Europe, Myriad Genetic Laboratories was also granted several patents on *BRCA1* and

BRCA2 genes between 2001 and 2004 by the European Patent Office (EPO). However, opposition procedures against the granting of these patents were initiated in October 2001, first by genetic societies and followed by several European governments. In May 2004, Myriad's first patent¹² on *BRCA1* covering "a method for diagnosing a predisposition for breast and ovarian cancer" was revoked. In January 2005, the EPO ruled that the breadth of the second¹³ and third¹⁴ patents were to be significantly reduced¹⁵. As for *BRCA2*, both Myriad Genetic Laboratories¹⁶ and the Cancer Research UK¹⁷ charity were awarded patents. The patent owned by the Cancer Research UK charity covers more applications, placing the organization in a powerful position. Following opposition procedures regarding Myriad's patent on *BRCA2*, its scope was voluntarily reduced and the EPO thus maintains a reduced version of that patent as of June, 2005. This patent now covers only the use of the *BRCA2* sequence that applies to one of the Ashkenazi Jewish founder mutations. It seems that these patents should no longer interfere with DNA testing for *BRCA1* in Europe (nor *BRCA2*, in the non-Ashkenazi Jewish population). Indeed, all reference to "diagnostic method" seems to have disappeared from these patents.

12. EP699754.

13. EP705903.

14. EP705902.

15. Originally, the second patent claimed a right to one of the Ashkenazi Jewish founder mutations (the larger claim to the sequence and a set of 34 mutations had been reduced by the patent owners prior to the oral hearings) and the third patent was a product patent which claimed the *BRCA1* sequence and all possible applications. These patents will now be restricted to just one probe, in addition to a vector and a host cell containing these isolated nucleic acids.

16. EP785216.

17. EP858467. The Cancer Research UK charity has declared publicly that its patent is available without any charge to public laboratories.

2.1.2 Mutations in the *BRCA1/2* genes

BRCA1 and *BRCA2* are very large genes¹⁸. Since the cloning of *BRCA1* and *BRCA2*, more than a thousand mutations have been identified in these genes, the majority being frameshift mutations¹⁹ that result in a non-functional, truncating protein²⁰ [Narod and Foulkes, 2004].

The Breast Cancer Information Core (BIC) was established in 1995 as a central repository for information regarding mutations in the *BRCA1/2* genes. Besides information on mutations with known deleterious effects, this database also contains information on polymorphisms (common alterations with no deleterious effects) and on variants of unknown clinical significance (VUCs)²¹. Over 300 VUCs have been reported to the BIC database and these pose a challenge for the interpretation of their effects, if any, at the clinical level [Narod and Foulkes, 2004].

In the past 10 years, much research has gone into the identification of families with breast or ovarian cancer which harbour a mutation in the *BRCA1/2* genes [Narod and Foulkes, 2004], and the characterization of mutations in different populations. Recurrent *BRCA1/2* mutations have been found in several ethnic groups. For example, as early as 1994, Simard and colleagues identified two of the three Ashkenazi Jewish founder mutations (*BRCA1* 185delAG and 5382insC) in a small sample of Quebec families [Narod and Foulkes, 2004; Simard *et al.*, 1994]. Due to the high prevalence of founder *BRCA1/2* mutations, the Ashkenazi Jewish community has been studied in depth,

particularly with respect to mutation penetrance [Narod and Foulkes, 2004].

2.1.3 Function of the *BRCA1/2* proteins

Slight homology exists between the *BRCA1* and *BRCA2* proteins, and both have a function in DNA repair. Various other cellular mechanisms are associated with the 220-kilodalton *BRCA1* protein such as control of the cell cycle, protein degradation, and transcription regulation [Narod and Foulkes, 2004]. *BRCA1/2* functions do not appear to be specific to breast tissue. Why cancer susceptibility associated with *BRCA1/2* is tissue specific is unclear, although some avenues such as the involvement of tissue responsiveness to estrogen are currently being examined [Hodgson *et al.*, 2004]. The study of *BRCA1/2* protein levels in normal and tumour tissues has been hampered by the lack of a reliable immuno-histological test [Narod and Foulkes, 2004].

BRCA1/2 genes are considered to be tumour suppressor genes. In general, tumour development related to this class of genes occurs when two copies of mutated alleles are present. This implies that carrying an inherited mutated allele is not a sufficient condition for abnormal cell growth and that inactivation of the second, normal allele is a necessary step for carcinogenesis [Schneider, 2002].

2.1.4 Clinical, pathological and prognostic aspects of cancers associated with *BRCA1/2* genes

HBOC is essentially defined by the pattern of occurrence of cancers in the family. The presence of multiple affected family members in several generations is a feature of a genetic susceptibility to breast cancer. In HBOC families, breast cancer tends to occur at an earlier age and more often affects both breasts than in sporadic breast cancer [Haite and Gregory, 2002]. In addition, *BRCA*-related breast cancer is associated with other tumours, such as ovarian cancer (more frequently associated with *BRCA1*) and male breast cancer (more frequently associated with *BRCA2*). These clinical features are statistically associated with HBOC and with *BRCA1/2* mutation carrier

18. *BRCA1* is a large gene that includes more than 100 kb of DNA, its 24 exons coding for a protein of 1,863 amino acids. *BRCA2* is still larger, with 27 exons encompassing about 200 kb and coding for a protein of 3,418 amino acids [Chappuis and Foulkes, 2002; Nathanson *et al.*, 2001].

19. Some mutations cause the substitution of one amino acid by another (i.e. missense mutations), whereas others cause premature protein synthesis termination (i.e. nonsense mutations) or a shift in codon reading (i.e. frameshift mutations). Nonsense and frameshift mutations are associated with a more severe phenotype, since they lead to the absence of a protein or to a truncated protein.

20. Breast Cancer Information Core Web site: <http://research.nhgri.nih.gov/bic/>, accessed February 2005).

21. These are usually a missense mutation that substitutes one amino acid for another, leaving the protein intact. This type of mutation could be a neutral polymorphic variant or deleterious.

status, but do not allow precise individual predictions as to which families carry *BRCA1/2* mutations.

BRCA1-related breast tumours appear to have pathological features that are distinct from those in non-hereditary tumours (that is, tumours considered to be unrelated to a genetic susceptibility). First, *BRCA1*-related breast tumours are generally high-grade, infiltrating ductal carcinomas and have characteristics of a 'basal phenotype',²² which is often associated with a poor outcome [Narod and Foulkes, 2004]. Second, there is only a weak relationship between the size of a *BRCA1*-related breast tumour and how many axillary lymph nodes are involved [Narod and Foulkes, 2004]. Third, *BRCA1*-related breast tumours seem to be responsive to estrogen blockage as a primary or a secondary preventive measure, even though most are estrogen receptor negative [Narod and Foulkes, 2004]. Finally, prognosis for women with *BRCA1*-related tumours appears to be worse than for women with sporadic breast cancer [Narod and Foulkes, 2004]. The identification of pathological features that typify *BRCA2*-related breast tumours has been more challenging. In comparison with *BRCA1*-related tumours, it is difficult to differentiate *BRCA2*-related and non-hereditary breast cancer and evidence regarding prognosis is less clear [Narod and Foulkes, 2004]. For ovarian cancer, there seem to be no major pathological differences between *BRCA1/2*-related and non-hereditary tumours. Most *BRCA1/2*-related ovarian tumours are of the invasive epithelial type and a large proportion have serous histology [Narod and Boyd, 2002].

Molecular profiling of tumours is an active field of research which is likely to improve the characterisation of a distinctive pathological phenotype for *BRCA1/2*-related cancers, as well as prognostic predictions for affected patients. Based on micro-array technology, molecular profiling yields either the pattern of gene

expression or the pattern of copy numbers for a whole range of genes in tumour cells. These studies suggest that a distinctive molecular profile for *BRCA1*- and *BRCA2*-related breast tumours, compared to non-hereditary breast cancer, is likely to exist [Narod and Foulkes, 2004; Wooster and Weber 2003]. In contrast, the gene expression profiles of almost all ovarian carcinomas are similar to those in tumours associated with *BRCA1* or *BRCA2* mutations [Narod and Foulkes, 2004].

The use of pathological markers to guide prognosis, clinical management and testing indications²³ is thus a promising avenue for the future [P. Chappuis, personal communication, June 3, 2005; J. Hopper, personal communication, June 1, 2005; Turner *et al.*, 2004].

2.2 OTHER GENES IDENTIFIED IN HEREDITARY BREAST AND OVARIAN CANCER

Other susceptibility genes that confer an increased risk of breast or ovarian cancer have been identified, as summarised in Tables 1 and 2. Some of these genes (*TP53*, *STK11/LKB1*, *PTEN*, and *ATM*) are associated with other clinical manifestations as part of a syndrome. Their contribution to the burden of breast cancer as a whole is very low. Mismatch repair genes (such as those involved in hereditary nonpolyposis colorectal cancer syndrome) are also associated with an increased risk of ovarian cancer [Olschwang *et al.*, 2005; Chappuis and Foulkes, 2002; Chang-Claude, 2001]. The above genes have in common an involvement in DNA repair and maintenance of genome integrity [Narod and Foulkes, 2004]. The complex pathway involving their encoded proteins is a challenging field of research.

The most important discovery since the *BRCA1/2* genes has been the identification of the

22. such as oestrogen receptor and tyrosine kinase receptor negative expression.

23. In fact, the French National Ad Hoc Committee, an expert panel which recently updated its criteria for referral and testing indications, discusses the contribution to risk assessment of various pathological features of breast and ovarian tumours [Eisinger *et al.*, 2004].

cell cycle-checkpoint kinase gene (*CHEK2* or *CHK2*). The *CHEK2* 1100delC²⁴ variant is an example of a polymorphism that confers susceptibility to breast cancer with low penetrance. Its association with breast cancer was tested in a large collaborative analysis of 10,860 cases of breast cancer and 9,065 controls in five countries [CHEK2, 2004]. The analysis showed that *CHEK2* 1100delC is associated with a moderately increased breast cancer risk (odds ratio of 2.34; 95% confidence interval: 1.72-3.20). *CHEK2* appears to explain about 1.9% of all breast cancer cases.

2.3 SEARCH FOR ADDITIONAL BREAST CANCER SUSCEPTIBILITY GENES

A substantial proportion of familial breast cancer cases do not harbour a *BRCA1* or *BRCA2* mutation. Since the cloning of the *BRCA1/2* genes, efforts have been made to map these families to a third major *BRCA* gene of high penetrance (“*BRCA3*”). A variety of approaches have been used, such as linkage analysis, analysis of sib-pairs with breast cancer, and combining linkage analysis with tumour expression profiling, but no evidence of such a gene has been found to date [Hodgson *et al.*, 2004; Narod and Foulkes, 2004]. No distinctive clinical phenotype characterizes *BRCA1/2*-mutation negative families. In addition, conventional histopathology and molecular profiling based on micro-arrays in *BRCA1/2*-negative tumours do not suggest the existence of a single pattern, but instead show a large variety of profiles [Hodgson *et al.*, 2004; Narod and Foulkes, 2004].

Modeling exercises exploring which patterns of inheritance most closely predict the residual familial clustering of breast cancer once

BRCA1/2 has been accounted for also support the idea of genetic heterogeneity in *BRCA1/2*-negative families. Segregation analysis suggests a polygenic model, implying that multiple susceptibility genes with low penetrance would affect risk in a multiplicative way [Antoniou *et al.*, 2002; Pharoah *et al.*, 2002]. A model involving a recessive gene cannot be excluded, particularly in early-onset breast cancer [Antoniou *et al.*, 2002; Pharoah *et al.*, 2002; Cui *et al.*, 2001]. Taken together, these studies suggest that a combination of different genetic pathways is likely to confer breast cancer susceptibility in *BRCA1/2*-negative families, rather than a single major gene.

The knowledge of the human genome sequence and the available computational methods for finding coding information are powerful tools for the identification of previously unknown breast cancer susceptibility genes [Wooster and Weber 2003]. Nevertheless, the isolation of genetic variants with lower penetrance poses an important challenge as these rarely produce a familial pattern that is striking enough for traditional linkage analysis [Houlston *et al.*, 2004; Pharoah *et al.*, 2004]. The identification of *CHEK2* is a successful example of discovery of a low penetrance gene. Case-control studies are feasible, but require large sample sizes to achieve sufficient statistical power [Wooster and Weber 2003; Nathanson *et al.*, 2001]. An alternative approach is to study potential cancer risk modifier genes, in *BRCA1/2* carriers, as candidates for low penetrance genes [Houlston *et al.*, 2004; Pharoah *et al.*, 2004; Nathanson *et al.*, 2001]. Candidate modifiers involved in different cellular pathways, such as hormone metabolism and DNA repair, are being examined in this manner.

24. 1100delC is a variation in the genetic sequence of the *CHEK2* gene.

3. Molecular methods

3.1 OVERVIEW OF MOLECULAR TECHNIQUES

Many factors influence the choice of a molecular technique to detect mutations (see Box 1).

Box 1.

Factors that influence the choice of molecular technique (adapted from Hosking *et al.* [1998])

- the biological material (genomic DNA or messenger RNA)
- the size of the gene
- the expected nature of the mutations (e.g., point mutations, large rearrangements, protein truncating mutations)
- known mutation in family versus no known mutation in family
- the test performance
- the available laboratory resources and expertise
- costs
- existing patents and related licensing practices

Genomic DNA is often preferred over RNA because it is more stable and because it allows mutations in the splice site junctions to be detected. However, if the mutation is unknown, using genomic DNA requires more polymerase chain reactions (PCRs) since all exons must be amplified [Hosking *et al.*, 1998].

Considering that *BRCA1* and *BRCA2* are very large genes with many exons and a wide spectrum of mutation types, several different analytical methods are used for mutation detection in these genes. However, no unique testing protocol has been adopted world-wide. In this section, we have summarised commonly used methods for *BRCA1/2* analysis, including consideration of their strengths and weaknesses. We have classified these methods in three categories, depending on the type of mutation(s) they are able to detect. Technical details for each method are summarised in Appendix A.

The first category of techniques detects point mutations and small insertions/deletions in the exons and splice site junctions of the *BRCA1/2* genes (we refer to this as “EX/SP screening”). The second category includes techniques that detect large genomic rearrangements and mutations in the non-coding regions.²⁵ Finally, we classify PTT in a third category because it detects truncated proteins resulting from point mutations, small insertions/deletions or large genomic rearrangements. We include cDNA²⁶ analysis in the same category as PTT because both use RNA as the starting biological material.

The choice of a molecular method for *BRCA1/2* mutation testing is often a compromise between achieving the highest detection rate and the lowest cost [Iau *et al.*, 2001].

3.1.1 Techniques that detect point mutations and small insertions/deletions in the exons and splice site junctions (EX/SP screening)

The techniques which are able to detect these types of mutations are direct sequencing and “gene scanning” techniques. These methods rely on PCR amplification of the exons and splice site junctions, starting from genomic DNA [Hosking *et al.*, 1998].

Direct sequencing:

Direct sequencing is considered to be the “gold standard” technique to detect point mutations and small insertions/deletions because it determines the exact nucleotide sequence of a DNA fragment. However, analysis of the large, multi-exonic *BRCA1/2* genes by direct sequencing is labour-intensive and takes a lot of time; it is usually chosen when there is a limited study population and the maximum detection rate is required [Andrulis *et al.*, 2002; Iau *et al.*,

25. Non-coding region mutations include mutations in introns and in the 3' and 5' untranslated regulatory regions.

26. cDNA stands for complementary DNA, which is generated from RNA by reverse transcription.

2001; Eng *et al.*, 2001]. Sequencing can be carried out according to a number of modalities in terms of which DNA segments are sequenced (complete sequencing of a gene, sequencing of all exons and intron/exon junctions, sequencing restricted to a number of exons or segments thereof), the instruments and laboratory techniques used (fully versus partially automated sequencing), the methods used to reveal the results, the degree of observer involvement in reading these, and the software used for analysis in the case of fully automated reading. Automation of sequencing with fluorescent detection technologies has improved its efficiency but direct sequencing remains a very expensive technique with respect to both equipment and materials [Andrulis *et al.*, 2002; Iau *et al.*, 2001]. Myriad Genetic Laboratories, which hold a series of patents on the *BRCA1/2* genes in the USA, Canada, and elsewhere, has developed a robotic sequencing technique to screen for point mutations and small deletions/insertions in the EX/SP regions of the *BRCA1/2* genes, marketed as the “Comprehensive” BrACAnalysis®²⁷.

“Gene scanning”:

“Gene scanning” techniques have advantages over direct sequencing in that they are faster and can be performed at lower cost to detect point mutations and small insertions/deletions in the *BRCA1/2* genes [Narod and Foulkes, 2004; Eng *et al.*, 2001; Iau *et al.*, 2001]. The “gene scanning” techniques (see Box 2) make use of the different mobility of the mutant versus the wild type (non-mutated) DNA molecule (either in single or double strand form) on a gel matrix, the methods for subsequently revealing the presence of a mutation being different.

27. Two other types of *BRCA1/2* analysis are also offered: Single Site BRACAnalysis®: DNA sequence analysis for a specified mutation in *BRCA1* and/or *BRCA2*. Analysis for a specified large genomic rearrangement includes analysis for five rearrangements. Multisite 3 BRACAnalysis®: DNA sequence analysis of specific portions of *BRCA1* exon 2, *BRCA1* exon 20 and *BRCA2* exon 11 designed to detect the mutations 187delAG and 5385insC in *BRCA1* and 6174delT in *BRCA2* (Myriad Genetic Laboratories technical note, http://www.myriadtests.com/providerdoc/tech_specs_brac.pdf, accessed February 1, 2005).

Box 2.

Examples of “gene scanning” techniques used for *BRCA1/2* analysis

- SSCP / SSCA
- HDA
- DHPLC
- CDGE
- DGGE
- CSGE

SSCP/SSCA (single-strand conformation polymorphism/analysis), which studies the altered structure of a single DNA strand with gel electrophoresis, has in the past been one of the most widely used methods for detection of *BRCA1/2* mutations. SSCP is often combined with HDA (heteroduplex²⁸ analysis of DNA fragments) for *BRCA1/2* analysis: the two methods identify mutant alleles using different principles and are used in a complementary fashion. [Iau *et al.*, 2001] CSGE (conformational sensitive gel electrophoresis) is also based on analysis of heteroduplexes that form between normal and mutant DNA. CDGE (constant denaturant gel electrophoresis) and DGGE (denaturing gradient gel electrophoresis) detect point mutations that change the melting characteristics of double-stranded DNA.

“Gene scanning” techniques are only a first step of the testing protocol, however. Indeed, if an anomaly is detected by one of these methods, the altered DNA fragment has to be sequenced to determine the mutation’s position and the exact change in nucleotide sequence. Once the mutation has been precisely characterised, it is possible to examine its clinical significance (i.e., whether it is a deleterious mutation or not). A second limitation of some of the “gene scanning” techniques (SSCP and CSGE, for example) is that they tend to miss some types of

28. Heteroduplexes are double-strand DNA molecules that form between wild type (normal) and mutant DNA.

small mutations, particularly single nucleotide substitutions such as non-sense mutations [Eng *et al.*, 2001; Iau *et al.*, 2001; Serova *et al.*, 1996].

In comparison, the DHPLC (denaturing high performance liquid chromatography) technique, which is based on heteroduplex analysis of DNA fragments, should be able to detect all types of point mutations and small insertions/deletions and has a high throughput (able to analyse many samples at the same time); the disadvantage of DHPLC is its high cost [Eng *et al.*, 2001; Xiao and Oefner, 2001]. “Gene scanning” techniques, like SSCP or CSGE, can be especially useful for fast and cost-effective screening of specific mutations, such as common mutations in populations with a founder effect or when testing for a known mutation in family members of a carrier [Eng *et al.*, 2001].

3.1.2. Techniques that detect large genomic rearrangements and mutations in non-coding regions

One limitation of the sequencing and “gene scanning” techniques is that they are not able to detect large genomic rearrangements. As we discuss in the chapter on clinical validity in this monograph (chapter 8), large genomic rearrangements (deletions or insertions) represent, in some ethnic groups, an important proportion of mutations in the *BRCA1* gene. Different techniques exist to detect deletions and insertions that stretch beyond a few nucleotides and sometimes involve one or several exons.

Southern blot:

Southern blot has been the most common procedure used to detect large genomic rearrangements in molecular genetics to date, and has the advantages of being simple and robust [Sellner and Taylor, 2004; Hosking *et al.*, 1998]. However, Southern blot analysis has several limitations, especially for complex genes such as *BRCA1/2*: it is labour-intensive, time-consuming, and only a few samples can be analysed at the same time [Sellner and Taylor, 2004; Hogervorst *et al.*, 2003].

Quantitative/semi-quantitative-fluorescent PCR (QF-PCR):

Gene dosage (number of copies of a gene or gene segment) can be measured using a type of PCR technique based on “competitive PCR”, for which a control target of known concentration is co-amplified with a target of unknown concentration. Quantitative/semi-quantitative-fluorescent PCR (QF-PCR) is less labour intensive than Southern blot but the number of PCRs necessary to cover enough of the gene sequence can be difficult to optimise, and the large number of fluorescently-labelled probes potentially needed can be prohibitive [Sellner and Taylor, 2004].

Multiplex ligation-dependent probe amplification (MLPA):

MLPA is considered one of the most promising recent developments for detecting large genomic rearrangements in the *BRCA1/2* genes. This technique relies on comparing the quantities of probes that are specifically bound to certain targets and that are subsequently amplified by PCR using ‘universal’ primers. MLPA can detect genomic rearrangements in the *BRCA1/2* genes with relatively high throughput. The introduction of universal primers has allowed laboratories to quantify many targets (e.g., exons) at the same time and, in MLPA, the complete *BRCA1* gene can be screened in a single reaction [Sellner and Taylor, 2004; Bunyan *et al.*, 2004; Hogervorst *et al.*, 2003]. Furthermore, the cost of operation is low since only one fluorescent primer is required for detecting mutations (rather than several fluorescent probes, one for each target, as it is the case for quantitative PCR) [Sellner and Taylor, 2004].

For a long time, EX/SP screening has been the major focus of *BRCA1/2* analysis and the first step used before screening for large genomic rearrangements. However, with the recent development of MLPA and its many advantages in detecting large genomic rearrangements, some researchers suggest that laboratories opting for gene dosage analysis have to consider whether this should be performed before or after

conventional PCR-based EX/SP techniques. This decision depends, in part, on the frequency of large genomic rearrangements in the target population [Bunyan *et al.*, 2004].

Techniques that detect mutations in the non-coding regions:

Screening for point mutations and small insertions/deletions in the non-coding regions of genes, such as the promoter, untranslated 5' and untranslated 3' regions, is not frequently seen in *BRCA1/2* mutation research. Such mutations appear to be rare, but knowledge about the spectrum of mutations is dependent on the techniques used to date. When a search for such mutations has taken place, investigators have started from either genomic DNA or cDNA and the ensuing techniques have usually been similar to those used for screening in the EX/SP regions [Puget *et al.*, 1999a].

Another technique, LHR (loss of heterozygosity at RNA level) can be used to identify mutations that affect mRNA expression. LHR is based on identifying heterozygosity at polymorphic sites on the cDNA molecule. Investigators infer loss of RNA from the preferential presence of the normal allele in the cDNA [Puget *et al.*, 1999b; Serova *et al.*, 1996].

3.1.3 Protein truncation test (PTT)

PTT has been widely used in *BRCA1/2* mutation analysis due to the high frequency of truncating mutations with clinical relevance [Narod and Foulkes, 2004; Iau *et al.*, 2001]. PTT, which is also PCR-based, is an *in vitro* translation technique that detects truncated (shortened) proteins. Its ability to analyse large amplified fragments such as the cDNA is an attractive feature for genes as large and complex as *BRCA1/2*, but this approach requires a preliminary step to obtain cDNA from the more unstable RNA material. PTT can also be used to screen genomic DNA but is not effective for small exons: the majority of exons in the *BRCA1/2* genes are small [Narod and Foulkes, 2004; Iau *et al.*, 2001]. Some laboratories have therefore restricted the use of PTT to the analysis of the large exons of *BRCA1* (exon 11) and *BRCA2* (exons 10 and 11); these regions

represent only approximately 60% of the coding sequence [Narod and Foulkes, 2004; Iau *et al.*, 2001].

Another limitation of PTT is that it may not detect mutations at the 5' end of the amplified DNA fragment because the synthesised protein products are too small. It may also be difficult to differentiate truncated proteins from the wild type (normal) products when the mutation is at the 3' end of the gene and the difference in size between them is small. Furthermore, some *BRCA1/2* mutations do not cause premature termination of the protein product (e.g., missense mutations) and are thus not detectable by PTT [Eng *et al.*, 2001].

3.1.4 *BRCA1/2* mutation testing protocols

Molecular techniques have evolved considerably since the identification of the *BRCA 1/2* genes. Although there is overlap, techniques used in research and in clinical practice are not necessarily identical. In countries where patents on the *BRCA1/2* genes have been granted, most of the clinical testing is performed by direct sequencing at the Myriad Genetic Laboratories. In the research context and in other countries a variety of techniques are in use. The search for large genomic rearrangements and for mutations in non-coding regions is still predominantly performed in research laboratories, although techniques such as MLPA have recently been introduced in clinical laboratories and their use is rapidly expanding around the world.²⁹ It is significant to note that Myriad Genetic Laboratories currently includes specific testing for five large *BRCA1* rearrangements if a comprehensive analysis is requested (see section 8.4).

The “gene scanning” techniques are performed as a pre-screen to identify altered DNA segments to which direct sequencing will then be applied to identify the precise nature of the mutation. Among these scanning techniques, SSCP and CSGE were initially quite popular, but it has gradually become clear that these techniques detect fewer mutations than

29. J. Simard, personal communication, May 30, 2005.

sequencing or than some of the more recent techniques such as DHPLC. DHPLC is increasingly being used in clinical laboratories in Canada and in Europe.³⁰ PTT has also been widely used because truncating protein mutations are thought to be deleterious in all cases, while this is not so for missense mutations for example. PTT is most often used for the largest exons, in combination with other “gene scanning” techniques for the analysis of the rest of the coding sequence. In fact, the combination of different techniques is almost the rule rather than the exception. As a result, comparisons between studies are rarely feasible and the assessment of test performance for single techniques (or for specific combinations of techniques) is very difficult. This situation could change if future studies focus on the utilization of particular combinations of different techniques to detect *BRCA1/2* mutations, such as DHPLC coupled with MLPA.

3.2 TEST PERFORMANCE

Despite intensive investigation in the field of mutation detection technology, several problems still hamper molecular analysis for HBOC:

- 1) *BRCA1/2* are very large genes with, in most populations, a wide spectrum of mutations (in terms of location and mutation type);
- 2) conventional “gene scanning” techniques (including sequencing) miss some types of mutations;
- 3) the discovery of a number of variants of unknown clinical significance poses problems for the interpretation of test results;
- 4) current knowledge on the full spectrum of mutations is incomplete in most populations and is dependent on the evolution of molecular techniques used; and
- 5) the genetic heterogeneity of the disease means that genes other than *BRCA1/2* may lead to HBOC [Montagna *et al.*, 2002].

These sources of complexity not only render the execution and interpretation of molecular tests more difficult, but also represent additional challenges when it comes to assessing test performance.

A molecular test can be assessed for both its technical and clinical performance. There are thus two types of validation to consider. Analytical validity³¹ relates to the technical performance of a molecular test (with respect to detecting mutations for which it is designed) and reflects the comparison of test results with the genotype, or the DNA sequence. Clinical validity²⁴ refers to the comparison of test results with the phenotype, i.e., the clinical expression of disease. The evaluation of molecular tests poses – perhaps more acutely than for other biological tests – the challenge of choosing a suitable reference or “gold standard”, both for the genotype and for the phenotype.

3.2.1 Analytical validity

Sequencing is often considered as the “gold standard” molecular technique. As mentioned previously, different types of equipment and technical protocols can be used to perform direct sequencing. In addition, it has become clear that even if sequencing can be considered as an appropriate technique to detect point mutations and small insertions/deletions in the exons and splice site junctions in the *BRCA1/2* genes, it is not a suitable method to detect large genomic rearrangements and non-coding region mutations. The fact that sequencing misses such mutations affects its clinical validity, and this issue will be considered further in this monograph when assessing clinical sensitivity.

In line with the most common clinical practice (deferring the search for large rearrangements to families in which the search for point mutations

30. J. Simard, personal communication, May 30, 2005; P. Chappuis, personal communication, June 3, 2005.

31. Both analytical and clinical validity are measured by the following parameters: sensitivity, specificity, positive predictive value and negative predictive value.

has been unproductive) and in line with the predominant perception that direct sequencing is the gold-standard molecular method, we consider analytical validity as the ability of a number of techniques (gene scanning methods and PTT) to identify point mutations and small insertions/deletions in the exons and splice site junctions, taking sequencing as the reference test.

For the purposes of this assessment, analytical validity data have not been systematically reviewed. From our reading of review articles, it appears that clear differences in analytical sensitivity are to be expected from one gene scanning technique to the next. For SSCP and CSGE for example, analytical sensitivity figures around 60 to 70% are frequently reported, whereas DHPLC is sometimes cited as having a technical performance comparable to that of sequencing. A thorough analysis of the nature and quality of the evidence on which these estimates are based is essential. Unfortunately, despite the multitude of techniques in clinical use, rigorous comparative studies meeting epidemiological standards of quality for studies of molecular tests seem to be scarce. At the time of writing, a number of systematic reviews are underway which are expected to address these issues.³²

3.2.2 Clinical validity

Clinical validity refers to the comparison of the test results with the phenotype, or clinical expression of disease. A clear phenotypic distinction between hereditary forms of breast and ovarian cancer and sporadic (non-inherited) cancers does not exist on clinical grounds. Likewise, a clinical distinction between the phenotype for *BRCA1/2*-associated hereditary cancer and hereditary cancer related to other

genes is also unavailable. Distinctive features on pathological grounds are being intensively studied, but their potential use in targeting molecular testing is still the object of research. The choice of a reference phenotype thus remains problematic. In addition to incorporating the technical performance of the molecular test (the analytical validity), clinical validity reflects both the distribution of mutations and other risk factors in the population under study and the correlation between the genotype and the phenotype. Since clinical validity varies according to population, inclusion criteria used to select the study population need to be scrutinised carefully, whether the target population is families defined as having hereditary breast and ovarian cancer (HBOC) or high risk founder populations. Difficulties related to the definition of study population are outlined in the section on methodological issues in clinical validity studies (section 8.1).

The ideal investigation of clinical validity would require not only high quality study design and methods (including the systematic use of the test in all study subjects and blind assessment of results, for instance), but also sufficient follow-up to allow for disease expression and adequate documentation thereof. Such a study would allow for the computation of all the parameters of interest: sensitivity, specificity, positive predictive value and negative predictive value. However, a follow-up of several decades would be necessary to document the occurrence of an outcome such as breast and ovarian cancer, and prospective studies are the exception.³³ One therefore has to rely on studies with alternative designs from which data related to either sensitivity or positive predictive value can be extracted.³⁴ Data on specificity and negative predictive value are scarce.

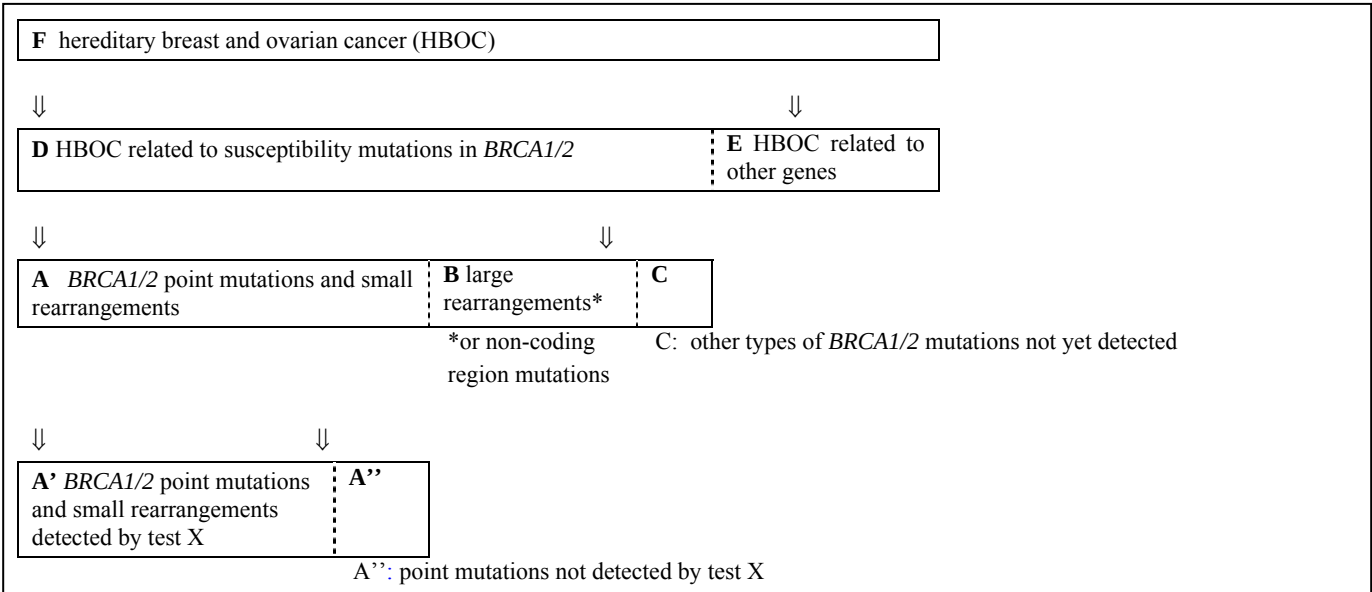
32. Foundation for Blood Research (FBR). Family history and *BRCA1/2* testing for identifying women at risk for inherited breast/ovarian cancer (preliminary report) (www.cdc.gov/genomics/gtesting/ACCE/FBR.htm); CCOHTA (Canadian Coordinating Office for Health Technology Assessment). Systematic review of *BRCA1* and *BRCA2* genetic testing for breast and ovarian cancer susceptibility (in preparation); US Preventive Services Task Force/Agency for Healthcare Research and Quality (USPSTF/AHRQ). Genetic risk assessment and *BRCA* mutation testing for breast and ovarian cancer susceptibility. Evidence Synthesis no. 37, 2005 (www.ahrq.gov/clinic/upstf/usprgen.htm).

33. In addition, carrying out a prospective study without providing preventive measures raises ethical questions.

34. A study designed to document clinical validity would yield data in all cells of the following 2x2 table, whereas most available studies yield data either in the upper row (PPV= $a/a+b$) or in the left column (sensitivity= $a/a+c$).

	Phenotype +	Phenotype -
Test +	a	b
Test -	c	d

Figure 1.
Schematic representation of the distribution of hereditary breast and ovarian cancer and susceptibility mutation types (note: figure is not to scale)



Penetrance, the cumulative probability of disease expression at a specific age given the presence of a mutation, is the major determinant of the clinical positive predictive value (assuming that the analytical validity of the tests used is reasonably good). Penetrance data will be presented in section 5.2, and the chapter on clinical validity will focus on clinical sensitivity. If the distribution of all mutations conferring susceptibility to hereditary breast and ovarian cancer were well documented in a given population, a theoretical estimate of clinical sensitivity could be derived from the proportion of cases carrying detectable susceptibility mutations. This would correspond to the upper limit of clinical sensitivity, irrespective of the technique used and its analytical performance. To provide a coherent approach to the assessment of analytical and clinical validity, we will consider the clinical sensitivity of EX/SP screening methods as the proportion of cases carrying point mutations and small insertions/deletions, as opposed to large

rearrangements or non-coding region mutations.³⁵

Figure 1 illustrates how our definitions of clinical and analytical sensitivity are related to the distribution of *BRCA1/2* mutations conferring susceptibility to hereditary breast and ovarian cancer. According to the definitions proposed above, analytical sensitivity for a given “gene scanning” technique X represents an estimate of A’/A. If a phenotypic distinction between D and E had existed, clinical sensitivity of EX/SP screening methods in general could have been defined as A/D. Since this is not the case, the reference phenotype is HBOC (F).

The lack of a well defined HBOC phenotype means that clinical sensitivity represents an estimate of A/F where F itself is poorly defined. As will be discussed in more depth in chapter 8, the choice of these definitions was constrained by the nature of the available evidence for both analytical and clinical validity.

35. As discussed in chapter 8, more restricted definitions of clinical sensitivity will also be used, either to look at a particular molecular technique (PTT for instance) or for founder populations where, in practice, molecular analyses may be limited to a search for common mutations rather than a screen for all point mutations.

4. Methods of systematic literature review

A systematic literature review was carried out on the following topics: (i) prevalence and penetrance of *BRCA1/2* mutations; (ii) risk assessment (predictive models); (iii) indications for *BRCA* mutation testing (guidelines); and (iv) clinical validity of molecular methods used to test for *BRCA1/2* mutations.

4.1 LITERATURE SEARCH

A comprehensive search strategy using key words was designed in order to identify published, 'grey' and unpublished literature for each topic. The search was limited to human studies, and there were no language restrictions. A draft set of key words was tested for its precision before finalisation. Searches of the following databases were completed to the end of June, 2004: PubMed, Cochrane Library, Dialog® OneSearch® on MEDLINE®, CANCERLIT®, EMBASE®, Biosis Previews®, and PASCAL. In addition, PubMed, CANCERLIT®, and EMBASE® were searched from July to end of December, 2004.

A preliminary investigation revealed extensive literature, particularly on prevalence and penetrance, subsequent to the cloning of *BRCA1* and *BRCA2* in 1994 and 1995, respectively. The following strategies were therefore used:

- 1) The literature search for primary research articles was restricted to January 1999 onwards. An additional search for review articles published from 2001 to 2002 was conducted, and the reference lists of these articles were examined to identify key primary research articles published before 1999.
- 2) For the risk assessment chapter, review articles published from 2001 and 2002 were used to describe models developed before 1999. Contact was also made with clinical practice experts in cancer genetics from Quebec, Ontario, and British Columbia to

confirm that the models and tools most often used in clinical care were included in this chapter.

- 3) Clinical practice guidelines on test indications were examined from 1996 onwards, as the clinical use of *BRCA* genetic testing was first discussed in guidelines in that year. Guidelines listed in the InheritBRCA database [INHERIT BRCA, 2001] were examined.
- 4) For the clinical validity chapter, besides studies on mutation prevalence, articles on the performance of molecular tests for *BRCA1/2* were also examined to identify studies on large genomic rearrangements and non-coding mutations.

Grey literature was identified by searching the INAHTA (International Network of Agencies for Health Technology Assessment) websites, other health agency websites, clinical trial registries, clinical practice guideline databases, websites of relevant societies and associations (for conference abstracts), and other specialized databases. The reference lists of primary research studies, review articles, and reports were searched to identify other relevant articles.

4.2 STUDY SELECTION CRITERIA

The selection criteria used for each topic are listed below.

Prevalence and penetrance

The selection criteria for material on prevalence and penetrance were the following:

1. Primary research study published since 1999.
2. Sample size ($n \geq 100$) for samples selected for family history.

- Families could be a mixture of breast cancer only, ovarian cancer only, or breast-ovarian families, or the sample could be entirely made up of one of these groups.
 - No sample size cut-off was used for Canadian studies of this type.
3. Sample size ($n \geq 500$) for samples not selected for family history.
 - These were usually persons affected with cancer, and selection for age at onset/diagnosis was allowed.
 - No sample size cut-off was used for Canadian studies, no studies of this type from Iceland (due to the small number of studies available for the Icelandic population).
 - “Key” articles (i.e., frequently cited primary research) published before 1999 were identified using 33 review articles from January 2001 to June 2002 and using reference lists from primary research articles which met the selection criteria. For key articles which studied persons unselected for family history, the sample size cut-off was lowered to at least 100 persons.
 - For samples not selected on the basis of family history nor age at onset/diagnosis, the sample size cut-off was lowered to at least 100 persons if the study sample was from USA, United Kingdom or France (the two last countries being major founding regions for the Canadian and American populations).
 4. For key articles on Ashkenazi Jewish persons, testing for all 3 frequent *BRCA1/2* founder mutations was necessary (i.e., *BRCA1* 185delAG, *BRCA1* 5382insC, *BRCA2* 6174delT).
 5. Abstracts were excluded, due to the large number of peer-reviewed primary research articles in this area, except for those

presented at a recent large genetics conference (American Society of Human Genetics, Toronto, October 2004).

Review articles and additional primary research articles that were suggested by a genetics expert were used in the discussion of variability of penetrance and in the section on other cancers. These issues were not the primary focus of the systematic review on prevalence and penetrance.

Risk assessment

No sample size restrictions were used as criteria for this topic. To be included in the data extraction, a primary research study (published since 1999) had to develop and/or test predictive risk models (these models were developed using *BRCA1/2* mutation testing or estimates of *BRCA1/2* prevalence/penetrance and cancer incidence). Simulation studies and segregation analyses used to develop explanatory models and purely methodological papers were excluded.

Testing indications

No sample size restrictions were used as criteria for material on testing indications. Guidelines were selected that specifically mentioned indications for *BRCA* genetic testing or referral to family cancer clinics, clinical geneticists, genetic counsellors, etc.; some of these also discussed clinical management (management details were not summarized).

Recommendations for referral to different levels of specialized care (within which a decision to test for mutations may or may not be made) were included since these also gave an idea of the risk levels in use in clinical practice.

Clinical validity

No sample size restrictions were used as criteria for clinical validity material. A search was carried out for primary research on the prevalence of *BRCA1/2* mutations or on *BRCA* mutation testing methods that potentially contained information on the distribution of susceptibility mutations. Additional specific details about the articles selected for data extraction are presented at the beginning of the clinical validity chapter, since relatively extensive background information is required for

the explanation of our numeric calculations. Due to the relative scarcity of material, abstracts were not excluded in the search for this topic.

4.3 METHODS OF STUDY SELECTION AND DATA EXTRACTION

Two reviewers independently made the final selection of studies to be included in the review based on the selection criteria. Study selection forms were developed and pilot tested for this purpose. The decision to order an article was based on the title and abstract, where available. In cases where these offered insufficient information, the article was ordered for further information. The degree of agreement between

reviewers was noted and any persisting differences were resolved by consensus. Flow charts were constructed to summarise the selection process for each topic (Appendices B1-B4).

For articles containing quantitative data, forms that were specifically developed and pilot tested for this project were used to extract data. Various procedures were put in place to ensure quality control, including checking of extracted data by a second reviewer, discussion of all particularly complex articles by several reviewers, and reading of text and tables by a scientific reviewer not directly involved in data extraction.

5. Prevalence and penetrance

Literature searching results

Primary research articles on penetrance and prevalence were identified from the original electronic search strategy (January 1999 to January 2003), from other sources (key articles published before 1999 identified from reference lists and cited in review papers), and from an update of the literature from February 2003 to end of December 2004. In total we selected 106 reports. For a description of the flow of documents, please refer to the chart in Appendix B1.

5.1 PREVALENCE

A precise estimate of the prevalence of mutations in the *BRCA1* and *BRCA2* genes is important in more than one respect. *BRCA1/2* mutation prevalence is used to estimate the contribution of these genes to the etiology of breast cancer. The familial and personal risk factors associated with specific levels of mutation prevalence are used to establish testing indications. As well, the frequency of these mutations needs to be determined in order to better plan clinical services, to assess the need and potential demand for testing, and to predict the impact that the offer of testing will have on the healthcare system.

BRCA1/2 mutation prevalence values have been estimated in studies with various designs and various populations. For the purpose of synthesising this vast and heterogeneous literature, prevalence studies were categorized into four main areas: those involving (1) individuals with a family history of breast or ovarian cancer, (2) individuals with breast or ovarian cancer unselected for family history, (3) the general population and (4) founder populations. The majority of studies have examined prevalence in individuals with a family history of breast or ovarian cancer or in founder populations. Within each category, additional diversity stems from the use of different study designs and molecular methods

as well as the enrolment of participants from a wide variety of ethnic groups. Results from the prevalence studies reviewed and some of their key sample characteristics are presented in Tables 3 to 8. We have not pooled data in order to summarize because of heterogeneity between studies, as will become clear in the sections that follow. Instead, we present a range of values corresponding to the lowest and highest estimates from the studies that pertain to a particular section. Confidence intervals for estimates were not available in many prevalence studies we reviewed (Tables 4-8). When we report point estimates of prevalence from a single study, we include confidence intervals whenever these were provided by the authors. For a detailed description of the individual characteristics of the prevalence studies, please refer to Tables C1 to C5 in Appendix C.

5.1.1 Individuals with a family history of breast or ovarian cancer

Approximately one-third of breast cancers are thought to be familial, that is, occurring in families in which at least two members are diagnosed with breast cancer at any age [Nathanson *et al.*, 2001]. Therefore, as a first step in research, the contribution of *BRCA1/2* mutations to the familial clustering of breast and ovarian cancers was explored. The frequency of *BRCA1/2* mutations was first estimated from families that were used to identify the genes by genetic linkage analysis. Subsequently, the prevalence of these mutations was determined by direct mutation analysis in families referred to familial cancer clinics or ascertained in population-based studies. Data on prevalence are presented in Tables 3 and 4 for linkage studies and clinic-based studies, respectively, along with key study characteristics. The studies presented in Table 4 cover heterogeneous populations worldwide and populations where a founder effect for certain *BRCA1/2* mutations exists (e.g., AJ, French Canadian and Dutch). The details pertaining to each individual study and

results of sub-group analyses can be found in Table C1 in Appendix C.

5.1.1.1 STUDIES OF FAMILIES SELECTED FOR THE PURPOSE OF GENETIC LINKAGE ANALYSIS

The first data on the frequency of *BRCA1/2* mutations are from studies by the Breast Cancer Linkage Consortium (BCLC) that served to identify the genes. Families in these studies included multiple individuals with early-onset breast cancer over several generations. In the study by Ford *et al.* [1998] (Table 3) that was part of the BCLC, data were pooled from 237 families, from various countries, in which there were at least 4 cases of breast cancer (female breast cancer diagnosis at or before 60 years or male breast cancer at any age), with or without cases of ovarian cancer. Genetic linkage analysis indicated that the *BRCA1/2* genes were implicated in most (84%) of these families. As shown in Table C1 of Appendix C, the contribution of the *BRCA1/2* genes was also determined in three sub-groups: 1) families where there were cases of both female and male breast cancer (92% were associated with *BRCA1/2*, but mostly with *BRCA2*), 2) families where there were cases of both breast cancer and ovarian cancer (95% were associated with *BRCA1/2*, mostly with *BRCA1*) and 3) families where there were at least 4 cases of female breast cancer only (i.e., no ovarian cancer and no male breast cancer). The contribution of the *BRCA1/2* genes to the last subgroup was reported to be about 58% and decreased to 33% for families with four or five

cases of breast cancer. This suggests that the clustering in breast cancer-only families is more heterogeneous and can be explained in part by factors other than the *BRCA1/2* genes (e.g., other genes, environment).

5.1.1.2 STUDIES OF FAMILIES EVALUATED AT CLINICS

Due to the inclusion criteria used, the families in the BCLC studies were not necessarily representative of those seen at family cancer clinics. Most families referred to these clinics contain only two or three breast cancer cases, with or without cases of ovarian cancer [Chang-Claude, 2001]. In Table 4, all of the studies included individuals who were referred to cancer clinics because of a family history of breast or ovarian cancer. The estimates of prevalence in these studies were based on the analysis of one individual per family. The frequencies of the mutations detected by direct mutation analysis varied from 3.5 to 45.0% for *BRCA1* and 1.2 to 22.5% for *BRCA2*, which are, in general, lower point estimates than those observed in the BCLC study (based on proportion of linked families, with partial *BRCA1* mutation analysis). Some possible reasons for the between-study variability are the diverse family inclusion criteria for the molecular analysis and the different mutation detection techniques used (see Table C1 in Appendix C for details). Furthermore, the between-study variability can also be due to mutation frequency distribution varying according to the population (e.g., due to founder effects).

Table 3.
Estimated frequency of germline *BRCA1/2* mutations in individuals selected on the basis of family history of breast or ovarian cancer (linkage studies*)

REFERENCE	SAMPLE CHARACTERISTICS		PROPORTION OF LINKED FAMILIES, % (95% CI)	
	Country or ethnic origin	Sample size	<i>BRCA1</i>	<i>BRCA2</i>
Ford <i>et al.</i> , [1998]	Europe and North America (multi-centre)	237 families	52 (42-62)	32 (22-43)

*presented figures incorporate partial mutation analysis of *BRCA1*

Table 4.
Prevalence of germline *BRCA1/2* mutations in individuals selected on the basis of breast or ovarian cancer (clinic-based studies)

REFERENCE	SAMPLE CHARACTERISTICS		PREVALENCE OF MUTATIONS, % (95% CI, IF AVAILABLE)	
	Country or ethnic origin§	Sample size	<i>BRCA1</i>	<i>BRCA2</i>
Simard <i>et al.</i> , [1994]	Caucasian (principally residing in Canada)	30 fam	40.0	nd
Shattuck-Eidens <i>et al.</i> , [1995]	North America and UK	372 cases	16.9	nd
Durocher <i>et al.</i> , [1996]	Canada	23 fam	21.7	nd
Phelan <i>et al.</i> , [1996]	majority of European descent (all but one fam residing in Canada)	49 fam	nd	16.3
Tonin <i>et al.</i> , [1996]	AJ in North America (multi-centre)	220 fam	41.4* *185delAG or 5382insC	4.1** **6174delT
Couch <i>et al.</i> , [1997]	USA: majority White (15% AJ)	169 fam	16.0	nd
Gayther <i>et al.</i> , [1997]	UK and Eire	290 fam	22.1	8.6
Stoppa-Lyonnet <i>et al.</i> , [1997]	France: AJ; Italy, Poland, Spain, Northern and Western Africa	160 fam	23.8	nd
Vehmanen <i>et al.</i> , [1997a, b]	Finland	100 fam	10 (5-18)	11 (6-19)
Frank <i>et al.</i> , [1998]	USA: 20% AJ	238 fam	26.5	13.0
Tonin <i>et al.</i> , [1998]	French Canadian	97 fam	24.7	17.5
Gayther <i>et al.</i> , [1999]	UK	112 fam	35.7	7.1
Arver <i>et al.</i> , [2001]	Sweden	160 fam	15.6	1.2
Martin <i>et al.</i> , [2001]	USA	100 fam	45.0	11.0
Verhoog <i>et al.</i> , [2001]	Dutch	517 fam	19.0	4.1
Meindl <i>et al.</i> , [2002]	Germany	989 fam	21.4	9.1
Nedelcu <i>et al.</i> , [2002]	Canada: at least one parent with Italian ancestry	116 fam	19.8	13.8
Phelan <i>et al.</i> , [2002]	AJ in Canada	245 fam	24.5* *185delAG or 5382insC	10.2** **6174delT
Shih <i>et al.</i> , [2002]	USA: 95% White; 27% AJ; 2% Af-Am; 2% Hispanic	164 fam	17.1	5.5
Velasco Sampedro <i>et al.</i> , [2002]	Spain	153 fam	5.2	7.2
Diez <i>et al.</i> , [2003]	Spain	410 fam	13.9	12.4

Table 4 (continued).

REFERENCE	SAMPLE CHARACTERISTICS		PREVALENCE OF MUTATIONS, % (95% CI, if available)	PREVALENCE OF MUTATIONS, % (95% CI, if available)
	Country or ethnic origin§	Sample size	<i>BRCA1</i>	<i>BRCA2</i>
Menkiszak <i>et al.</i>, [2003]	Polish	177 fam	32.8 (5382insC, C61G, 4153delA)	nd
Rostagno <i>et al.</i>, [2003]	France	140 fam	3.5	nd
Cipollini <i>et al.</i>, [2004]	Italy	1758 fam	14.3	8.8
Claes <i>et al.</i>, [2004]	Belgium	291 fam	15.8	8.6
Oros <i>et al.</i>, [2004]	French Canadian (index cases reported grand-parental ancestry)	169 fam	21.3	22.5
Simard <i>et al.</i>, [2004]	French Canadian	251 fam (5 fam had 2-sided family history)	11.3	12.9

§ In this table, as throughout the document, ethnic groups are designated as reported by authors.
nd: not determined; fam: families; AJ: Ashkenazi Jewish; Af-Am: African-American

Analysing prevalence by subgroups of families with different characteristics allows for a determination of the risk factors that increase the probability of *BRCA1/2* mutation involvement in the family history. Results of the subgroup analysis for each study are presented in Table C1 (Appendix C) and the results of the multivariate analyses performed in a limited number of studies can be found in Table C2 in Appendix C. The following summary provides highlights of the data analysis stratified according to various risk factors.

▪ Ethnic origin

Two studies were identified that involved only hereditary breast and ovarian cancer families of AJ origin [Phelan *et al.*, 2002; Tonin *et al.*, 1996]. The prevalence of the three founder *BRCA1/2* mutations varied from 34.7 to 45.5% in these families (Table 4). Although such comparisons should be interpreted with caution, these point estimates are comparable to the prevalence of all the *BRCA1/2* mutations detected in coding regions in American families unselected for ethnic origin (although 15-27% of

these samples were AJ) but with similar family inclusion criteria [Shih *et al.*, 2002; Frank *et al.*, 1998; Couch *et al.*, 1997].

▪ Ovarian cancer

On the basis of pedigree analysis, a distinction has been made between families with breast cancer and ovarian cancer and families with ovarian cancer only, as well as those families with an individual with both breast and ovarian cancer. In families with both breast cancer and ovarian cancer, the prevalence of mutations varied from 16.7 to 68.3% for the *BRCA1* gene and from 0 to 22.2% for *BRCA2* (Table C1)³⁶. The presence of individuals who have developed both breast cancer and ovarian cancer increases the likelihood of a *BRCA1* mutation. The frequency of mutations varied from 60.5 to 76.2% for *BRCA1* in this case, except in one particularly small study where no *BRCA1*

36. However, the Swedish study did not detect any *BRCA2* mutations in BrO families, but it is known that there is a lower prevalence of *BRCA2* mutations in the Stockholm area [Arver *et al.*, 2001].

mutations were found [Diez *et al.*, 2003; Shih *et al.*, 2002; Phelan *et al.*, 2002; Martin *et al.*, 2001; Couch *et al.*, 1997]. The frequency of *BRCA2* mutations in patients with both cancers varied from 0 to 14.3% [Diez *et al.*, 2003; Shih *et al.*, 2002; Martin *et al.*, 2001]. Lastly, two studies analyzed the sub-group of ovarian cancer-only families where the prevalence of mutations was 0 to 27.3% (*BRCA1*) and 0 to 9.1% (*BRCA2*) [Arver *et al.*, 2001; Verhoog *et al.*, 2001].³⁷

Results of the multivariate analyses in Table C2 confirm that presence of ovarian cancer is strongly associated with *BRCA1* mutations and less so with *BRCA2* mutations [Shih *et al.*, 2002; Arver *et al.*, 2001; Couch *et al.*, 1997; Tonin *et al.*, 1996].

▪ **Breast cancer**

The importance of the number of cases of breast cancer³⁸ in families as a predictive factor of the presence of a *BRCA1/2* mutation is still a subject of much debate (see Table C2, Appendix C). A number of studies report that there is no significant difference (i.e., in terms of the number of breast cancer cases) between families with and families without a *BRCA1/2* mutation [Shih *et al.*, 2002; Arver *et al.*, 2001; Couch *et al.*, 1997; Tonin *et al.*, 1996]. The families included in these studies, however, are a mixture of breast cancer-only families and breast and ovarian cancer families. In the one study involving families with both breast and ovarian cancer, a significant association with number of cases was found [Gayther *et al.*, 1999].

According to statistical analyses, the age at diagnosis of breast cancer is significantly lower in some families with a *BRCA1/2* mutation (see Table C2; Shih *et al.* [2002], Arver *et al.* [2001], Couch *et al.* [1997]). It is interesting to note that

in a Dutch study, no *BRCA1/2* mutations were detected in the subgroup of younger women with breast cancer (diagnosed before 40 years, with no family history; data not shown) [Verhoog *et al.*, 2001]. This finding is in contrast to those from population-based studies of cases of early-onset breast cancer (see section 5.1.2 on *Individuals with a personal history of breast or ovarian cancer*).

▪ **Male breast cancer**

According to genetic linkage studies, most families with cases of male breast cancer are linked to the *BRCA2* gene (Table C1) [Ford *et al.*, 1998]. Results from seven studies in which prevalence in this sub-group of families was estimated using direct mutation analysis also suggest that the prevalence of mutations may be higher in the *BRCA2* gene (23.4 to 100%) than in the *BRCA1* gene (0-12.5%), although results vary substantially [Claes *et al.*, 2004; Oros *et al.*, 2004; Diez *et al.*, 2003; Meindl *et al.*, 2002; Phelan *et al.*, 2002; Shih *et al.*, 2002; Phelan *et al.*, 1996]. In contrast, a Dutch study involving 12 families with at least one case of male breast cancer and referred to two cancer genetic clinics over a 5-year period suggested that the prevalence of *BRCA1* mutations may be higher than that of *BRCA2* mutations [Verhoog *et al.*, 2001]. In a Spanish study, no *BRCA1/2* mutations were found in the 4 males in the study [Velasco Sampedro *et al.*, 2002].

▪ **Bilateral breast cancer**

A bivariate analysis conducted in one study demonstrated no significant association between a family history of bilateral breast cancer and presence of *BRCA1* mutations (Table C2) [Couch *et al.*, 1997]. Patients in the study had been referred to a clinic due to their personal history of breast cancer (unselected for age) and a family history of breast or ovarian cancer. It is possible that an association depends upon the age of onset of the cancer. One study showed, for example, that among women who developed bilateral breast cancer before the age of 50 years and who had a family history, 51% were found to be *BRCA1/2* mutation carriers (Table C1) [Frank *et al.*, 1998].

37. The Swedish study [Arver *et al.*, 2001] (Stockholm) did not find any *BRCA1/2* mutations in the 12 families with at least 2 ovarian cancer cases (apparently very few Ov-only families were analyzed in this area, with the result that it is difficult to draw any conclusions).

38. In general, these studies enrolled families with female breast cancer only; however, in a study from Sweden, there may also have been cases of male breast cancer [Arver *et al.*, 2001].

A number of limitations of the studies based on family history of breast or ovarian cancer were identified:

- Genetic linkage studies are a measure of probability and do not provide a direct measure of prevalence [Ford *et al.*, 1998]. Families were highly selected and are not representative of the entire range of families seen at clinics.
- Some articles did not indicate the number of eligible individuals, but instead, only the number of individuals in whom molecular analysis was performed for *BRCA1/2* mutations [Velasco Sampedro *et al.*, 2002; Tonin *et al.*, 1998; Durocher *et al.*, 1996; Phelan *et al.*, 1996; Tonin *et al.*, 1996]. This, in turn, limits the generalisability of the results.
- In some studies, it was impossible to determine if the family history of breast or ovarian cancer was confirmed by medical reports or death certificates, which could have lead to errors in classifying cancer cases (especially ovarian cancer cases) and may affect the prevalence estimates for sub-groups of families [Phelan *et al.*, 2002; Shih *et al.*, 2002; Velasco Sampedro *et al.*, 2002; Martin *et al.*, 2001; Frank *et al.*, 1998].
- Lastly, prevalence estimates are influenced by the validity of the techniques used.³⁹ In addition, when the same molecular techniques were not used for all the families in a given study, the prevalence estimate could be biased if technical performance or test sensitivity was different for certain sub-groups [Claes *et al.*, 2004; Durocher *et al.*, 1996].

39. In most studies, mutations were screened in exons and splicing junctions (i.e. EX/SP screening). In some studies, mutation detection using this approach was not optimal, especially when use was not made of sequencing or a combination of PCR-based techniques [Shih *et al.*, 2002; Velasco Sampedro *et al.*, 2002; Martin *et al.*, 2001; Couch *et al.*, 1997; Stoppa-Lyonnet *et al.*, 1997; Phelan *et al.*, 1996]. Furthermore, only two studies screened for types of mutations that are not detected by EX/SP screening, such as genomic rearrangements [Claes *et al.*, 2004; Nedelcu *et al.*, 2002].

5.1.1.3 STUDIES OF FAMILIES WITH BREAST OR OVARIAN CANCER CASES ASCERTAINED IN AN ENTIRE POPULATION

In studies based in familial cancer clinics, the selection and referral criteria can vary. In an effort to obtain more representative data from families with a familial form of breast cancer in an entire population, some studies considered all the cancer cases in a given region and then examined their family history. Two studies, based on a series of index breast cancer patients diagnosed before age 46 in one study and in the other before age 55, indicated that *BRCA1/2* mutations could explain only 16-17% of the familial clustering of breast cancer [ABC Study Group, 2000; Peto *et al.*, 1999]. Furthermore, in a study of relatives of breast cancer cases diagnosed before 40 years, *BRCA1/2* mutations accounted for less than 1/3 of the familial clustering of breast cancer⁴⁰ [Dite *et al.*, 2003]. Such estimates are probably not valid for founder populations (e.g., three founder mutations can explain most of the familial clustering of breast cancer in the AJ population) [Hopper, 2001]. In comparison, *BRCA1/2* mutations have been reported to explain 55-60%⁴¹ of the familial clustering of ovarian cancer [Chappuis and Foulkes, 2002] (D. Easton).⁴² However, a study using the national Swedish cancer registry indicated that the contribution of *BRCA1/2* to familial ovarian cancer was smaller in that population: the excess risk for ovarian cancer patients that was attributed to familial factors was estimated as 26% [Hemminki and Granstrom, 2003]. We did

40. The results were based on the excess risk of breast cancer attributable to *BRCA1/2* mutations in the mothers and sisters (and in aunts for Dite *et al.*, 2003) of the index cases who were mutation carriers, compared to the relatives of the index cases who were non-carriers [Dite *et al.*, 2003; ABC Study Group, 2000; Peto *et al.*, 1999].

41. For these results, we do not know the specific ethnicity or country of origin of the study samples but we presume that estimation has been carried out in heterogeneous populations from the UK or US.

42. Evaluating the contribution of *BRCA1*, *BRCA2* and *BRCAX*: modelling and modifying risks; oral presentation at the Joint conference on inherited susceptibility to breast and ovarian cancer: Second annual INHERIT BRCA meeting and first national hereditary cancer task force, Quebec City, November 24-26th, 2002.

not evaluate the methods used to arrive at the values presented.

5.1.2 Individuals with a personal history of breast or ovarian cancer

Recently, several population-based studies have directly measured the prevalence of *BRCA1/2* mutations in a series of patients with breast and/or ovarian cancer who were unselected for family history. On closer examination, however, it was found that some women in these studies had a family history of such cancers. Details of the study characteristics are found in Table C3 for breast cancer and in Table C4 for ovarian cancer, Appendix C. In total, 9 studies examining the prevalence of *BRCA1/2* mutations in women with breast cancer were identified (Table 5). The frequency of deleterious mutations varied, for *BRCA1*, from 0.7% (95% CI: 0.3-1.3) to 6.0% (95% CI: 3.8-8.8) and from 1.1% (95% CI: 0.4-2.2) to 3.9% (95% CI: 2.2-6.3) for *BRCA2*. In the study reporting the highest point estimates of prevalence, a fraction of the sample was selected on the basis of a family history of breast cancer [Malone *et al.*, 2000]. Most of the studies used early age at diagnosis as an inclusion criterion (usually pre-menopause, although it varied). We identified three American studies and one from China that measured prevalence in patients diagnosed with breast cancer both before and after menopause [Suter *et al.*, 2004; Whittemore *et al.*, 2004; Anton-Culver *et al.*, 2000; Newman *et al.*, 1998]. For these four studies the prevalence of *BRCA1* mutations was found to be between 1.1% (95% CI: 0.4-2.2) and 2.6% (95% CI: 0-5.5) and that for *BRCA2* was 1.1% (95% CI: 0.4-2.2; only the Chinese study analysed both *BRCA1* and *BRCA2*).

A total of ten studies which were either population-based or examined cases ascertained at hospitals were identified for women with ovarian cancer (Table 6). There were no restrictions for early age of diagnosis in these

studies. The prevalence of mutations varied from 1.9% (no CI provided) to 9.6% (95% CI: 6.7-13.5) for *BRCA1* and from 0.9 to 4.1% (no CIs provided) for *BRCA2*. There may be several reasons for the variability in results between studies on series of breast or ovarian cancer. These include the ascertainment of subjects through prevalent cases as opposed to incident cases, the inclusion criteria (e.g., first cancers only or not), selection based on the age at diagnosis (in the case of the breast cancer studies), population- versus hospital-based selection, the histological type (in the case of the ovarian cancer studies), and the mutation screening techniques used.

Of all the studies presented in Tables 5 and 6, two were Canadian. One of these studies, conducted by Wolpert *et al.* [2000], examined male breast cancer cases. The frequency of *BRCA1/2* mutations was estimated to be 14.3%. The second study was population-based and involved a series of ovarian cancer cases [Risch *et al.*, 2001].

A prevalence analysis by subgroups (e.g., age at diagnosis of cancer or family history) again allows for an assessment of the risk factors associated with detecting a *BRCA1/2* mutation, this time among cancer cases. Results of the analysis by subgroups are presented in Tables C3 and C4 in Appendix C but highlights of the data analysis, grouped by various risk factors, are presented below. Difficulties were encountered in summarising this stratified analysis since the identified subgroups could vary from study to study and because the number of cases with mutations was low in absolute numbers in some studies [Anton-Culver *et al.*, 2000; Janezic *et al.*, 1999; Berchuck *et al.*, 1998; Newman *et al.*, 1998; Rubin *et al.*, 1998; Stratton *et al.*, 1997; Takahashi *et al.*, 1996; Takahashi *et al.*, 1995].

Table 5.
Prevalence of germline *BRCA1/2* mutations in breast cancer cases

REFERENCE	SAMPLE CHARACTERISTICS			PREVALENCE, % (95% CI OR # CASES WITH MUTATION / TOTAL # CASES)	
	Country or ethnic origin	Age at diagnosis (years)	Sample size (cases)	<i>BRCA1</i>	<i>BRCA2</i>
Newman <i>et al.</i> , [1998]	USA: 57% White; 42% Black; 1% Native Am	20-74	211	2.6 (0-5.5)	nd
Hopper <i>et al.</i> , [1999]	Australia	<40	388	4.6 (18/388)	
Peto <i>et al.</i> , [1999]	UK: all Caucasian	<46	617	2.6 (16/617)	2.3 (14/617)
ABC Study Group [2000] ⁴³	UK	<55	1435	0.7 (0.3-1.3)	1.3 (0.8-2.1)
Anton-Culver <i>et al.</i> , [2000]	USA: 83.5% White, 4.5% AJ, 6.2% Hisp, 5.2% Asian, 0.6% Af-Am	no age selection	673	1.6 (0.8-2.9)	nd
Malone <i>et al.</i> , [2000]	USA: 96% White; 1% Af-Am; 1.6% Asian	<45 ¹	386	6.0 (3.8-8.8)	3.9 (2.2-6.3)
Wolpert <i>et al.</i> , [2000]	Canada: predominantly White, mixed ancestry (1 AJ)	no age selection	14 (males)	0	14.3 (2/14)
Dite <i>et al.</i> , [2003]	Australia	<40	788	5.3 (42/788)	
Suter <i>et al.</i> , [2004]	China	no age selection	645	1.1 (0.4-2.2)	1.1 (0.4-2.2)
Whittemore <i>et al.</i> , [2004]	USA: non-Hispanic White (6.9% AJ)	20-64	525	2.4 ² (0.8-6.9)	nd

1. a fraction of the sample was selected for family history of cancer (225/428 originally recruited)

2. prevalence was 2.2% (95% CI: 0.6-7.4) in patients without Ashkenazi Jewish heritage; results are weighted estimates to account for differential testing rates among cases thought to be hereditary or non-hereditary

nd=not determined; AJ=Ashkenazi Jewish; Am=American; Hisp=Hispanic; Af-Am=African American

43. At the time the ABC Study Group published their results, 1435/1486 patients provided blood for testing among whom 8 cases were positive for *BRCA1* mutations and 16 cases were positive for *BRCA2* mutations. In a recent publication that used the same study sample, the frequency of mutation positive *BRCA1/2* cases was slightly different (8 cases with a *BRCA1* mutation and 15 cases with a *BRCA2* mutation) because all 1486 patients were finally tested for *BRCA1/2* mutations and there was a reclassification of the number of deleterious mutations [Antoniou *et al.*, 2002].

Table 6.
Prevalence of germline *BRCA1/2* mutations in ovarian cancer cases

REFERENCE	SAMPLE CHARACTERISTICS			PREVALENCE, % (95% CI OR # CASES WITH MUTATION / TOTAL # CASES)	
	Country or ethnic origin	Age at diagnosis (years)	Sample size (cases)	<i>BRCA1</i>	<i>BRCA2</i>
Takahashi <i>et al.</i>, [1995], [1996]¹	USA	no age selection	<i>BRCA1</i> : 115 <i>BRCA2</i> : 130	6.1 (7/115)	3.1 (4/130)
Stratton <i>et al.</i>, [1997]	UK	<70	374	3.5 (2-6)	nd
Berchuck <i>et al.</i>, [1998]	USA: 87% White; 11% African-American, 2% Native American	no age selection	103	3.9 (4/103)	nd
Rubin <i>et al.</i>, [1998]¹	USA	no age selection	116	8.6 ³ (10/116)	0.9 ³ (1/116)
Janezic <i>et al.</i>, [1999]²	USA: 85% non-Hispanic White; 9% Hispanic; 6% Asian	no age selection	107	1.9 (2/107)	nd
Anton-Culver <i>et al.</i>, [2000]²	USA: 82.5% non-Hispanic White; 3.3% AJ; 10% Hispanic; 4.2% Asian	no age selection	120	3.3 (0.8-8.3)	nd
Risch <i>et al.</i>, [2001]	Canada	20-79	649	6.0 (39/649)	3.2 (21/649)
			515 *invasive	7.6* (39/515)	4.1* (21/515)
Malander <i>et al.</i>, [2004]	Sweden	no age selection	161	8.1 (13/161)	2.5 (40/161)
Whittemore <i>et al.</i>, [2004]	USA: non-Hispanic White (9.7% AJ)	20-64	290	9.6 ⁴ (6.7-13.5)	nd

1. these studies appear to have cases in common (according to the sample characteristics)

2. these studies have cases in common

3. one patient had both a *BRCA1* and a *BRCA2* mutation

4. prevalence was 8.4% (95% CI: 5.7-12.4) in patients without Ashkenazi Jewish heritage; results are weighted estimates to account for differential testing rates among cases thought to be hereditary or non-hereditary

AJ= Ashkenazi Jewish; nd: not determined

Age at diagnosis of cancer

In women who developed breast cancer prior to 46 to 55 years of age (depending on the study), the prevalence of *BRCA1* mutations varied from 0.4 (95% CI: 0.01-2.2) to 2.6% (no CI

provided), and for *BRCA2*, mutation prevalence varied from 1.6 (95% CI: 0.4-4.0) to 2.3% (no CI provided) (Table 5 and C3) [Suter *et al.*, 2004; Anton-Culver *et al.*, 2000; ABC Study Group, 2000; Peto *et al.*, 1999; Newman *et al.*, 1998]. If cancer was diagnosed before the age of

35 to 40 years (depending on the study), the prevalence of *BRCA1* mutations was reported to vary from 3.5 (no CI provided) to 7.6% (95% CI: 3.5-15.9) and *BRCA2* mutation prevalence varied from 2.4 (no CI provided) to 8.3 (95% CI: 2.3-19.8) [Whittemore *et al.*, 2004; ABC Study Group, 2000; Anton-Culver *et al.*, 2000; Malone *et al.*, 2000; Peto *et al.*, 1999] (Table C3). Two studies tested for both *BRCA1* and *BRCA2* mutations in cases diagnosed before the age of 40 years for both *BRCA1* and *BRCA2* and the combined prevalence varied from 4.6 to 5% (no CIs provided) [Dite *et al.*, 2003; Hopper *et al.*, 1999] (Table 5).

The mean age of onset of breast cancer tends to be earlier in women who carry a *BRCA1* mutation than in non-carriers; however, this difference was either not statistically significant or borderline in the identified studies [Anton-Culver *et al.*, 2000; Peto *et al.*, 1999; Newman *et al.*, 1998] (Table C3). For ovarian cancer, the prevalence of *BRCA1* mutations in women who develop cancer at or prior to age 50 years varied from 6.5 to 36.8%, and in those who develop ovarian cancer after age 50, this varied from 1.4 to 9.5% (Table C4) [Risch *et al.*, 2001; Anton-Culver *et al.*, 2000; Berchuck *et al.*, 1998]. The prevalence of *BRCA2* mutations in ovarian cancer was estimated at 2.2% at or prior to age 50 and 3.8% thereafter [Risch *et al.*, 2001]. The mean age of onset of ovarian cancer appears to be lower in women who are *BRCA1* mutation carriers, but the difference when compared with non-carriers was not significant or borderline (data not shown) [Risch *et al.*, 2001; Anton-Culver *et al.*, 2000; Stratton *et al.*, 1997].

Ovarian cancer

The frequency of *BRCA1* mutations in breast and ovarian cancer families varied from 24 to 33.3% (no CIs provided; Table C3) [Malone *et al.*, 2000; Newman *et al.*, 1998]. In the one study reviewed that tested *BRCA2*, no mutations were identified [Malone *et al.*, 2000]. Two studies reported a combined point estimate for both *BRCA1* and 2 and this varied from 5.1 to 45.0% (no CIs provided) [ABC Study Group, 2000; Peto *et al.*, 1999]. In ovarian cancer-only families, prevalence of *BRCA1* mutations varied

from 14.3 to 20.0% (no CIs provided; Table C3) [Anton-Culver *et al.*, 2000; Malone *et al.*, 2000]. In the one study that measured *BRCA2* mutation frequency in families with only ovarian cancer, no mutations were identified [Malone *et al.*, 2000].

Bilateral breast cancer

In the only study identified with stratification by this risk factor, no significant difference in mutation prevalence was observed between the families with and without bilateral breast cancer cases [Malone *et al.*, 2000]. It may be that such an association depends on the age of onset of bilateral breast cancer.

Absence of family history in carriers

In some studies, up to 36.4% of the women who developed breast cancer and who were *BRCA1/2* mutation positive reported no family history of breast or ovarian cancer [ABC Study Group, 2000; Anton-Culver *et al.*, 2000]. The absence of a family history of these cancers can be explained, in part, by incomplete penetrance, a high proportion of men in the families and small family size.

A number of limitations were identified in the studies based on individuals with breast or ovarian cancer:

- The use of prevalent cases can lead to selection bias [Risch *et al.*, 2001; ABC Study Group, 2000; Malone *et al.*, 2000; Peto *et al.*, 1999; Stratton *et al.*, 1997].
- In some articles, it was not clear if the authors confirmed the family history of cancer by examining medical reports [Malone *et al.*, 2000; Newman *et al.*, 1998]. In others, the authors stated that this confirmation was not done for all the cancer cases in the family [Anton-Culver *et al.*, 2000; Peto *et al.*, 1999]. This practice can lead to errors in classifying cancer cases (especially for ovarian cancer) and can affect the prevalence estimate for *BRCA1/2* mutations.

- Lastly, prevalence estimates are influenced by the validity of the techniques used⁴⁴ [Anton-Culver *et al.*, 2000; Malone *et al.*, 2000; Peto *et al.*, 1999]. In some studies, the same mutation analysis techniques were not used for all the families [Dite *et al.*, 2003; Anton-Culver *et al.*, 2000] which can bias the prevalence estimate.

5.1.3 Individuals in the general population

It would be a difficult and expensive undertaking to directly measure the prevalence of *BRCA1/2* mutations in heterogeneous populations, such as those in the US or Canada, due to the present limitations of molecular techniques, the large size of these genes and the wide spectrum of mutations. Thus far, prevalence values for *BRCA1/2* mutations in the general population have been extrapolated from studies of these mutations in series of female breast cancer patients. The estimated frequency of carriers in the UK general population was 0.09 to 0.11% (*BRCA1*) and 0.12 to 0.22% (*BRCA2*) [ABC Study Group, 2000; Peto *et al.*, 1999]. *BRCA1* prevalence has also been estimated in the US, among non-Hispanic White individuals with no Ashkenazi Jewish origin from Northern California [Whittemore *et al.*, 2004]. Researchers have extrapolated a general population prevalence figure by combining prevalence data from two population-based studies (one from a series of invasive breast cancer cases and one from a series of ovarian cancer cases) with published penetrance data in *BRCA1* carriers. A prevalence of 0.24% (95% CI: 0.15-0.39) was estimated when assuming a 90% analytical sensitivity for the molecular techniques. When analytical sensitivity was assumed to be lower (65%), the prevalence estimate increased to 0.34% (95% CI: 0.21-0.55). As noted by Whittemore and colleagues [2004], these point estimates are higher than what has been estimated in the UK population (i.e., 0.09 to 0.11% for *BRCA1* mutations), and

the authors suggest this could reflect different ethnic backgrounds between the US and UK populations, if not due to chance or bias.

5.1.4 Populations with founder effects

Several mutations have been identified that are unique to specific countries or ethnic groups, suggesting they are founder mutations. Founder effects give rise to a higher frequency of one or more specific mutations in the descendants of a group of common ancestors. In haplotype analysis, individuals with the same founder mutation will share the same alleles at polymorphic markers within the gene or adjacent to the gene. It is important, however, to distinguish between founder mutations and mutations that are recurrent because they occur at “hot spots” but do not segregate with the same alleles [Evans *et al.*, 2004a].

The AJ, Icelandic and Polish populations are the three ethnic groups found to date where there is a major founder effect of *BRCA1/2* mutations; Table 7 presents the results of different prevalence studies for these three groups. In Table 8, prevalence data are presented for other populations with founder effects (Finnish, Hungarian, Dutch, Norwegian, and French Canadian); based on the point estimates, the effects in these groups appear to be less, in general, than for AJ, Icelandic and Polish populations. Again, such comparisons must be interpreted with caution. For some populations (e.g., Finnish, Hungarian), it is not clear whether the common mutations which were screened in the reviewed studies represent all founder mutations. Information on the design of the founder population studies is presented in Table C5, Appendix C.

In the AJ population, the three founder mutations, *BRCA1* 185delAG, *BRCA1* 5382insC and *BRCA2* 6174delT, reportedly account for up to 90% of the *BRCA1/2* mutations detected [Srivastava *et al.*, 2001; Taylor, 2001]. Studies in the general AJ population have estimated that between 1.9 and 2.7% of individuals are carriers of one of the three mutations (Table 7) [Bahar *et al.*, 2001; Struewing *et al.*, 1997; Roa *et al.*, 1996]. Using point estimates, this represents a

44. As noted earlier, most studies used EX/SP screening but mutation detection using this approach was not optimal, especially when use was not made of sequencing or a combination of PCR-based techniques. Furthermore, no studies screened for types of mutations that are not detected by EX/SP screening, such as genomic rearrangements.

prevalence approximately 10 times higher than that for all *BRCA1/2* mutations detected in heterogeneous populations such as those in the US. Despite the distinct study designs and the different origins of the AJ communities represented in Table 7 (e.g., Israel, Australia and the US), there are no statistically significant differences in the prevalence results of these three studies according to Bahar and colleagues [2001].

In AJ individuals with additional risk factors, the point estimates of the prevalence of the three common *BRCA1/2* mutations are even higher⁴⁵: 24.2 to 43.3% of AJ women who develop breast cancer before age 40-45 years and 35.7 to 53.3%⁴⁶ of AJ patients who develop ovarian cancer were carriers of one of the three founder mutations (Table 7) [Hirsh-Yechezkel *et al.*, 2003; Menczer *et al.*, 2003; Moslehi *et al.*, 2000; Fodor *et al.*, 1998; Abeliovich *et al.*, 1997; Karp *et al.*, 1997]. Approximately 57% of Jewish Israeli women who had a tumour both in the breast and the ovary were founder mutation carriers [Fishman *et al.*, 2000] (the proportion of women in this study who were of AJ descent was not reported) (Table 7). As seen with other case series, the variability in prevalence in the studies of breast cancer cases is likely to be mostly attributable to design differences. Factors such as the ascertainment of prevalent versus incident cases, the inclusion of women who had undergone conservative surgical treatment only, selection on the basis of age at diagnosis, and the combination of population-based and hospital-based sampling all contribute to this variability.

Studies of patients with a family history of breast cancer strongly suggest that only a single founder mutation in each of the *BRCA1* and *BRCA2* genes exists in the Icelandic population [Rafnar *et al.*, 2004; Johannesdottir *et al.*, 1996; Thorlacius *et al.*, 1996]. The *BRCA2* 999del5 mutation is the most common one and has been

found in 40% of men with breast cancer and in 15.8-16.6% of women with breast cancer diagnosed before 50 years (Table 7). The *BRCA1* G5193A mutation is much rarer and was found in less than 1% of female breast cancer cases (data not shown; reported by Rafnar *et al.* [2004]). In Poland, three recurrent *BRCA1* mutations are founder mutations. They have been estimated to be present in 0.3% of the general population (Table 7) [Gorski *et al.*, 2004b].

In other ethnic groups (i.e., Finnish, Hungarian, Dutch, Norwegian), the prevalence of common mutations varied from 7.1 to 9.9% in women who developed breast cancer before the age of 40 years (Table 8) [Papelard *et al.*, 2000; Syrjakoski *et al.*, 2000]. The frequency of common mutations varied from 2.9 to 11% in those who developed ovarian cancer [Van Der Looij *et al.*, 2000; Dorum *et al.*, 1999a]. In the French-Canadian population, certain *BRCA1/2* mutations recur in families with multiple cases of breast and/or ovarian cancer; several of these have been shown to be founder mutations by haplotype analysis (J. Simard⁴⁷) [Oros *et al.*, 2004; Simard *et al.*, 2004]. We identified three studies that measured the prevalence of six to eight common *BRCA1/2* mutations in different cancer case series (Table 8) [Chappuis *et al.*, 2001; Tonin *et al.*, 2001; Tonin *et al.*, 1999]. A total of 3.1% of the women with breast cancer and 8.1% of those with ovarian cancer were carriers of one of the common mutations. Among women who had developed breast cancer at or before the age of 40, the frequency of the common mutations was 13.1%.

45. In this section, prevalence data for AJ are summed data across the *BRCA1/2* genes.

46. In the study of ovarian cancer reporting a 53.4% prevalence [Abeliovich *et al.*, 1997], this figure may represent an overestimation as part of the sample was selected on the basis of a family history of cancer.

47. Familial breast/ovarian cancer in the French Canadian founder population; oral presentation at the Oncogenetics: Achievements and challenges. *Les 17ièmes Entretiens du Centre Jacques Cartier* meeting, Montréal, Québec, Oct 7-8, 2004.

Table 7.
Prevalence of founder germline *BRCA1/2* mutations in Ashkenazi Jewish (AJ), Icelandic and Polish populations

ETHNICITY	FOUNDER MUTATIONS	BREAST CANCER CASES (UNSELECTED FOR FAMILY HISTORY)			OVARIAN CANCER CASES (UNSELECTED FOR FAMILY HISTORY AND AGE AT DIAGNOSIS)			GENERAL POPULATION (UNSELECTED FOR PERSONAL OR FAMILY HISTORY OF CANCER)		
		Reference	Age at diagnosis (years)	Prevalence, % (# cases with mutation / total # cases)	Reference	Prevalence, % (# cases with mutation / total # cases or 95% CI)		Reference	Prevalence, % (# cases with mutation / total cases or 95% CI)	
				<i>BRCA1</i> <i>BRCA2</i>		<i>BRCA1</i>	<i>BRCA2</i>		<i>BRCA1</i>	<i>BRCA2</i>
AJ	<i>BRCA1</i> 185delAG and 5382insC, <i>BRCA2</i> 6174delT	Abeliovich <i>et al.</i>, [1997]	ascertainment was hetero-geneous ³	11.3 (18/160) (unilateral)	Abeliovich <i>et al.</i>, [1997]	53.3 (8/15) ⁴ (unknown if epithelial)		Roa <i>et al.</i>, [1996]²	185delAG : 1.1 (0.7-1.5) 5382insC: 0.2 (0-0.3)	6174delT: 1.4 (0.9-1.8)
		Karp <i>et al.</i>, [1997]	<65 <45 ¹	14.9 (21/141) 24.2 (8/33)	Fishman <i>et al.</i>, [2000]	57.1 (28/49) (previous primary Br ca)		Struewing <i>et al.</i>, [1997]²	185delAG: 1.0 (0.7-1.2). 5385insC: 0.3 (0.1-0.4)	6174delT: 0.6 (0.4-0.8)
		Fodor <i>et al.</i>, [1998]	No age selec	6.7 (18/268)	Moslehi <i>et al.</i>, [2000]	41.3 (86/208) (epithelial)		Bahar <i>et al.</i>, [2001]	185delAG: 1.25 (0.6-1.9) 5382insC: 0.25 (0.0-0.5)	6174delT: 1.1 (0.5-1.7)
		Robson <i>et al.</i>, [1999]	No age selec <50 ¹	9.2 (28/305) 22.2 (22/99)	Menczer <i>et al.</i>, [2003]; Hirsh-Yechezkel <i>et al.</i>, [2003]	35.7 (201/563) (epithelial invasive)				
		Warner <i>et al.</i>, [1999]	No age selec <50 ¹ <40 ¹	11.7 (48/412) 22.0 (36/164) 43.3 (13/30)						

Table 7 (continued).

ETHNICITY	FOUNDER MUTATIONS	BREAST CANCER CASES (UNSELECTED FOR FAMILY HISTORY)				OVARIAN CANCER CASES (UNSELECTED FOR FAMILY HISTORY AND AGE AT DIAGNOSIS)			GENERAL POPULATION (UNSELECTED FOR PERSONAL OR FAMILY HISTORY OF CANCER)		
		Reference	Age at diagnosis (years)	Prevalence, % (# cases with mutation / total # cases)		Reference	Prevalence, % (# cases with mutation / total # cases or 95% CI)		Reference	Prevalence, % (# cases with mutation / total cases or 95% CI)	
				<i>BRCA1</i>	<i>BRCA2</i>		<i>BRCA1</i>	<i>BRCA2</i>		<i>BRCA1</i>	<i>BRCA2</i>
Icelandic		Johannesdottir <i>et al.</i>, [1996]	No age selec <50 ¹	nd	8.5 (39/459) 15.8 (24/152)	Johannesdottir <i>et al.</i>, [1996]	nd	7.9 (3/38)	Johannesdottir <i>et al.</i>, [1996]	nd	0.4 (2/499)
	<i>BRCA2</i> 999del5	Thorlacius <i>et al.</i>, [1997]	No age selec <50 ¹	nd nd	7.7 ⁵ (49/632) 16.6 (32/193)				Thorlacius <i>et al.</i>, [1997]	nd	0.6 (0.1-1.7)
			No age selec	nd	40.0* (12/30) *male Br						
	<i>BRCA1</i> G5193A; <i>BRCA2</i> 999del5	ni				Rafnar <i>et al.</i>, [2004]	1.2 (0.1-3.3) invasive	6.0 (3.1-11.1) invasive	Rafnar <i>et al.</i>, [2004]	nd	0.3 (0.1-0.7)
Polish	3 <i>BRCA1</i> : 5382insC, C61G, 4153delA	Gorski <i>et al.</i>, [2004b]	No age selec (21-80)	2.9 (59/2012)	nd	Menkiszak <i>et al.</i>, [2003]	13.5† (49/364)	nd	Gorski <i>et al.</i>, [2004b]	0.3 (6/2000)	nd
						Gorski <i>et al.</i>, [2004b]	13.5† (49/364)	nd			

nd=not determined; selec: selection; ni=not identified by our search; †appear to be the same sample

1. Subgroup analysis according to age of diagnosis; 2. The presentation of data of Struewing [1997] and Roa [1996] are from Bahar [2001]; 3. Some Br ca cases were unselected for age and others were selected for family history or other risk factors such as young age at diagnosis (proportion among AJ cases not reported); 4. Some Ov ca cases may have been selected for family history of cancer;

5. 95% CI: 5.7-9.7

Table 8.
Prevalence of common germline *BRCA1/2* mutations in populations with a founder effect

ETHNICITY	<i>BRCA1/2</i> COMMON MUTATIONS	BrEAST CANCER CASES (UNSELECTED FOR FAMILY HISTORY)				OvARIAN CANCER CASES (UNSELECTED FOR FAMILY HISTORY AND AGE AT DX)		
		Reference	Age at diagnosis (years)	Prevalence % (# cases with mutation /total cases and/or 95% CI)		Reference	Prevalence % (# cases with mutation / total cases or 95% CI)	
				<i>BRCA1</i>	<i>BRCA2</i>		<i>BRCA1</i>	<i>BRCA2</i>
Finnish	11 recurrent + 8 mutations found only once ¹	Syrjakoski <i>et al.</i> , [2000]	No age selection <40 ²	1.8 (1.1-2.9) (19/1035) 9.9 (7/71)		ni		
Hungarian	<i>BRCA1</i> : 185delAG, 5382insC, 300T>G; <i>BRCA2</i> : 6174delT†, 9326insA	Van der Looij <i>et al.</i> , [2000]	No age selection	3.4 (2.1-5.4)	0.2 (0.04-1.1)	Van der Looij <i>et al.</i> , [2000]	11.1 (6.1-19.1)	0 (0-4.1)
Dutch	<i>BRCA1</i> : 35 mutations; (including 3 founder large deletions); DSDI ³	Papelard <i>et al.</i> , [2000]	No age selection <40 ²	1.6 (0.8-2.9) 7.8 (2.2-18.9)	nd	ni		
Norwegian	2 <i>BRCA1</i> : 1675delA and 1135insA	ni				Dorum <i>et al.</i> , [1999a]	2.9 (18/615)	nd
French Canadian	6-8 mutations ⁴	Chappuis <i>et al.</i> , [2001]	<80	0 (0/127)	3.1 (4/127)	Tonin <i>et al.</i> , [1999]	8.1 (8/99) ⁵	
		Tonin <i>et al.</i> , [2001]	≤40	13.1 (8/61)				

ni=not identified by our search methodology; nd: not determined; selec: selection; †no mutations found

1. In addition to 11 common *BRCA1/2* mutations in the Finnish population, Syrjakoski *et al.*, [2000] have also tested the sample for 8 *BRCA1/2* mutations identified once in Finnish families.

2. subgroup analysis according to age of onset of cancer

3. DSDI (detection of small deletions and insertions): mutation analysis performed for 35 mutations in *BRCA1* [Papelard *et al.*, 2000]

4. *BRCA1*: 5221delTG (only tested by Tonin *et al.*, [2001]), 2953del3+C, 3768insA, 4446C>T; *BRCA2*: 2816insA, 6085G>T, 6503delTT (not tested by Tonin *et al.*, [1999]), 8765delAG

5. invasive ovarian carcinoma (sample also included borderline ovarian carcinoma)

Some common mutations are present in more than one ethnic group. The *BRCA1* 5382insC mutation is frequent in several populations other than the AJ [Ladopoulou *et al.*, 2002; Backe *et al.*, 1999]. In the German population, the prevalence of this mutation was estimated to be 4% in breast-ovarian cancer families, which is comparable to the frequency reported among high risk families in other Central European countries [Backe *et al.*, 1999]. In Greek and Polish populations, the 5382insC mutation appears to be the most frequent and has been found in 13.5% and 34.0% of hereditary breast and ovarian cancer families,⁴⁸ respectively [Gorski *et al.*, 2004a; Ladopoulou *et al.*, 2002].⁴⁹

In the Sephardic Jewish population, the *BRCA1* 185delAG and Tyr978X mutations are founder mutations [Shiri-Sverdlov *et al.*, 2001]. The prevalence of the Tyr978X mutation has been estimated to be about 1% in the Iraqi Jewish population [Shiri-Sverdlov *et al.*, 2001].⁵⁰

A few limitations of the founder population studies were identified:

- The comparison between the characteristics of the eligible cases and those for whom molecular analysis was performed was not always available or complete, which limits the generalizability of the results [Shiri-Sverdlov *et al.*, 2001; Moslehi *et al.*, 2000; Van Der Looij *et al.*, 2000; Backe *et al.*, 1999; Thorlacius *et al.*, 1997].
- As noted for other reviewed studies, the use of prevalent cases can lead to selection bias [Moslehi *et al.*, 2000; Warner *et al.*, 1999].

5.1.5 Variants of unknown clinical significance (VUCs) in the *BRCA1/2* genes

The prevalence data reported above are for *BRCA1/2* mutations with confirmed deleterious

effect or where there is strong suspicion of a deleterious effect. Generally, these mutations are truncating and are predicted to result in a non-functional, shortened protein. Additionally, some studies with *BRCA1/2* mutation analysis report the frequency of variants of unknown clinical significance (VUCs), which can be either neutral polymorphisms or deleterious *BRCA1/2* mutations. Not all molecular techniques will identify VUCs, but direct sequencing, for instance, will detect any base substitution.

More than 300 distinct VUCs have been submitted to the BIC database and pose a challenge in interpreting the effects at the clinical level [Narod and Foulkes, 2004]. As of fall 2002, Myriad Genetics Laboratories had analysed more than 20,000 samples⁵¹ by direct sequencing for *BRCA1/2* EX/SP mutations and had detected a VUC in 12.8% of their samples [Abkevich *et al.*, 2004] (S. Tavtigian⁵²). This is not a negligible proportion since the prevalence of deleterious mutations in the same population has been estimated as 14.5% (S. Tavtigian⁵³). The frequency of VUCs could be ethnic specific. For instance, the prevalence of *BRCA1/2* VUCs in the African-American population has been reported to be approximately 40% (L.A. Burbidge, Myriad Genetics, personal communication, May 11, 2004). However, the proportion of reported VUCs may depend to a certain extent on how much research has been devoted to elucidating clinical significance of these variants.

Different types of investigations may help to classify VUCs, most of which are carried out in research [Goldgar *et al.*, 2004; Spurdle *et al.*, 2004; Deffenbaugh *et al.*, 2002]. Abkevich and colleagues [2004] have used the approach of

48. A mixture of breast-only and breast-ovarian cancer families.

49. Given the prevalence of the *BRCA1* 5382insC mutation in a number of ethnic groups, some authors have suggested screening for this mutation in all at-risk individuals [Backe *et al.*, 1999].

50. The study authors recommended that this mutation be included when screening for *BRCA1/2* mutations in high-risk Sephardic Jewish families and in patients of Iraqi or Iranian origin with ovarian cancer [Shiri-Sverdlov *et al.*, 2001].

51. Most individuals had a personal or a family history of breast or ovarian cancer but no further information is available regarding the inclusion criteria for genetic analysis [Abkevich *et al.*, 2004].

52. Classification of missense variants in high-risk cancer susceptibility genes; oral presentation at the Oncogenetics: Achievements and challenges. *Les 17ièmes Entretiens du Centre Jacques Cartier meeting*, Montréal, Québec, Oct 7-8, 2004.

53. Classification of missense variants in high-risk cancer susceptibility genes; oral presentation at the Oncogenetics: Achievements and challenges. *Les 17ièmes Entretiens du Centre Jacques Cartier meeting*, Montréal, Québec, Oct 7-8, 2004.

conservation of the variant amino acid across species and the analysis of the severity of the amino acid substitution to make a preliminary classification of *BRCA1* VUCs identified in the Myriad Genetics' sample. They tentatively classified about 50% of the VUCs identified in *BRCA1*: 52 VUCs as "probably deleterious mutations" and 92 VUCs as "probably neutral polymorphic variants". However, independent confirmation is needed for clinical applicability [Abkevich *et al.*, 2004]. Others have used a three-dimensional protein structure approach to predict deleterious effect of VUCs in the *BRCA1* C-terminus region [Mirkovic *et al.*, 2004].

A multifactorial approach that integrates results of different types of investigations may be more suitable for valid VUC classification, and epidemiological studies (examining co-segregation with the disease in pedigrees, co-occurrence with deleterious mutations, etc.) are very important components for the validation since they are directly related to cancer risk [Goldgar *et al.*, 2004]. However, such empirical studies will not always be feasible due to the rarity of many VUCs (J. Hopper, personal communication, June 1, 2005). Goldgar and colleagues [2004] provided a demonstration of the utility of an integrated evaluation by classifying a few *BRCA1/2* VUCs but this model needs to be validated prior to clinical use.

5.1.6 Conclusion

- Prevalence data are provided for *BRCA1/2* mutations in individuals with a family history of breast or ovarian cancer, individuals with these cancers unselected for family history, the general population and founder populations. It is notable that most of the reviewed studies do not provide confidence intervals for their point estimates of prevalence.
- Estimated frequency of *BRCA1/2* mutations in families with multiple cases of cancer is high (i.e., up to 84% of families are linked to these genes when at least 4 breast cancer cases are diagnosed prior to 60 years, with or without ovarian cancer).
- In individuals referred to cancer clinics due to family history, frequency of *BRCA1/2* mutations are, in general, lower than in highly selected families, at least on the basis of point estimates. Prevalence estimates vary in less highly selected samples from 3.5 to 45% (no CIs provided) for *BRCA1* and 1.2 to 22.5% (no CIs provided) for *BRCA2*. Variability could be attributed to differing inclusion criteria, mutation detection techniques and mutation frequency distributions. According to population-based studies, *BRCA1/2* mutations only explain 16-17% of clustering of breast cancer in families, compared to 55-60% for ovarian cancer. However, in some ethnic groups the contribution of *BRCA1/2* to familial breast cancer or familial ovarian cancer is different, possibly due to mutation frequency distribution (and also penetrance) varying according to the population (related to a founder effect).
- In female breast cancer cases unselected for family history, mutation prevalence estimates vary for *BRCA1* from 0.7% (95% CI: 0.3-1.3) to 6.0% (95% CI: 3.8-8.8) and, for *BRCA2*, from 1.1% (95% CI: 0.4-2.2) to 3.9% (95% CI: 2.2-6.3). In ovarian cancer series, estimates vary from 1.9% (no CI provided) to 9.6% (95% CI: 6.7-13.5) for *BRCA1* and 0.9 to 4.1% (no CIs provided) for *BRCA2* in ovarian cancer series. Variability could be attributed to ascertainment of prevalent as opposed to incident cases, differing inclusion criteria, selection based on age at diagnosis (for breast cancer), population-based versus hospital-based selection (for ovarian cancer) or mixtures of molecular techniques.
- No studies directly measure *BRCA1/2* mutation prevalence in the general population (i.e., heterogeneous population). As prevalence is expected to be low, this approach would require a very large number of samples to be analyzed, a difficult and expensive undertaking. Few studies have determined prevalence in women with breast cancer unselected for both age and family history: estimates vary from 1.1% (95% CI: 0.4-2.2) to 2.6% (95% CI: 0-5.5) for *BRCA1*

mutations, and prevalence for *BRCA2* mutations has been estimated in one study as 1.1% (95% CI: 0.4-2.2).

- Stratified analyses show that mutation prevalence is associated with various risk factors: breast cancer diagnosed at an early age, specific ethnic origin (e.g., AJ), ovarian cancer and male breast cancer. Cut-off values for defining 'early' age at diagnosis of breast cancer vary widely in the literature, and there is no general agreement as to the value that would justify age as the only risk factor for ordering tests. This matter will be explored further in chapter 7 on testing indications. The issue is not as controversial if there is also a family history of breast and/or ovarian cancer. The discriminating power of the number of breast cancer cases and the presence of bilateral cancer as risk factors appears to depend on the presence of ovarian cancer in the family history and age of onset of cancer, respectively.
- The fact that recurrent mutations have been discovered in certain populations makes the molecular analysis of the *BRCA1/2* genes easier, since one can screen for common mutations first rather than proceeding directly to screening all exons. However, the testing strategy should take the importance of the founder effect into account. The three most striking examples of founder mutations are those in the Ashkenazi Jewish, Icelandic and Polish populations. Between 1.9 and 2.7% of the AJ population have been estimated to carry one of three common mutations and 36-53% of AJ women who develop ovarian cancer are estimated to be carriers of one of these mutations. In the Icelandic population, 40% of men who develop breast cancer during their life are estimated to be *BRCA2* 999del5 mutation carriers. Screening for selected founder mutations is a common clinical procedure for specific ethnic groups (e.g., AJ), while optimal approaches have not yet been established in other founder populations.

- There are a number of uncertainties that affect the use of molecular testing for *BRCA1/2* mutations. For example, between 44-96% of individuals referred to specialised clinics due to family history of cancer test negative for *BRCA1/2* mutations. On the other hand, up to 36.4% of women who develop breast cancer and are mutation carriers report no family history of breast or ovarian cancer. Identification of a fairly large proportion of *BRCA1/2* variants of unknown clinical significance (VUCs) raises the problem of classifying and interpreting these test results, deciding whether or not to include them in prevalence calculations, and choosing appropriate clinical follow-up strategies.

5.2 PENETRANCE

For *BRCA1/2* mutation carriers, penetrance is defined as the cumulative risk of developing cancer, either up to a specific age or during lifetime. The penetrance of the *BRCA1/2* genes is less than 100% and is therefore said to be incomplete. For breast and ovarian cancer risks, the majority of studies evaluated were retrospective cohort or case-control studies. Results are presented in Tables 9 to 12. The presentation of the data and synthesis of information from these studies will be organized according to three main groups, similar to the case for prevalence data: i) families selected for genetic linkage analysis (Table 9); ii) founder populations (Tables 10 and 11); and iii) heterogeneous populations (Table 12). Here, however, breast and ovarian cancer data will be featured separately. The details pertaining to each individual study can be found in Table D1 in Appendix D. Although other cancers, such as prostate and male breast cancer, have been associated with *BRCA1* and *BRCA2* mutations, the highest risks are for breast and ovarian cancer and therefore the selected studies primarily focus on these two conditions. Possible explanations for the variability in penetrance of *BRCA1/2* mutations in the

literature, such as methodology,⁵⁴ will also be discussed.

5.2.1 Breast Cancer

5.2.1.1 STUDIES OF FAMILIES SELECTED FOR THE PURPOSE OF GENETIC LINKAGE ANALYSIS

The magnitude of the risk of breast cancer associated with *BRCA1/2* mutations is the subject of much debate in the literature. As seen for prevalence figures, the first penetrance estimates arose from the BCLC studies (Table 9). It was reported that the cumulative breast cancer risk at age 70 years is similar for the two genes: 85 to 87% (95% CI: 72-95 for the latter estimate) for *BRCA1* and 84% for *BRCA2* mutations (95% CI: 43-95) [Ford *et al.*, 1998; Easton *et al.*, 1995; Ford *et al.*, 1994]. However, the cumulative risk curve as a function of age was estimated to be different for the two genes: the mean age of onset of breast cancer was observed to be lower in *BRCA1* mutation carriers whereas the risk of cancer was found to be higher in older women who carried a *BRCA2* mutation [Easton *et al.*, 1995; Ford *et al.*, 1994]. One study found no statistically significant difference in cumulative risk by gene, regardless of age [Ford *et al.*, 1998].

5.2.1.2 FOUNDER POPULATIONS

Penetrance estimates have been made for specific ethnic groups, including several with founder effects. In Table 10, six studies reported the risk of breast cancer associated with the *BRCA1* 185delAG, *BRCA1* 5382insC and *BRCA2* 6174delT founder mutations in the AJ population. Breast cancer risk at 70 to 75 years (depending on the study) varied from 43.8% (no CI provided) to 69% (95% CI: 59-79) for the *BRCA1* 185delAG and the 5382insC mutations, and from 26% (95% CI: 14-50) to 74% (95% CI:

58-90) for the *BRCA2* 6174delT mutation⁵⁵ [King *et al.*, 2003; Satagopan *et al.*, 2001; Moslehi *et al.*, 2000; Warner *et al.*, 1999]. Struewing and colleagues [1997] presented a combined risk of 56% (95% CI: 40-73) by age 70 for carriers of any of the three mutations, while Fodor and colleagues [1998] estimated a risk of 36% (no CI provided) for carriers of either the *BRCA1* 185delAG or *BRCA2* 6174delT mutation. The variability of the penetrance estimates can be attributed, in part, to the case ascertainment process and the method used to calculate penetrance, both of which differed across studies. As for case ascertainment, four studies used breast cancer cases [King *et al.*, 2003; Satagopan *et al.*, 2001; Warner *et al.*, 1999; Fodor *et al.*, 1998], while others used ovarian cancer cases [Satagopan *et al.*, 2002; Moslehi *et al.*, 2000] or volunteers unselected for personal history of cancer [Struewing *et al.*, 1997]. Furthermore, some studies used prevalent cases [Moslehi *et al.*, 2000; Warner *et al.*, 1999] while others used incident cases (e.g., Satagopan *et al.* [2001]).

For the calculation of penetrance, the two main approaches are based on disparate study designs and differ in terms of whether or not familial clustering of cancer is taken into account. In the pedigree approach, the family history of breast cancer in the *BRCA1/2* mutation-carrying index cases is compared to that in non-carriers [King *et al.*, 2003; Moslehi *et al.*, 2000; Warner *et al.*, 1999; Struewing *et al.*, 1997]. In the case-control approach, the frequency of *BRCA1/2* mutations in a series of cancer cases is compared to their frequency in a control group without cancer [Satagopan *et al.*, 2001 and 2002; Moslehi *et al.*, 2000 (for Ov only); Fodor *et al.*, 1998]. Of note, studies that use family history of cancer for the risk analysis are subject to recall bias, especially if the cancer cases in the family

54. The different calculation methods used for the different designs are presented in Table 9-12 and in Appendix D (Table D1), but we did not analyse these in detail.

55. According to several studies, penetrance of the *BRCA2* 6174delT mutation for breast cancer could be lower than that of *BRCA1* 185delAG. Furthermore, as pointed out by Narod and Foulkes [2004], lower prevalence of the 6174delT mutation has been observed in cases of breast cancer or in families with multiple cases of breast cancer, despite similar frequency in the general Jewish population. Of note, the 6174delT mutation is situated in the ovarian cancer cluster region (see section 5.2.3.2 Genotype/Phenotype Correlation: Mutation Position).

have not been confirmed by pathology reports [Moslehi *et al.*, 2000; Warner *et al.*, 1999; Struewing *et al.*, 1997]. In addition, the accuracy of the estimate is limited by the fact that molecular analysis is not performed in all relatives. In one study of this kind, cancer cases were confirmed by pathology reports and carrier status of family members was documented to the best extent possible; however, only relatives of known *BRCA1/2* mutation status were included in the analysis and whole nuclear families were excluded if carrier status could not be inferred in a member of a sibship [King *et al.*, 2003]. As a result, it is possible that a greater proportion of unaffected carriers compared with affected carriers were excluded from the analysis, resulting in the overestimation of penetrance [CHEK2, 2004; Wacholder *et al.*, 2004].

Breast cancer risk has been estimated in other founder populations besides the AJ and results from selected studies are presented in Table 11. In the Icelandic population, a study was carried out that involved women and men with breast cancer ascertained from the national cancer registry.⁵⁶ The penetrance of the *BRCA2* 999del5 founder mutation was estimated as 37.2% at age 70 years (95% CI: 22.4-53.9) [Thorlacius *et al.*, 1998]. In Norwegian women, the risk of breast or ovarian cancer (whichever was detected first) associated with the two founder mutations *BRCA1* 1675delA and *BRCA1* 1135insA was estimated as 70 to 90% (no CIs provided) at 70 years, depending on assumptions about untested subjects [Dorum *et al.*, 1999b]. Heimdal *et al.* [2003] extended the study done by Dorum *et al.* [1999b] and estimated that the risk of breast and/or ovarian cancer associated with four founder *BRCA1* mutations (1675delA, 1135insA, 816delGT, 3347delAG) in the Norwegian population was a minimum of 62% and a maximum of 84% at 70 years (no CIs provided).

In the French-Canadian population, penetrance of *BRCA1/2* mutations has been measured in families with multiple cases of breast and/or

ovarian cancer [Durocher *et al.*, 2004].⁵⁷ Risk of breast cancer at 70 years was estimated as 72% (95% CI: 0-93) for *BRCA1* mutations and 75% (95% CI: 0-97) for *BRCA2* mutations, but the confidence intervals for these figures are particularly large (Table 11). Penetrance specific to the two French-Canadian founder mutations *BRCA1* R1443X and *BRCA2* 8765delAG was estimated as 86% (n=18 families) and 71% (n=23 families), respectively (no CIs provided).

5.2.1.3 HETEROGENEOUS POPULATIONS

Breast cancer risk in *BRCA1/2* mutation carriers has been estimated in heterogeneous populations, such as those in Great Britain, US, Australia and Canada (Table 12). In studies that sampled cancer cases unselected for family history, three reported data separately for *BRCA1* and 2 [Antoniou *et al.*, 2003; Risch *et al.*, 2001; ABC Study Group, 2000]. The cumulative risk of breast cancer varied from 45% (95% CI: 22-76) at age 70 to 68% (no CI provided) at age 80 for *BRCA1* mutations and was estimated as 56% (95% CI: 5-80; only one study) at age 70 for *BRCA2*. Two studies estimated penetrance for *BRCA1/2* mutations combined and the breast cancer risk at 70 years varied from 36% (95% CI: 15-65) to 50.2% (95% CI: 14.9-100) [Loman *et al.*, 2003; Hopper *et al.*, 1999]. Possible reasons for the inter-study variability in penetrance values are the different mutation detection techniques employed, use of prevalent versus incident cases and the ascertainment of breast cancer cases versus those with ovarian cancer. For the calculation of penetrance, all identified studies used family history of cancer in relatives; two considered both first- and second-degree relatives [Antoniou *et al.*, 2000; Sutcliffe *et al.*, 2000], while others considered first-degree relatives only [Antoniou *et al.*, 2003; Loman *et al.*, 2003; Risch *et al.*, 2001; ABC Study Group, 2000]. In one study, the genotype of certain family members was analysed and carrier status was inferred in other relatives [Hopper, 2001].

56. All male breast cancer diagnosed during 1955–1996; time period was not specified for female breast cancer.

57. Updated information was provided by J. Simard and F. Durocher, personal communication, September 2 and 8, 2005.

Very few studies have assessed cancer risk in families referred to clinics as a result of their family history. Yet, most individuals in whom a *BRCA1/2* mutation search is performed attend these clinics. In one study that determined the penetrance of *BRCA1* mutations in families ascertained at two high-risk cancer clinics in the US, the cancer risk was found to be 72.8% at 70 years (95% CI: 67.9-77.7) (Table 12) [Brose *et al.*, 2002]. For this risk calculation, affected individuals whose age at diagnosis of cancer was not known were considered unaffected, which could have resulted in an underestimation of penetrance.

In addition, one study selected families from a national ovarian cancer registry [Sutcliffe *et al.*, 2000]. The authors report a relatively low risk of breast cancer in women from *BRCA1* or *BRCA2* mutation positive families (about 11%) and they suggest that this low risk could be due to study design limitations (the proportion of unaffected relatives that were mutation carriers is not clear). An alternative explanation relates to the fact that all *BRCA1/2* mutations identified lie in regions previously associated with low risk of breast cancer [Sutcliffe *et al.*, 2000].

Table 9.
Estimation of breast (Br) and ovarian (Ov) cancer risks in *BRCA1/2* carriers from families selected for linkage studies

REFERENCE	CHARACTERISTICS OF STUDY SAMPLE FOR RISK ESTIMATION			PENETRANCE AT 70 YEARS % (95% CI, IF AVAILABLE)		DESIGN/ METHODOLOGY
	Country / region	Mode of ascertainment	Sample size (families)	<i>BRCA1</i>	<i>BRCA2</i>	
Ford <i>et al.</i>, [1994]	NA and Western Europe	multiple case (≥ 4) families with Br/Ov ca†	33	Br: 87 (72-95) Ov: 44 (28-56)	nd	lifetime incidence of 2 nd ca in FDR carriers with ca (contralateral Br or Ov ca)
Easton <i>et al.</i>, [1995]	NA and Western Europe	multiple case (≥ 4) families with Br/Ov ca†	33	Br: 85.0 Ov: 63.3	nd	max of Lod score over different penetrance functions
Ford <i>et al.</i>, [1998]	NA and Western Europe	multiple case (≥ 4) families with Br/Ov ca†	32	nd	Br: 84 (43-95) Ov: 27 (0-47)	max of Lod score over different penetrance functions
BCLC [1999]	NA and Western Europe	multiple case (≥ 2 or more restrictive in some centres) families with Br/Ov ca†	173	nd	Br: 52.3 (41.7, 61.0) Ov: 15.9 (8.8-22.5)	lifetime incidence of 2 nd ca in FDR carriers with ca (contralateral Br or Ov ca)

NA: North America; ca: cancer; nd: not determined; FDR: first degree relative; max: maximization

†see Table D (Appendix D) for more details on selection criteria

Table 10.

Estimation of breast (Br) and ovarian (Ov) cancer risks associated with the three founder *BRCA1/2* mutations in the AJ population

REFERENCE	CHARACTERISTICS OF STUDY SAMPLE			PENETRANCE AT 70 y, %(95% CI, IF AVAILABLE)		DESIGN/ METHODOLOGY
	Country or region	Mode of ascertainment	Sample size*	<i>BRCA1</i> (185delAG, 5382insC)	<i>BRCA2</i> (6174delT)	
Struewing <i>et al.</i>, [1997]	USA	volunteer men and women >20 y (n=5318)	306 FDR of carriers; 13018 FDR of non-carriers	Br: 56 (40-73) Ov: 16 (6-28)		Based on fam hx of ca in FDR of <i>BRCA1/2</i> mutation carriers versus non-carriers; risk of disease among relatives determined using Kaplan-Meier
Fodor <i>et al.</i>, [1998]	USA	cases: hospital-based Br ca; controls: men and women referred for prenatal testing	268 cases; 1715 controls	Br: 36 (for 185delAG only)	Br: 36	Case-control approach: Risk for a carrier (R_C), calculated with the formula: $R_C = C_{BC} \times R_G / F$; C_{BC} is the proportion of mutation carriers among the patients with Br ca, R_G is the risk of Br ca in the gp (0.125), and F is the carrier frequency in the AJ control sample
Warner <i>et al.</i>, [1999]	Canada	hospital-based Br ca cases (n=412)	128 FDRs of 48 carrier index cases	Br: 59.9	Br: 28.3	Cumulative risk calculated for female FDR of carrier cases based on fam hx of Br ca using life table analysis; PN estimates by kin-cohort method
Moslehi <i>et al.</i>, [2000]	North America and Israel	cases: hospital and population-based Ov ca (n=208); controls: female hospital employees and community volunteers (n=386)	253 FDRs of 86 carrier index cases; 1130 FDRs of 386 controls	Br at 75 y, method 2: 43.8 (for the 2 mutations) Ov at 75 y, method 1: 51.2 (for 185delAG); 29.1 (for 5382insC); Ov at 75 y, method 2: 10.1 (for 185delAG); 21.0 for 5382insC	Br at 75 y, method 2: 36.8 Ov at 75 y, method 1: 19.9 Ov at 75 y, method 2: 26.6	1 (for Ov): compared freq of mutations in cases with AJ gp data (Struewing <i>et al.</i> , [1997]) & used baseline gp (SEER) lifetime risk; 2 (for Br and Ov): cumulative risks for female FDR of cases compared to female FDR of controls based on hx of Br and Ov ca, using actuarial survival method and Cox model table analysis; PN estimates by kin-cohort method

Table 10 (continued).

REFERENCE	CHARACTERISTICS OF STUDY SAMPLE			PENETRANCE AT 70 y, %(95% CI, IF AVAILABLE)		DESIGN/ METHODOLOGY
	Country or region	Mode of ascertainment	Sample size*	<i>BRCA1</i> (185delAG, 5382insC)	<i>BRCA2</i> (6174delT)	
Satagopan <i>et al.</i>, [2001]	USA and Canada	cases: hospital- based Br ca; controls: unaffected volunteer men and women >20 y	782 cases; 3434 controls	Br: 46 (31-80)	Br: 26 (14-50)	Case-control approach: PN determined by age- specific incidence rates of Br ca for AJ female carriers of <i>BRCA1/2</i> mutations using: 1) age specific prevalence of the 3 mutations in AJ gp using controls; 2) age- specific RR of Br ca in carriers versus controls; 3) gp incidence rate (using SEER data)
Satagopan <i>et al.</i>, [2002]	USA, Canada, and Israel	cases: hospital- based Ov ca; controls: unaffected volunteer men and women >20 y	382 cases; 3434 controls	Ov: 37 (25-71)	Ov: 21 (13-41)	Case-control approach: PN determined by age- specific incidence rates of Ov ca for AJ female carriers of <i>BRCA1/2</i> mutations using: 1) age specific prevalence of the 3 mutations in AJ gp using controls; 2) age- specific RR of Ov ca in carriers versus controls; 3) gp incidence rate (using SEER data)
King <i>et al.</i>, [2003]	USA	hospital-based Br ca cases (n=1008)	212 carrier relatives	Br†: 69 (59-79) Ov†: 46 (33-58)	Br†: 74 (58-90) Ov†: 12 (0-26)	Based on fam hx of ca in relatives of <i>BRCA1/2</i> mutation carriers (all probands were excluded); carrier status in relatives was inferred or determined by molecular analysis; risk calculation by Kaplan-Meier

y: years; ind: individuals; gp: general population; FDR: first degree relatives; freq: frequency; RR: relative risk; PN: penetrance; pop: population; fam hx: family history; molec: molecular; SEER: National Cancer Institute's Surveillance, Epidemiology and End Results registry, USA

*sample used to estimate penetrance figures presented in chapter 5

†calculated (and rounded) using reported standard errors and assuming a normally distributed sampling distribution of cumulative risk

Of note, breast cancer risk was recently the subject of a meta-analysis that pooled raw data from 22 studies from around the world⁵⁸ [Antoniou *et al.*, 2003]. The meta-analysis combines different ethnic groups, including both heterogeneous and founder populations. Inclusion criteria for the meta-analysis included: 1) availability of the analysis of *BRCA1* or *BRCA2* mutations (by a systematic screen) in breast cancer cases (male or female) or ovarian cancer cases unselected for family history, and 2) availability of a detailed family history of breast or ovarian cancer in the first-degree relatives of carriers of deleterious mutations, with information on age at diagnosis and age at last observation. This analysis has the advantage of having assembled the largest sample of cancer cases unselected for family history (i.e., 498 *BRCA1/2* mutation carriers) whose family histories of breast or ovarian cancer were available. However, in addition to differences in ethnicity, the included studies varied widely in terms of sample ascertainment methods, timing of data collection, and, in particular, with respect to techniques used to detect mutations.⁵⁹ The risk of breast cancer at 70 years was estimated to be 65% (95% CI: 51-75) for *BRCA1* mutations and 45% (95% CI: 33-54) for *BRCA2* mutations. Antoniou and colleagues also present relative risks of breast cancer for mutation carriers by decade of age, compared to the population of England and Wales in 1973-77. These results—with significant relative risk estimates in the order of about 10 to 30—demonstrate that breast cancer risk for carriers is clearly elevated over that of the general population.

58. Several of these studies are detailed in Tables 8 and 10 of the present section [Risch *et al.*, 2001; ABC Study Group, 2000; Moslehi *et al.*, 2000; Warner *et al.*, 1999; Thorlacius *et al.*, 1998]. In addition, some of these studies were included in our synthesis of results for the prevalence section [Risch *et al.*, 2001; ABC Study Group, 2000; Anton-Culver *et al.*, 2000; Moslehi *et al.*, 2000; Syrjäkoski *et al.*, 2000; Van Der Looij *et al.*, 2000; Peto *et al.*, 1999; Warner *et al.*, 1999]. The other studies in the meta-analysis either did not meet our sample size selection criteria or were unpublished.

59. Antoniou and colleagues test for heterogeneity of risk according to geographic region (i.e., UK, Canada, Poland [for *BRCA1* only], Iceland [for *BRCA2* only], and “Other”) rather than by centre, but find no significant evidence.

5.2.2 Ovarian cancer

Many of the studies in Tables 9 to 12 also include data on the cumulative risk of ovarian cancer; five studies did not [Loman *et al.*, 2003; Hopper *et al.*, 1999; Warner *et al.*, 1999; Fodor *et al.*, 1998; Thorlacius *et al.*, 1998]. One study provided values for ovarian cancer only [Satagopan *et al.*, 2002].

5.2.2.1 STUDIES OF FAMILIES SELECTED FOR THE PURPOSE OF GENETIC LINKAGE ANALYSIS

As Table 9 shows, the cumulative risk of ovarian cancer at 70 years was estimated in genetic linkage studies of BCLC families and varied from 44% (95% CI: 28-56) to 63.3% (no CI provided) for *BRCA1* mutations and from 15.9% (8.8-22.5) to 27% (95% CI: 0-47) for *BRCA2* mutations [Breast Cancer Linkage Consortium, 1999; Ford *et al.*, 1998; Easton *et al.*, 1995; Ford *et al.*, 1994].

5.2.2.2 FOUNDER POPULATIONS

Ovarian cancer risk has been estimated in carriers of one (or more) of the three *BRCA1/2* founder mutations in studies in the AJ population (Table 10). For carriers of the *BRCA1* 185delAG or 5382insC mutations, the cumulative risk at 70 years varied from 37% (95% CI: 25-71) to 46% (95% CI: 33-58), and for *BRCA2* 6174delT mutation carriers, risk estimates varied from 12% (95% CI: 0-26) to 21% (95% CI: 13-41) [King *et al.*, 2003; Satagopan *et al.*, 2002]. Struewing and colleagues [1997] presented a combined risk of 16% (95% CI: 6-28) at age 70 for carriers of any of the three founder mutations. Moslehi and colleagues [2000], in contrast, provide penetrance estimates at age 75 for the three mutations separately, and according to two different methods of risk calculation. As mentioned earlier, one study from Norway [Dorum *et al.*, 1999b] reported a combined cumulative risk of breast and ovarian cancer associated with the two *BRCA1* founder mutations in the Norwegian population (see Table 9). In the French-Canadian population, the ovarian cancer risk at age 70 was estimated in high risk families as 38% (95% CI: 0-78) for *BRCA1* mutations and 49% (95% CI: 0-81) for

BRCA2 mutations [Durocher *et al.*, 2004].⁶⁰ Penetrance specific to the two French-Canadian founder mutations *BRCA1* R1443X and *BRCA2* 8765delAG was estimated as 54% (n=18 families) and 51% (n=23 families), respectively (no CIs provided).

5.2.2.3 HETEROGENEOUS POPULATIONS

In heterogeneous populations (Table 12), the ovarian cancer risk at 70 years estimated from cancer cases unselected for family history varied, depending on the study, from 36% (95% CI: 4-99) to 66% (95% CI: 36-92) for *BRCA1* and was reported as 10% (95% CI: 1-55) for *BRCA2* [Risch *et al.*, 2001; ABC Study Group, 2000; Antoniou *et al.*, 2000]. According to a study that involved families ascertained at two high-risk clinics in the US, penetrance in *BRCA1* mutation carriers at age 70 was 40.7% (95% CI: 35.7-45.6) [Brose *et al.*, 2002]. In a study that selected families from a national ovarian cancer registry, the risk of ovarian cancer was estimated to be about 11.5% (no CI provided) at 70 years in women from *BRCA1* or *BRCA2* mutation positive families [Sutcliffe *et al.*, 2000].

Lastly, a 2003 meta-analysis reported ovarian cancer risk at age 70 to be 39% (95% CI: 22-51) for *BRCA1* mutations and 11% (95% CI: 4.1-18) for *BRCA2* mutations [Antoniou *et al.*, 2003]. Relative risk estimates for ovarian cancer varied widely from about 6 to 68, depending on decade of age, and were statistically significant. As previously noted, this meta-analysis combines different ethnic groups, including both heterogeneous and founder populations.

An important assumption of penetrance research in general is that the risks of breast and ovarian cancer are independent of each other among carriers of *BRCA1/2* mutations. Recently, Chatterjee *et al.* [2003] determined, by means of a simulation model taking the presence of non-independent censoring events⁶¹ into account, that the cumulative risk of ovarian cancer in *BRCA* mutation positive families has presumably been underestimated [Chatterjee *et al.*, 2003]. On the

basis of the Washington Ashkenazi Study [Struwing *et al.*, 1997], the authors estimated that the cumulative risk of ovarian cancer at age 70 should be increased from 10.9% (95% CI: 3.2-15.9) to 14.4% (95% CI: 4.1-22.6), to account for the fact that the competing risk of breast cancer is related to the presence of a *BRCA1/2* mutation. Given the high risk of breast cancer for *BRCA1/2* mutation carriers, previous cumulative risk estimates for ovarian cancer could be directly applicable only to women who have some kind of intervention (e.g., bilateral mastectomy) that removes most or all of the risk of breast cancer, but does not change the risk of ovarian cancer.

5.2.3 Penetrance variability

Penetrance point estimates for *BRCA1/2* mutations vary substantively from study to study. They appear to be highest in families selected for the purpose of genetic linkage analysis and generally lower in population studies, although the confidence intervals associated with these estimates are large. Point estimates for individuals referred to specialised clinics as a result of their family history fall between these two extremes. A number of reasons for this variability likely exist (related to wide confidence intervals, study design, etc.) and this is a subject of much debate among those conducting genetic breast cancer research. Since penetrance point estimates obtained in the BCLC studies were higher than those obtained subsequently (despite overlapping confidence intervals), the question has been posed whether the high risk values obtained in the early studies were due to biases inherent to the methodology used for the statistical analysis and sampling, or if they reflect specific characteristics of a highly selected population. Further, are these specific characteristics genetic in nature (i.e., due to highly penetrant mutations or variations at other genetic loci) or are they environmental factors that happen to cluster in these families? The answers to these questions are still not clear [Narod and Foulkes, 2004]. Exploration of methodological issues in penetrance studies and other potential sources of variability was thus thought to be of benefit for the reader and follows in the next section.

60. Updated information was provided by J. Simard and F. Durocher, personal communication, September 2 and 8, 2005.

61. That is, the fact that people could leave the observation period of the study due to either breast cancer or ovarian cancer.

Table 11.

Estimation of breast (Br) and ovarian (Ov) cancer risks associated with common *BRCA1/2* mutations in populations with a founder effect

REFERENCE	CHARACTERISTICS OF STUDY SAMPLE			COMMON MUTATIONS	PENETRANCE AT 70 Y, % (95% CI)		DESIGN/METHODOLOGY
	Ethnicity	Mode of ascertainment	Sample size		<i>BRCA1</i>	<i>BRCA2</i>	
Dorum <i>et al.</i>, [1999b]	Norwegian	population based Ov ca cases (n=615) and clinic-based families with Br/Ov ca (n=222)	relatives of 30 carrier index cases	<i>BRCA1</i> 1675delA and 1135insA	Br or Ov ca, whichever came first ¹ : 90; 70; 76	nd	Kaplan-Meier analysis done in 3 ways using ca hx among relatives: MAX: included demonstrated, obligate and affected carriers; MIN: all untested unaffected sisters assumed to be carriers; INTERMEDIATE: assumed different proportions of carriers among untested unaffected sisters according to age.
Heimdal <i>et al.</i>, [2003]	Norwegian	population based Ov and Br ca cases (n=791) and clinic-based families with Br/Ov ca (n tested not specified)	962 FDR of 99 carrier index cases	<i>BRCA1</i> 1675delA, 1135insA, 816delGT, 3347delAG	Br or Ov ca, whichever came first ^{1,2} : 84; 62; 70	nd	Kaplan-Meier analysis done in 3 ways using ca hx among female FDR of carriers: MAX: included demonstrated, obligate and affected carriers; MIN: all untested relatives assumed to be carriers; INTER-MEDIATE: assumed different proportions of carriers among untested relatives according to age.
Thorlacius <i>et al.</i>, [1998]	Icelandic	population-based Br ca cases (541 females, 34 males)	252 FDRs of 56 carrier index cases; 2156 FDRs of 485 non-carrier cases	<i>BRCA2</i> 999del5	nd	female Br ca: 37.2 (22.4-53.9)	Based on hx of ca in FDR of mutation carriers versus non-carriers; the cumulative incidence of Br ca in each age group of FDR was estimated using Kaplan-Meier curves
Durocher <i>et al.</i>, [2004]³	French Canadian	families with multiple affected individuals recruited in Quebec (n=251)	25 <i>BRCA1</i> mutation + families; 27 <i>BRCA2</i> mutation + families	mixture of founder and unique mutations ⁴	Br: 72 (0-93) Br: 86 for R1443X Ov: 38 (0-78) Ov: 54 for R1443X	Br: 75 (0-97) Br: 71 for 8765delAG Ov: 49 (0-81) Ov: 51 for 8765delAG	Max likelihood using modified segregation analysis; only families for whom at least one carrier was identified, and at least one other family member was subsequently tested, were included in the PN analysis

y: years; min: minimum; max: maximum; nd: not determined; PN: penetrance; FDR: first degree relatives; SDR: second degree relatives

1. max; min; intermediate

2. For Br ca, no difference in PN between the 4 mutations. For Ov ca, 1675delA had significantly higher PN than the 816delGT and 3347del AG mutations. No differences in PN between families ascertained through the cancer family clinics and the population-based consecutive series.

3. Updated information was provided by J. Simard and F. Durocher, personal communications, September 2 and 8, 2005.

4. J. Simard, personal communication, January 19, 2005.

Table 12.
Estimation of breast (Br) and ovarian (Ov) cancer risks in BRCA1/2 mutation carriers in heterogeneous populations

REFERENCE	CHARACTERISTICS OF STUDY SAMPLE			PENETRANCE AT 70 Y, % (95% CI)		DESIGN/METHODOLOGY
	Country or ethnic origin	Mode of ascertainment	Sample size	BRCA1	BRCA2	
Hopper <i>et al.</i>, [1999]	Australia	population-based Br ca cases (n=388)	relatives of 18 carrier cases	1. Br: 40 (16-64) 2. Br: 36 (15-65)		Based on hx of ca in female FDR and SDR of carrier index cases by 1) maximum likelihood and 2) repeated sampling and Kaplan Meier. Carrier status was determined by molecular analysis in some relatives and probabilistically in others.
ABC Study Group [2000]	UK	population-based Br ca cases (n=1435)	FDR of 24 carrier index cases	Br: 47 (5-82) Ov: 36 (4-99)	Br: 56 (5-80) Ov: 10 (1-55)	Based on hx of ca in FDR and mutation analysis in proband. Max likelihood of pedigree given phenotypic and genotypic information of the index case to estimate RR for Br and Ov ca in carriers versus general population.
Antoniou <i>et al.</i>, [2000]	UK	hospital-based Ov cases (n=374)	FDR + SDR of 12 carrier index cases	Br: 45 (22-76) Ov: 66 (36-92)	nd	Based on hx of ca in FDR and SDR and mutation analysis in proband. Max likelihood of pedigree given phenotypic and genotypic information of the index case, to estimate RR for Br and Ov in carriers versus general population.
Sutcliffe <i>et al.</i>, [2000]	UK	families with ≥ 2 Ov ca in FDR identified in a national ovarian cancer registry (n=112)	54 carrier families for Ov ca risk, 55 for Br ca	Br: about 11% ⁶² Ov: about 11%		Based on lifetime incidence of ca in FDR and SDR of carriers or in affected family members who remained at risk of developing a 2 nd primary cancer.
Risch <i>et al.</i>, [2001]	Canada	population-based Ov ca cases (n=649)	451 FDRs of 60 carrier index cases; 4378 FDRs of 589 non-carrier index cases	Br at 80 y: 68 Ov at 80 y: 36	No significant increase in risk of Br or Ov ca in relatives of BRCA2 mutation carriers	Based on hx of ca in female FDR, according to mutation status of the proband; RR for Br and Ov ca calculated using proportional-hazards regression (baseline=ca in relatives of non-carrier cases). PN calculated by kin-cohort method.

62. Breast and ovarian cumulative risks were determined for both BRCA1/2 mutations. Cumulative risks presented above were extrapolated from data reported graphically by Sutcliffe and colleagues [2000].

Table 12 (continued).

REFERENCE	CHARACTERISTICS OF STUDY SAMPLE			PENETRANCE AT 70 Y, % (95% CI)		DESIGN/METHODOLOGY
	Country or ethnic origin	Mode of ascertainment	Sample size	<i>BRCA1</i>	<i>BRCA2</i>	
Brose <i>et al.</i>, [2002]	USA: 95% Caucasian	clinic-based families with Br/Ov ca (n=147)	483 carrier relatives of 147 carriers	Br: 72.8 (67.9-77.7) Ov: 40.7 (35.7-45.6)	nd	Based on cumulative age-adjusted ca risk in carrier relatives using lifetime data. The age-adjusted risk was calculated by decade to account for the small number of older individuals in the study.
Antoniou <i>et al.</i>, [2003]	many different countries and ethnic origins including founder populations (22 studies)	meta-analysis: <i>BRCA1/2</i> mutation carriers identified among hospital-based or population-based Br or Ov ca cases (n=8319)	relatives of 498 carriers	Br: 65 (51-75) Ov: 39 (22-51)	Br: 45 (33-54) Ov: 11 (4.1-18)	Meta-analysis. Based on hx of ca in FDR of identified carriers. Information on mutation status in relatives was incorporated when available. Max likelihood estimation, using modified segregation analyses.
Loman <i>et al.</i>, [2003]	Sweden	population-based Br ca cases dx before 41 y (n=262)	82 FDR of 22 carrier index cases	Br: 50.2* (14.9-100) *estimated for both <i>BRCA1/2</i> genes because of small sample size		Based on cumulative risk of Br ca for FDR of the index carriers. Index cases excluded.

nd: not determined; fam hx: family history; ca: cancer; dx: diagnosed; max: maximum; FDR: first degree relatives; SDR: second degree relatives; y: years

5.2.3.1 METHODOLOGY

Care should be exercised before rejecting penetrance values obtained from studies involving highly selected families [Devilee, 1999]. In their statistical analyses, the BCLC studies applied the lod-score method, which is independent of mutation type since it uses data from linkage analysis with gene-flanking markers. (This method is primarily used to close in on a susceptibility gene by looking for the co-occurrence of disease and genetic markers in a region thought to contain the gene of interest). Using this method, the genetic status of individuals in a given family could be determined with a degree of accuracy considered

valid and was then compared to clinical status confirmed by medical records or pathology reports [Devilee, 1999]. On the other hand, in the case of a population-based study involving 5,000 AJ volunteers, penetrance was estimated by comparing the number of cancer cases in first-degree relatives according to whether or not the index cases were *BRCA1/2* mutation carriers or non-carriers [Struwing *et al.*, 1997]. Cancer cases in the first-degree relatives were reported in interviews by the index cases and genetic status was determined only in the index cases. Therefore, it has been suggested that the link between carrier status in first-degree relatives and cancer in this particular study was not as well documented as in the BCLC studies

[Devilee, 1999]. It is still not certain if such methodological differences can explain the variation in estimations of penetrance. At the time of writing, a project being carried out by the International Agency for Research on Cancer is examining the advantages and the biases associated with the different sampling techniques and methods of statistical analysis for estimating cancer risk in *BRCA1/2* mutation carriers [IARC, 2004; Rebbeck, 2002; Devilee, 1999]. Besides methodological differences, other factors could contribute to penetrance variability, such as the nature of the particular mutation and the presence of genetic or environmental factors that can modulate cancer risk in carriers [Rebbeck, 2002; Hopper, 2001; Devilee, 1999]. These possible explanations will be discussed next.

5.2.3.2 GENOTYPE/PHENOTYPE CORRELATION

Phenotype (i.e., disease expression) is influenced by the type of mutation and its position in the genomic sequence. Some mutations cause the substitution of one amino acid by another (i.e., missense mutations), whereas others cause premature protein synthesis termination (i.e., nonsense mutations) or a shift in codon reading (i.e., frameshift mutations). In general, nonsense and frameshift mutations are associated with a more severe phenotype, since they lead to the absence of a protein or to a truncated protein. Some regions of a given protein are more important with regard to function and therefore, the risk of cancer may depend on where the mutation is located (i.e., mutation position).

▪ **Mutation type**

Since the nature of mutations could influence cancer risk, different mutation distributions in the population could, in part, explain variability in penetrance values [Rebbeck, 2002]. As an example, the estimated penetrance values in the BCLC studies might reflect the distribution of mutations associated with multiple-case families over several generations. Unfortunately, knowledge in this area is still incomplete and even though several hundred different *BRCA1/2* mutations have been identified, it is not clear to

what extent the mutations found in high-risk families differ from those identified in population studies [Hopper, 2001].

▪ **Mutation position**

Studies of families with multiple relatives with breast and/or ovarian cancer have shown that the position of *BRCA1/2* mutations in the genomic sequence can influence the risk of cancer in carriers. In the *BRCA1* gene, mutations found in the 3' region of exon 13 are associated with a lower proportion of ovarian cancers [Rebbeck, 2002; Iau *et al.*, 2001]. In addition, a study involving 356 high-risk families reported a 30% higher breast cancer risk in the women with a mutation in the 5' and 3' regions of the *BRCA1* gene, compared to those with a mutation in its central region (i.e., close to or in exon 11) [Rebbeck, 2002]. Some data suggest that the risk of breast cancer increases as the position of the mutation changes from the 5' end to the 3' end of the gene [Hopper, 2001].

Furthermore, nonsense and frameshift mutations in the ovarian cancer cluster region (OCCR) of the *BRCA2* gene (i.e., exon 11, nucleotides 1035-6629) are associated with a higher ovarian cancer to breast cancer ratio than such mutations in the other regions of the gene [Rebbeck, 2002; Borg, 2001; Iau *et al.*, 2001]. Mutations in the OCCR are associated with a lower risk of breast cancer and a higher risk of ovarian cancer compared to mutations outside this region [Antonioni *et al.*, 2005; Thompson *et al.*, 2001].

5.2.3.3 CANCER RISK MODIFIERS

Wide variability has also been observed in the age at diagnosis of breast cancer and in the site of occurrence of cancer in carriers of a particular *BRCA1/2* mutation. This variability is both inter- and intra-familial [Chang-Claude, 2001]. These observations support the hypothesis that other factors modulate the risk associated with *BRCA1/2* mutations such as other genes or environmental factors. The high penetrance values obtained in the BCLC studies may have resulted from cancer risk modification due to factors specific to high-risk families [IARC, 2004].

Several candidate genes are implicated in the metabolism of environmental carcinogens, metabolism of steroid hormones, cell cycle control and DNA repair. There remains a paucity of results in this area and little has been done to date to validate preliminary studies [Rebbeck, 2002].

Several reproductive factors (e.g., parity, age at first pregnancy, etc.) can modify the risk of sporadic breast cancer. Some studies suggest that these factors also influence breast cancer risk in *BRCA1/2* mutation carriers [Rebbeck, 2002], but the direction (i.e., increase or decrease in risk) and the extent of these effects are not well documented. It appears that for some of these factors (parity, pregnancy)⁶³, the effects are different from those observed in individuals with breast cancer who do not carry *BRCA1/2* mutations [Nkondjock and Ghadirian 2004; Tryggvadottir *et al.*, 2003; Rebbeck, 2002]. It has been reported that use of oral contraceptives may result in a slight decrease in the risk of ovarian cancer in *BRCA1/2* mutation carriers [Rebbeck, 2002].

Contradictory results have been reported for breast cancer risk. A large matched case-control study was recently carried out in 52 centers and 11 countries worldwide, including Canada, to measure the effect of oral contraceptive use on breast cancer risk [Narod *et al.*, 2002]. This study involved a total of 1,311 pairs of women with *BRCA1* or *BRCA2* mutations (981 and 330 matched pairs, respectively). Among *BRCA1* mutation carriers, it was found that oral contraceptive use modestly increased risk, especially of early-onset breast cancer, in women who first used contraceptives before 1975, in those who used them before age 30, or in those who used them for 5 or more years. In *BRCA2* mutation carriers, oral contraceptives do not appear to be associated with breast cancer [OR=0.94 (95% CI: 0.72-1.24)] but further confirmation of this finding is required.

In contrast, a population-based study of 1,156 early onset incident cases of breast cancer (diagnosed before age 40), and 815 controls in United States, Canada and Australia, found that using oral contraceptives for at least one year was associated with decreased breast cancer risk for *BRCA1* mutation carriers [adjusted OR=0.22 (95% CI: 0.10-0.49)] [Milne *et al.*, 2005]. Cases included only 47 *BRCA1* and 36 *BRCA2* mutation carriers (controls were not tested). This finding was not observed for *BRCA2* mutation carriers nor non-carriers; first use before 1975 was significantly associated with increased risk for non-carriers.

The effect of smoking on breast cancer risk in *BRCA1/2* mutation carriers is also a topic of debate, with some suggestion of a protective effect [Rebbeck, 2002]. A large international case-control study was recently conducted, including 1,097 women with breast cancer who were *BRCA1* or *BRCA2* mutation-positive and 1,097 matched women with a mutation in the same gene but without breast cancer [Ghadirian *et al.*, 2004]. The study did not show an association between breast cancer and smoking in carriers. However, a survival bias cannot be ruled out in this research because prevalent cases were used and an average of 8.9 years had elapsed from the time of diagnosis of breast cancer and the completion of the study questionnaire.

Preliminary data suggest there is an influence of birth year on breast cancer risk in *BRCA1/2* mutation carriers (i.e., a birth cohort effect). Breast cancer risk in AJ *BRCA1/2* mutation carriers born after 1940 was significantly higher than risk for carriers in the same family born before 1940 [King *et al.*, 2003]. In founder *BRCA1/2* mutation carriers identified from a cohort of AJ breast cancer cases diagnosed from 1980 to 1995, age of diagnosis decreased and prevalence of founder *BRCA1/2* mutations increased over the 15-year period [Foulkes *et al.*, 2002].

A better understanding of the factors that modulate breast cancer risk in *BRCA1/2* mutation carriers could help refine cancer risk at the clinical level, and could guide

63. Breast cancer diagnosed during pregnancy or within 1 year of giving birth [Nkondjock and Ghadirian, 2004].

recommendations concerning exposure to certain risk factors (e.g., oral contraceptives). In reality, however, knowledge of risk modifiers is still limited and so clinical impact remains marginal. A large part of this can be attributed to study limitations (i.e., insufficient sample size, retrospective recruitment of prevalent cases). In light of these limitations, an international prospective study entitled the International *BRCA1/2* Carrier Cohort Study (IBCCS), involving a cohort of more than 3,000 affected and unaffected *BRCA1/2* mutation carriers, is currently underway to specifically examine the factors that modulate cancer risk.⁶⁴ Nevertheless, breast cancer remains a complex disease and more precise cancer risk assessment may only be possible in certain groups of individuals or families. Research aimed at identifying the carcinogenic pathways of breast cancer may lead to the discovery of new therapeutic agents.

5.2.4 Other cancers associated with *BRCA1/2* mutations

There are other cancers that may be linked to *BRCA1/2* mutations in both women and men. In the reviewed studies, the risks in absolute terms appear to be lower and the levels are not as well defined as for ovarian and female breast cancer. Unfortunately, there remains a lack of data on the factors that contribute to the development of other types of cancer in mutation carriers (e.g., genotype-phenotype correlations, the role of other genes or environmental factors).

With regard to other cancers, the BCLC studies of multiple-case families did not find a statistical significantly elevated risk of colorectal cancer in *BRCA2* mutation carriers; they did, however, find a 2-fold increase of colon cancer (but not rectal cancer) in *BRCA1* carriers [Thompson and Easton, 2002; Breast Cancer Linkage Consortium, 1999]. In contrast, two recent case-control studies among AJ individuals indicate that the three AJ founder mutations are not significantly associated with risk of colorectal cancer [Kirchhoff *et al.*, 2004; Niell *et al.*, 2004]. The first of these studies consisted of 586

consecutive patients with confirmed colorectal cancer and 5,012 healthy controls, and the analysis was adjusted for age at diagnosis and sex [Kirchhoff *et al.*, 2004]. The second study consisted of 1,012 cases and 1,038 individuals without colorectal cancer [Niell *et al.*, 2004].

The early BCLC studies found a 3- to 4-fold higher risk of prostate cancer for *BRCA1* mutation carriers and a 4.5-fold higher risk associated with *BRCA2* mutations [Iau *et al.*, 2001]. More recent BCLC studies with larger samples do not show an increased risk in *BRCA1* mutation carriers, overall, but there is weak evidence for a modestly elevated risk at younger ages [Thompson and Easton, 2002]. However, *BRCA2* mutations appear to be responsible for a significant fraction of early-onset prostate cancer in men. In a study on 263 men with prostate cancer diagnosed before 56 years and identified regardless of family history of cancer, Edwards *et al.* [2003] have estimated a relative risk (RR) of 23 (95% CI: 9-57) in men with a *BRCA2* mutation. Edwards and colleagues report that this risk is significantly higher ($p=0.025$) than the RR of 7.3 (95% CI: 4.66-11.52) estimated from the BCLC study published in 1999, for patients diagnosed before 65 years [Breast Cancer Linkage Consortium, 1999]. The latter study was based on 173 high risk families with a *BRCA2* mutation [Breast Cancer Linkage Consortium, 1999].

In the AJ population, in comparison, it appears that the risk of prostate cancer associated with the three founder *BRCA1/2* mutations is not significantly increased. Hamel *et al.* [2003] analysed the three mutations in 146 AJ men with prostate cancer, combining their mutation frequency results with previous mutation data from a smaller sample of men with prostate cancer and from population controls. The combined data showed no statistically significant excess of mutations in cases compared to controls in either gene. Larger studies will be needed to confirm these results. Interestingly, it is possible that mutations in the *BRCA2* OCCR are associated with a lower risk of prostate cancer compared to mutations outside this region [Edwards *et al.*, 2003; Hamel *et al.*, 2003; Thompson *et al.*, 2001].

64. Web site of the International Agency for Cancer Research, <http://www-gep.iarc.fr/>, accessed on July 28, 2005.

The lifetime risk of male breast cancer is estimated to be 6% in *BRCA2* mutation carriers [Lynch *et al.*, 2004; Frank and Critchfield, 2001; Srivastava *et al.*, 2001]. The risk is also thought to be elevated in male *BRCA1* mutation carriers, but its magnitude has not been clearly determined [Frank and Critchfield, 2001]. Compared to the *BRCA1* gene, it appears that there is a wider range of cancers associated with *BRCA2* mutations (e.g., melanoma, stomach, pancreatic, gall bladder, pharyngeal, oral cavity, cervical and prostate cancer). The risk is relatively low in absolute terms for these cancers, with the exception of prostate cancer. Both *BRCA1* and *BRCA2* mutation carriers are found to be at increased risk for fallopian tube and peritoneal cancer [Casey and Bewtra, 2004; Swisher, 2001].

5.2.5 Clinical significance of polymorphisms

In general, polymorphisms consist of DNA sequence alterations that are of no consequence in terms of protein function and are found in more than 5% of the general population. Recently, however, the possibility has been raised that some common polymorphisms, such as N372H (H genotype) in the *BRCA2* gene, are associated (in the homozygous state) with a moderate cancer risk. In Australia and the UK, case-control studies suggest a 1.3- to 1.5-fold higher risk of breast and ovarian cancer in homozygous H genotype carriers of the N372H polymorphism [Auranen *et al.*, 2003; Spurdle *et al.*, 2002]. Despite this association, it has yet to be determined whether the N372H polymorphism is a genetic marker in linkage disequilibrium with another functional change in the *BRCA2* gene. In one case-control study from North Carolina, US, no association was found between ovarian cancer and the N372H polymorphism [Wenham *et al.*, 2003]. We did not find any association studies of the N372H polymorphism in breast cancer cases in the US population.

5.2.6 Conclusion

- Penetrance data for *BRCA1/2* mutations are provided in detail in this chapter for breast and ovarian cancer; penetrance variability, other associated cancers, and polymorphisms are also discussed. In comparison with the prevalence data, confidence intervals are more frequently reported in the published analyses of cancer risks. In general, these confidence intervals are wide.
- *BRCA1/2* mutations are associated with a high risk of breast and ovarian cancer, clearly elevated over that of the general population. Absolute risks of cancers other than breast or ovarian appear to be relatively small. In studies on high risk families (at least four cases), estimated risk of breast cancer by age 70 is similar for mutations in *BRCA1* (85-87%; 95% CI: 72-95 for the latter estimate) and *BRCA2* (84%; 95% CI: 43-95), but risk of ovarian cancer appears to be higher for mutations in *BRCA1* (44% [95% CI: 28-56] to 63% [no CI provided]) than in *BRCA2* (27% [95% CI: 0-47]). According to the only meta-analysis published in which data from 22 studies on populations unselected for family history were pooled, risk of breast cancer was lower based on point estimates (i.e., 65% [95% CI: 51-75] for *BRCA1* mutations and 45% [95% CI: 33-54] for *BRCA2* mutations). Point estimates for individuals referred to specialized clinics due to family history fall in between the above (i.e., 73% [95% CI: 68-78] for *BRCA1*).
- In one study, penetrance of *BRCA1/2* mutations has been estimated in French-Canadian high-risk families and is in line with point estimates of penetrance in high risk families among other ethnic groups, but confidence intervals are particularly large.
- Penetrance estimates with respect to breast and ovarian cancer vary widely from study to study. It is difficult to compare values since the design, study populations, and methods of calculating penetrance differ. Analyses are underway by the International Agency for Research on Cancer to differentiate between

the effects of disparate designs and analytical methodology, on the one hand, and the actual differences between populations, on the other.

- In addition to inter-study variability, there is a great deal of inter- and intra-familial variability in cancer risk in *BRCA1/2* mutation carriers, and different factors can play a role in modulating this risk (e.g., type of *BRCA1/2* mutation, and cancer risk modifiers such as other gene alterations, reproductive and environmental factors). With the exception of certain populations (e.g., AJ, Icelandic, Norwegian) where penetrance has been determined for specific founder mutations, estimates to date are a mean value for a broad range of *BRCA1/2* mutations. The knowledge of the specific penetrance values for each *BRCA1/2* mutation is limited and may never be known with any degree of accuracy, due to small numbers of carrier families and limited

availability of relatives for testing. Contradictions exist between studies on cancer risk modifiers and these cannot be used presently at the clinical level.

- Given the number of different factors that influence the phenotypic expression of hereditary breast cancer and the resulting inter- and intra-familial variability, a more precise estimate of individual breast and ovarian cancer risk in *BRCA1/2* mutation carriers is likely to be unrealistic, especially in heterogeneous populations. At the moment, as discussed in chapter 9, the information on penetrance is conveyed to patients and families as a range of values derived from empirical data on groups of families with similar risk factors. No biological markers are currently available to predict which carriers will develop cancer. In practice, family pedigree information and ethnicity are used to modulate risk assessment.

6. Risk assessment

6.1 INTRODUCTION

Chapter 5 demonstrates that the available evidence on prevalence and penetrance of *BRCA1* and *BRCA2* mutations is evolving over time as different populations are studied in different settings. There have been efforts to interpret and summarise this information for clinical purposes, in order to make recommendations on (1) who should be tested for *BRCA* mutations, and (2) how to manage counselling and/or testing, particularly with respect to interpretation of test results and assessment of probable disease risk.

Several tools currently exist to aid the clinician or genetic counsellor in predicting the probability (prior to testing) of carrying a *BRCA1/2* mutation, and in predicting the lifetime risk of breast cancer, given personal and family history of breast and/or ovarian cancer. For the probability of a mutation, one approach is to consult frequency tables that present the prevalence of *BRCA1/2* mutations according to categories that combine a patient's personal history of breast or ovarian cancer, if ever affected, and the patient's family history of these cancers. Categories containing larger proportions of carriers can be used to indicate priorities for testing. One of the most important sources of data of this kind comes from Myriad Genetic Laboratories. These data are similar to clinic-based information since the mutation frequencies arise from patients referred for genetic testing; however, the clinical referral criteria used are not specified, and are likely to be extremely varied.⁶⁵ As of January 2005, data were available for 23,317 non-Ashkenazi Jewish persons and 9,029 individuals with Ashkenazi Jewish (AJ) ancestry (tables available at www.myriadtests.com/provider/doc/mutprev.pdf).

A related approach to predicting the risk of having a mutation is to statistically test for associations between personal or family history factors and presence of *BRCA1/2* mutations. In this monograph, we refer to such analyses as statistical modelling for risk assessment. We identified a number of studies that have carried out this work using population, disease registry, laboratory or clinic-based samples; these studies often involved multivariate analysis to search for the 'best' predictors. Statistical models have also been developed to predict cancer risk, with or without knowledge of *BRCA1/2* mutation status.

The early statistical models, which have been widely used and amply discussed in the literature, will be succinctly described first. Subsequently, studies published since 1999 that have led to the development of new tools or models will be reviewed, with details as to the best predictors of mutation status as well as performance of the models, when available. The methodological approaches and results of studies intended to validate other statistical predictive models (for mutation or cancer risk) are then discussed. Finally, new trends in modelling cancer risk that account for all aspects of familial aggregation—in addition to *BRCA1/2* mutations—will be presented before concluding this chapter.

LITERATURE SEARCHING RESULTS

As described in the methods chapter of this monograph, we searched for primary research studies on risk assessment using electronic databases starting at 1999; we also consulted reference lists of relevant material. To be included in the data extraction, studies had to develop and/or test predictive risk models (incorporating *BRCA1/2* mutation testing or estimates of *BRCA1/2* prevalence/penetrance and cancer incidence). For models developed before 1999 ("early statistical models"), we used review articles from 2001 and 2002 to identify and describe the most-often cited studies. We

65. It is also reasonable to assume that there could be related persons in the tables which could impact the estimates.

also contacted care providers working in cancer genetics in Quebec, Ontario, and British Columbia to ensure that we included the most-often used tools.⁶⁶ A total of 59 articles were selected; some of these were used as complementary material only (see Appendix B2 for flow chart).

6.2 EARLY STATISTICAL MODELS

Tables 13 and 14 summarise the most commonly cited models, published prior to 1999, in the review articles we examined. For each model, a short description is provided of the data sources that contributed to the development of the model, the risk factors included, and the nature of the information provided. No models were found to predict risk of ovarian cancer.

Table 13 presents the early models predicting *risk of breast cancer*. The models by Gail and Claus were developed on the basis of samples that did not receive genetic testing for *BRCA* [Claus *et al.*, 1994; Gail *et al.*, 1989]; thus these models are not intended to be applied for risk prediction if an individual tests positive for a mutation. The Gail model is widely used for general risk screening for breast cancer [Euhus *et al.*, 2002a], but is limited in its incorporation of information about ethnicity and about family history, using number of affected first degree relatives only and excluding factors such as age at cancer diagnosis and presence of ovarian cancer. It may underestimate risk if there are highly penetrant susceptibility genes in a family or an autosomal dominant pattern of inheritance (this potential for underestimation also applies to the Claus model). Thus, the Gail model may be inappropriate for many women referred to high risk clinics on the basis of familial cancer.

The Claus model takes better account of the family cancer history (to the second degree level) and diagnostic age of the affected members, but cannot be used in the absence of breast cancer in first and second degree relatives

[Euhus *et al.*, 2002a] and does not consider the complete family structure [Chang-Claude *et al.*, 1999]. It excludes factors other than family history, and may underestimate risk under certain conditions related to distribution of cases and family structure.⁶⁷ The BRCAPRO model uses published data on population mutation prevalence, age-specific and cumulative penetrance (for *BRCA* carriers) and cancer incidence (for non-carriers) to estimate the probabilities of carrying a *BRCA1* or *BRCA2* mutation (calculated for each gene separately), based on an individual's personal and family history of cancer, if any [Parmigiani *et al.*, 1998]. BRCAPRO uses a Bayesian approach and an autosomal dominant inheritance model. Lifetime risks of disease to age 85 can then be calculated using the age-specific cancer probabilities.

In the clinical setting, BRCAPRO can be used to assist decisions about *BRCA* mutation testing (based on disease or mutation risk; it is also used in research as a substitute for testing or to validate other tools, see Tables 16 and 17). BRCAPRO has the advantage of using information from all first and second degree relatives, whether affected with breast or ovarian cancer or not. It relies on the specification of prevalence and penetrance values taken from the published literature. One set of input values, for example, uses prevalence figures of 1.22% and 1.36% for *BRCA1* and *BRCA2* mutations, respectively, for Ashkenazi Jewish individuals, and figures of 0.12% and 0.044% for *BRCA1* and *BRCA2* mutations, respectively, for non-Ashkenazi Jewish persons. Irrespective of ethnic groups, penetrance at age 70 is specified as 69.1% and 67.1% for breast cancer (for *BRCA1* and 2, respectively) and as 29.6% and 19.1% for ovarian cancer (for *BRCA1* and 2, respectively).⁶⁸ Berry and colleagues [2002] note that the model would be particularly inaccurate if high risk susceptibility genes other than

66. W. Foulkes (McGill University), C. Gilpin (Children's Hospital of Eastern Ontario), and K. Panabaker (BC Cancer Agency), personal communication, September 4, 15, and 23, 2003, respectively.

67. These include having more than two affected close relatives, cancer history on the paternal side, and estimating risk for an individual who does not have any sisters.

68. Penetrances at other ages are not necessarily set the same for Ashkenazi Jewish and non-Ashkenazi Jewish persons.

BRCA1/2 are segregating in a family [Berry *et al.*, 2002].

For the risk of carrying a *BRCA* mutation, the first three models in Table 14 are based on studies in which *BRCA* mutation testing was carried out among samples of persons fulfilling particular eligibility criteria. Both the Couch and Shattuck-Eidens models are limited since only *BRCA1* is considered. Couch and colleagues [1997] classify families according to average age at diagnosis of breast cancer and as containing (1) ‘breast cancer only’, (2) ‘breast and ovarian cancer’, (3) ‘with breast and ovarian cancer in a single member’, or both (2) and (3), rather than using information about the number of affected relatives⁶⁹ [Couch *et al.*, 1997]. The final model by Shattuck-Eidens and colleagues [1997] classifies family history slightly differently, as the number of relatives with breast cancer only, ovarian cancer only, or both [Shattuck-Eidens *et al.*, 1997]. The Frank model is only applicable to women with breast cancer diagnosed before age 50, or ovarian cancer at any age, and at least one female relative with breast cancer before age 50 or ovarian cancer [Frank *et al.*, 1998]. The Frank model does not provide separate estimates for Ashkenazi Jewish individuals. BRCAPRO is the only one that includes male breast cancer [Parmigiani *et al.*, 1998]. BRCAPRO may overestimate risk for those of Ashkenazi Jewish ancestry. The *CancerGene* computer software program can be used to calculate breast cancer risks according to the Gail, Claus and BRCAPRO models (Table 13) as well as *BRCA* mutation probabilities using the Couch, Shattuck-Eidens, Frank and BRCAPRO models (Table 14).⁷⁰

6.3 NEW STATISTICAL MODELS AND TOOLS: DEVELOPMENT AND PRELIMINARY TESTING

We identified 14 cross-sectional studies published in 1999 or later which developed (and sometimes tested) new predictive models or tools. Appendices E1 and E2 present the design of these studies according to ethnic group (Ashkenazi Jewish and non-Ashkenazi Jewish, respectively), with information on the origin and characteristics of the samples, the molecular methods used to test for *BRCA1/2* mutations, and the statistical analyses. Tables 15 and 16 summarise the results of these studies (according to ethnic group), and include the risk factors that were found to be the best predictors of mutation status. When available, data on the performance of these models and results of comparisons of various models are presented. Thirteen studies addressed the risk of having a *BRCA* mutation, whereas one incorporated the risk of developing breast or ovarian cancer in order to decide whether to refer for genetic counselling, and possibly mutation testing, based on a score [Gilpin *et al.*, 2000].

69. The model is not usable for ovarian cancer only.

70. Available free to health care providers at www3.utsouthwestern.edu/cancergene (accessed January 4, 2005).

Table 13.
Early models predicting risk of breast cancer

MODEL	DATA SOURCE	FACTORS IN THE MODEL	WHAT THE MODEL PROVIDES
Gail <i>et al.</i>, [1989]	large case-control study of women 35-79 years within large USA mammography project, and validated in other large cohorts of Caucasian women in USA	• age, age at menarche, and age at first live birth • number of previous biopsies • number of first degree relatives with breast cancer • presence of atypical ductal hyperplasia on biopsy • race (White, Black, Hispanic, or Asian)	cumulative risk estimates for breast cancer according to decade up to age 90 It is best used for women without a family history of breast cancer
Claus <i>et al.</i>, [1994]	large population-based case-control USA study of White women 20-54 years (developed using segregation analysis)	• age • number of breast cancer diagnoses among first and second degree relatives from both sides of family • age(s) of onset of breast cancer in relative(s)	lifetime breast cancer risk estimates by decade from 29-79 years Can also be used for women with a first-degree family history of ovarian cancer or of both breast and ovarian cancer
Parmigiani <i>et al.</i>, [1998]	BRCAPRO See Table 14; model can be used for both types of risk prediction		

Sources: NICE [2004]; Domchek *et al.*, [2003]; Ang and Garber, [2001]; Newman *et al.*, [2001]; Pinsky *et al.*, [2001]; Srivastava *et al.*, [2001]; Armstrong *et al.*, [2000]; Weitzel [1999]

Table 14.
Early models predicting risk of carrying a *BRCA* mutation

MODEL	DATA SOURCE	FACTORS IN MODEL	WHAT THE MODEL PROVIDES
Couch <i>et al.</i>, [1997]	263 mostly White breast cancer patients in high risk USA clinics (CSGE)	• presence of breast or ovarian cancer in the family • average age at breast cancer diagnosis (this may tend to include non-genetic cases) • presence of AJ ancestry	tables that can be used to estimate the probability of identifying a <i>BRCA1</i> mutation in families of particular types
Shattuck-Eidens <i>et al.</i>, [1997]	798 high risk persons in multi-site study (sequence analysis at Myriad Genetic Laboratories); <i>sometimes referred to as the "Myriad I" model</i>	• family history (# cases) and personal history of breast and ovarian cancer • age at diagnosis • presence of bilateral disease • ethnicity (AJ versus non-AJ)	multivariate equation that calculates the probability of identifying a <i>BRCA1</i> mutation for an affected individual diagnosed at no younger than age 30 (or for an unaffected relative of a case)
Frank <i>et al.</i>, [1998]	238 mostly White women with breast or ovarian cancer, and ≥ 1 affected relative (sequence analysis at Myriad Genetic Laboratories); <i>sometimes referred to as the "Myriad II" model</i>	• family history of breast and ovarian cancer (# cases) • age at breast cancer diagnosis • presence of bilateral disease	tables of mutation prevalences based on personal/family history and modelled probabilities of identifying a <i>BRCA1/2</i> mutation
Parmi-giani <i>et al.</i>, [1998]	published estimates of mutation prevalence, age-specific penetrance and cancer incidence (Claus <i>et al.</i> , 1994; Ford <i>et al.</i> , 1998; Streuwing <i>et al.</i> 1997, SEER† data) BRCAPRO	• first/second degree family history (# cases) of breast or ovarian cancer • ages at diagnosis and death, and ages of unaffected relatives • presence of other cancers and bilateral disease • whether genetic test results are available • AJ ethnicity	probability for a particular individual that a <i>BRCA1/2</i> mutation accounts for the family disease pattern (maternal or paternal) Also provides risk of cancer (using age-specific incidence estimates)

Sources: NICE [2004]; Domchek *et al.*, [2003]; Ang and Garber, [2001]; Newman *et al.*, [2001]; Srivastava *et al.*, [2001]; Farrell [1999]; Weitzel [1999]; Frank *et al.*, [1998]; Parmigiani *et al.*, [1998]; Couch *et al.*, [1997]; Shattuck-Eidens *et al.*, [1997]

†SEER: National Cancer Institute's Surveillance, Epidemiology and End Results, 1996

Table 15.

Development and preliminary testing of mutation risk models among Ashkenazi Jewish persons (since 1999): results

REFERENCE	SAMPLE SIZE / % CARRIERS†	BEST PREDICTORS OF MUTATION STATUS	MODEL PERFORMANCE VERSUS FOUNDER MUTATION TESTING OR VERSUS OTHER MODELS
Hodgson <i>et al.</i>, [1999] <i>Breast and ovarian cancer cases in the population</i>	184 / 15.2	<u>For 171 women with Br ca:</u> • age at dx • personal or family hx (1°, 2°) of Ov ca (dx at any age) • 1° relative with Br or Ov ca dx <age 60 • age at dx × personal hx of Ov ca	23/27 carriers had predicted risk ≥ 10% using model Sens=85%, Spec=59%, PPV*=28%, NPV*=95%
Hartge <i>et al.</i>, [1999] <i>Population volunteers</i>	5318 / 2.3	• Br or Ov ca in proband <u>For cases:</u> dx <age 50; little additional information from family hx <u>For non-cases:</u> family hx (mutation prob always <20%)	Lower mutation frequencies observed than Couch and Shattuck-Eidens estimates for the risk categories of women in the study
Hopper and Jenkins [1999] <i>Re-analysis of Hartge data</i>		<u>For women with same family hx,</u> mutation odds increase if affected & are also modified by age at dx or at testing Family hx effect seen in both cases & non-cases; average OR=2.7 per affected relative	
Foulkes <i>et al.</i>, [1999] <i>Re-analysis of Hartge data</i>		<u>For cases and non-cases,</u> family hx and age at dx important: OR for cases=2.6 with 1° family hx of Br ca (3.1 for non-cases) OR for cases=4.4 with ≥ 1 relative dx <age 50 (3.6 for non-cases)	
Apicella <i>et al.</i>, [2003] <i>Hodgson sample + Br and Ov cancer cases (or with family history)</i>	424 / 15.1	• Ov ca in proband (at any age) • Br ca in proband dx <age 50 • 1° relative with Br ca dx <age 60 or Ov ca (at any age) • 2° relative with Ov ca (at any age) • any bilateral Br ca in proband‡	<i>Note: the LAMBDA model (see Box 1) combines results with Hartge <i>et al.</i> information (mostly non-cases) to provide log odds for each predictor & total prob of carrying a founder mutation</i>

† BRCA founder mutation testing; ‡p=0.07

Br=breast; ca=cancer; dx=diagnosis; hx=history; Ov=ovarian; sens=sensitivity; spec=specificity; PPV, NPV=positive and negative predictive values, respectively; prob=probability

Box 3.**LAMBDA* model for predicting *BRCA* AJ founder mutation status [Apicella *et al.*, 2003]****Interpretation:**

Probability decreases as score decreases from 2.5 (>90% probability) to -3.75 (2% probability).

A table is provided to convert the final score to a probability in score increments of 0.25.

Scoring system:

baseline: -3.75

each age group at dx for consultand breast cancer: +3, +2 or +1 (for <40, 40-49, 50-59, respectively)

proband bilateral breast cancer at any age: +1

proband ovarian cancer at any age: +3

each current age group for unaffected consultand: -0.5, -1.0, or -1.5 (for 40-49, 50-59, 60+, respectively)

any ovarian cancer in family: +[# cases] × 1.5, 1 (for 1°, 2°, respectively)

1° relative with breast cancer: +[# cases] × 1.5, 1, 0.5 (for dx<40, 40-49, 50-59, respectively)

2° relative with breast cancer dx <40: +[# cases] × 0.5

*Log odds of the probability of carrying an Ancestral Mutation in *BRCA1* or *BRCA2* for a Defined personal and family cancer history in an Ashkenazi Jewish woman

6.3.1 Studies with Ashkenazi Jewish persons

The three studies among Ashkenazi Jewish (AJ) persons, which were all population-based, are presented in Table 15 [Apicella *et al.*, 2003; Hartge *et al.*, 1999; Hodgson *et al.*, 1999]. The Hartge data were re-analysed by two groups [Foulkes *et al.*, 1999; Hopper and Jenkins, 1999]. The most recent study, by Apicella and colleagues [2003], used the Hodgson sample [1999] from the United Kingdom along with a second group of women from Australia. The Hodgson sample was entirely composed of breast and/or ovarian cancer cases [Hodgson *et al.*, 1999], while the additional sample studied by Apicella comprised individuals with either personal or family history of breast or ovarian cancer [Apicella *et al.*, 2003]. The American women and men recruited by Hartge had a low frequency of cancer [Hartge *et al.*, 1999]. In all three studies genetic testing was completed for *BRCA1/2* AJ founder mutations only.

Common predictors of carrying a *BRCA* founder mutation were identified through multivariate analysis in the three main studies. One of these was presence of ovarian cancer, either in the proband alone [Hartge *et al.*, 1999] or in the proband or a close relative [Apicella *et al.*, 2003;

Hodgson *et al.*, 1999]. A second predictor was the proband's age at diagnosis, with somewhat different definitions of variables according to the study.⁷¹ The third common factor was other first degree relative history of cancer, in terms of breast or ovarian cancer before age 60 for Hodgson, and breast cancer before age 60 for Apicella. For the last of these factors, two re-analyses of Hartge data showed that family history factors were important for both affected and unaffected persons and that for cases, family history provided additional risk information even after considering age at diagnosis of the affected proband [Foulkes *et al.*, 1999; Hopper and Jenkins, 1999]. Apicella and colleagues also found that bilateral cancer in the proband was predictive at a borderline significant level [Apicella *et al.*, 2003]; in the other studies, this was either non-significant [Hodgson *et al.*, 1999] or not considered [Hartge *et al.*, 1999].

Using a carrier probability cut-off of 10%, the Hodgson predictive model for women with

71. In the Hodgson study [1999], age at diagnosis was a significant predictor of mutation status alone and in interaction with personal ovarian cancer; in the Hartge study [1999], early age at diagnosis was a significant predictor only in the analysis among cases; and in the Apicella study [2003], the significant predictor was breast cancer diagnosed before age 50 in the proband.

breast cancer was internally tested against mutation results,⁷² and was found to have fairly high sensitivity and excellent negative predictive value (NPV), but disappointing specificity and positive predictive value (PPV) [Hodgson *et al.*, 1999]. The LAMBDA model proposed by Apicella and colleagues [2003] appears to capture important factors and quantifies them in an easy-to-use format (see Box 3 for the scoring system). This model requires validation in independent samples of Ashkenazi Jewish persons but has already been favourably received by some cancer geneticists.⁷³

6.3.2 Studies with non-Ashkenazi Jewish persons

Table 16 presents the results for the nine studies among non-Ashkenazi Jewish persons. Three of these sampled persons from high risk cancer clinics in the Netherlands, Finland and Spain/Portugal on the basis of a family history of at least 3 cases of breast or ovarian cancer [de la Hoya *et al.*, 2002; Vahteristo *et al.*, 2001; Ligtenberg *et al.*, 1999 respectively], with the addition of at least one diagnosis before age 50 in the last study. Multivariate analysis identified similar predictive factors: ovarian cancer in the family (presence for Ligtenberg, number of cases for Vahteristo and de la Hoya; age at diagnosis for breast cancer (before age 40 for Ligtenberg, for youngest case for Vahteristo, mean age at diagnosis for de la Hoya; and bilateral breast cancer in the family, except in the Vahteristo study).

For two of these studies, the authors measured test performance by comparing estimated carrier probabilities in two groups (using a probability cut-off) with genetic testing results (either test positive or test negative for mutations). For a 10% carrier probability cut-off, the Vahteristo model performed extremely well in terms of sensitivity and NPV (and was more sensitive than both the Couch and Shattuck-Eidens models, which were designed with *BRCA1* data only), whereas specificity and PPV were fair

(and inferior to Couch). The de la Hoya model was able to achieve higher specificity and PPV, but its test performance was evaluated using a higher risk cut-off of 30% and a sample with higher prevalence of *BRCA1/2* mutations.

72. This is only a first step in evaluation of model performance, the next being validation with an independent sample.

73. W. Foulkes (McGill University), personal communication, September 4, 2003.

Table 16.
Development and preliminary testing of mutation risk models among non-Ashkenazi Jewish persons (since 1999): results

REFERENCE	SAMPLE SIZE / % CARRIERS	BEST PREDICTORS OF MUTATION STATUS	MODEL PERFORMANCE VERSUS MUTATION TESTING OR COMPARISON WITH OTHER MODELS
Ligtenberg <i>et al.</i>, [1999] <i>Families in a high risk clinic</i>	104 / 29.8	<u>For all families and for Br ca only families:</u> • Ov ca in family • ≥ 1 bilateral Br ca in family • ≥ 1 case with Br ca dx <age 40	
Vahteristo <i>et al.</i>, [2001] <i>Families in a high risk clinic</i>	148 / 19.6	• # Ov cases in family • age at dx of youngest Br ca case <i>Same factors significant for BRCA1 or 2</i> Can predict 28 of the 29 carriers using one criterion: Br ca dx <age 40 <u>or</u> Ov ca in family	28/29 carriers had predicted risk $\geq 10\%$ using model Sens=97%, Spec* =71%, PPV=44%, NPV*=99% Mean model prob of 55% for carriers, 11% for non-carriers Versus Couch and Shattuck-Eidens (S-E) models: higher sens; lower spec & PPV than Couch; higher spec & PPV than S-E; similar NPV
de la Hoya <i>et al.</i>, [2002] <i>Families in high risk clinics</i>	102 / 30.4	• # Ov cases • 1 case bilateral Br ca, male Br ca, or Br+Ov ca in 1 person† • ≥ 2 cases bilateral Br ca, male Br ca, or Br+Ov in 1 person • mean age at dx for Br ca	25/31 carriers had predicted risk $\geq 30\%$ using model Sens*=81%, Spec* =79%, PPV*=63%, NPV*=90% Mean model prob of 54% for carriers, 19% for non-carriers
Arver <i>et al.</i>, [2001] <i>Consecutive tested persons</i>	160 / 16.9	• # Ov cases in BrOv ca families • age of dx in Br ca-only families	
Frank <i>et al.</i>, [2002] <i>Consecutive tested persons (Myriad)</i>	10000 / 16.8*, 20.5* (for AJ)	<u>For 4716 non-AJ and for 2233 AJ: highest frequencies found for:</u> • personal and family hx of Ov ca (any age) and Br ca dx < age 50 • ≥ 2 cases in family with Br ca dx <age 50 • (a) early Br ca (b) Ov ca or (c) BrOv ca, no family hx of a, b (AJ)	

† p=0.07; * calculated by reviewer when either not reported or incorrectly reported in article
Ov=ovarian; Br=breast; ca=cancer; dx=diagnosis; sens=sensitivity; spec=specificity; PPV, NPV=positive and negative predictive values, respectively; prob=probability; hx=history

Table 16 (continued).

REFERENCE	SAMPLE SIZE / % CARRIERS	BEST PREDICTORS OF MUTATION STATUS	MODEL PERFORMANCE VERSUS MUTATION TESTING OR VERSUS OTHER MODELS
Ozelik <i>et al.</i>, [2003] <i>Breast cancer patients in registry</i>	314 / 9.9	<u>For <i>BRCA1</i>:</u> • age at dx for proband <36 • ≥ 1 1°, 2° or 3° relative with Br ca dx <age 36 or Ov ca dx <age 61 <u>For <i>BRCA2</i>:</u> • Br + Ov ca or multiple Br ca primaries in proband • ≥ 2 2° relatives with Br or Ov ca	
Aretini <i>et al.</i>, [2003] <i>BRCA1</i> or 2 status <i>Families in high risk clinics</i>	179 / 58.1 (<i>BRCA1</i>) & 41.9 (<i>BRCA2</i>)	<u>For carrying a <i>BRCA2</i> mutation (versus <i>BRCA1</i>):</u> • no Ov ca in proband nor family • later age at dx for proband • male Br ca in proband or family • prostate or pancreas ca in family	Overall prediction success=73% Prediction success for model in prob<0.3 & prob>0.7 regions=85% (63% of families in these regions) Maximum uncertainty region for model (where 0.4<prob<0.6) contains n=25 families (14% of total)
Gilpin <i>et al.</i>, [2000] Family History Assessment Tool <i>Tested with families referred for testing (clinic-based)</i>	184 / 19*		Using FHAT score ≥ 10 as a cut-off†: Sens=94%, Spec=51%, PPV=31%, NPV=97% Versus Claus ($\geq 22\%$ Br ca risk) and BRCAPRO (3 different cut-offs: $\geq 22\%$ Br ca risk; $\geq 3.2\%$ Ov ca risk; $\geq 20\%$ mutation prob): higher sens; higher or similar NPV; lower spec; mostly lower PPV Referral agreement with Claus 77%, and with BRCAPRO 61%*, 74%*, 71%* using the 3 cut-offs, respectively
Evans <i>et al.</i>, [2004b] Scoring tool <i>Validated with high and moderate risk‡ families (clinic- based)</i>	472 / 9.0‡		<u>Based on validation sample 2 (n=258) and 10% cut-off:</u> Sens=87%, Spec=65.5%, PPV=19.6%, NPV=98%; higher sens than BRCAPRO; same sens as Frank¶; higher spec, PPV, NPV than both <u>Based on validation sample 2 (n=175):</u> Area under ROC curve=0.772 for MSS, 0.714 for Frank¶, 0.596 for BRCAPRO

* calculated by reviewer when either not reported or incorrectly reported in article; † FHAT ≥ 10 corresponds to a doubling of the general population lifetime Br ca risk (0.11) or Ov ca risk (0.016)

‡ in validation sample #2 (n=258), where complete mutation testing was carried out; ¶ Frank *et al.*, [2002]

dx=diagnosis; Br=breast; Ov=ovarian; ca=cancer; prob=probability; sens=sensitivity; spec=specificity; PPV, NPV=positive and negative predictive values, respectively

The Arver *et al.* [2001] and Frank *et al.* [2002] studies are both based on consecutive samples referred for testing, in one city (Stockholm, Sweden; Arver *et al.*, 2001) or at one laboratory (Myriad; Frank *et al.*, 2002); neither study used multivariate analysis techniques. While Arver and colleagues specify the seven uniform testing criteria for their families [Arver *et al.*, 2001], the referral patterns for the Myriad data are not presented as they are certain to be extremely numerous and are possibly not recorded at the laboratory [Frank *et al.*, 2002]. Again, these studies identified the association of *BRCA1/2* mutations with number [Arver *et al.*, 2001] or presence [Frank *et al.*, 2002] of ovarian cancer cases in the family and age at diagnosis of breast cancer (for Frank *et al.*, [2002], specifically before age 50). In the Arver study, all *BRCA1* carrier families had at least one breast cancer diagnosis before age 36 [Arver *et al.*, 2001].

The Canadian study by Ozcelik and colleagues [2003] sampled women and men with breast cancer who met a series of testing criteria in the province of Ontario, and carried out multivariate analysis for each *BRCA* gene separately [Ozcelik *et al.*, 2003]. Both age at diagnosis and family history were associated with *BRCA1* mutation status.⁷⁴ The factor of ‘early age at diagnosis for breast cancer’ in the proband, which was significantly associated with *BRCA1* status, was defined as diagnosis before age 36. Ovarian cancer of some kind appeared in the final models for both *BRCA1* and *BRCA2*, either before age 61 in a first, second or third degree relative for *BRCA1*, or combined with breast cancer in the same person or another familial case of breast or ovarian cancer for *BRCA2*. In contrast, Aretini and colleagues [2003] found that one of the best predictors for

BRCA2 mutation status in their Italian sample of high risk families was the absence of ovarian cancer in the proband or her relatives. Male breast cancer was also associated with being a *BRCA2* carrier in the Italian study, as was observed by de la Hoya and colleagues [2002]. It should be noted that the Aretini study design differed in that only mutation carriers were sampled so that the comparison here was between *BRCA1* and *BRCA2* carriers [Aretini *et al.*, 2003], rather than for *BRCA1* carriers versus both *BRCA2* carriers and non-carriers, as in the Ozcelik study [Ozcelik *et al.*, 2003], for example.

Finally, two user-friendly tools have been developed to guide referral to cancer genetic services in one case and to facilitate triage in a busy cancer genetics clinic in the other. Gilpin and colleagues [2000] present the development and testing, in Ontario (Canada), of a Family History Assessment Tool (FHAT); this tool is intended to provide referring physicians with a way to quantify family cancer history and decide who to refer for genetic counselling/testing. Sensitivity was higher than for other tools with which FHAT was compared (Claus and BRCAPRO). The Claus model underestimated risk for some carriers with large affected families. BRCAPRO was more effective in reducing the number of unnecessary referrals but also underestimated risk for some families; the authors suggest that this may be due to too much weight being given in the model to unaffected members. As seen for other tools, the specificity and PPV of the FHAT were problematic. According to the first author (a practising genetic counsellor), this tool has not become part of standard practice in Ontario.⁷⁵

74. This was also seen in the re-analyses of the Hartge data among Ashkenazi Jewish persons (section 6.3.1).

75. C. Gilpin (Children’s Hospital of Eastern Ontario), personal communication, September 15, 2003.

Box 4.

Manchester scoring system to predict the likelihood of a pathogenic *BRCA 1* or *2* mutation in a lineage [Evans *et al.*, 2004b]

Interpretation:

A score of 10 corresponds to a 10% likelihood of a *BRCA1* or *BRCA2* pathogenic mutation being identified.

The lineage with the highest associated total score is used in the case of cancers on both sides of the family.

Scoring system (add across all cancers):

	<i>BRCA1</i>	<i>BRCA2</i>	respective ages at diagnosis
female breast cancer and age at diagnosis:	6, 4, 3, 2, 1	5, 4, 3, 2, 1	(<30, 30-39, 40-49, 50-59, 60+)
male breast cancer and age at diagnosis:	5*, 5*	8, 5	(<60, 60+)
ovarian cancer and age at diagnosis:	8, 5	5*, 5*	(<60, 60+)
prostate cancer and age at diagnosis:	0, 0	2, 1	(<60, 60+)
any pancreatic cancer:	0	1	

*if testing has already been completed for the other *BRCA* gene (otherwise 0)

Evans and colleagues [2004b] devised the Manchester Scoring System (MSS) as a simple tool to determine whether the likelihood of identifying a mutation in one lineage reaches the 10% threshold either for *BRCA1* or for *BRCA2*. Scores were derived on the basis of mutation frequencies in high risk families from northwest England stratified according to type of cancer (including male breast cancer, prostate and pancreatic cancer) and age at onset (see Box 4 for the scoring system). Subsequently, Manchester scores were computed for an independent sample of 258 affected individuals from moderate risk families in northwest England tested for mutations in both genes (following a scoring validation in a smaller independent sample of 192 cases from high risk families in southern England). At a 10% pre-test probability cut-off, the MSS showed a better trade-off between sensitivity and specificity than the Couch model, Frank (1998) model, Frank (2002) tables, and BRCAPRO. Likewise, the estimated area under the Receiver Operating Characteristic (ROC) curve was greatest for the Manchester scoring system, but confidence intervals were large. The MSS had high sensitivity and negative predictive value but poor specificity and positive predictive value, similar to that seen in the Hodgson AJ study [Hodgson *et al.*, 1999] also using a 10% probability cut-off. In contrast to BRCAPRO,

the MSS performed particularly well for *BRCA2* and the authors hypothesised that adjusting MSS weights to take tumour histopathology into account may improve prediction for *BRCA1*.

6.3.3 Study limitations

The methodological limitations for the studies presented in Tables 15 and 16 include the following:

- size of eligible population not reported, thus sample representativeness cannot be assessed (all studies except Ozcelik *et al.*, [2003]; Frank *et al.*, [2002]; Arver *et al.*, [2001])
- use of self-referred population samples, which may introduce selection bias [Apicella *et al.*, 2003; Hartge *et al.*, 1999; Hodgson *et al.*, 1999]
- family history of cancer apparently not fully validated or not at all validated, in terms of pathological or record confirmation (which can be particularly important for ovarian cancer); this is potentially both a reliability and validity issue, especially if validation was associated with mutation status [Apicella *et al.*, 2003; Aretini *et al.*, 2003; Ozcelik *et al.*, 2003; Frank *et al.*, 2002; Gilpin *et al.*, 2000; Hartge *et al.*, 1999; Hodgson *et al.*, 1999]

- incomplete mutation screen which could have resulted in misclassification of true carriers as non-carriers [Aretini *et al.*, 2003; Ozcelik *et al.*, 2003; de la Hoya *et al.*, 2002; Arver *et al.*, 2001; Vahteristo *et al.*, 2001]; not all study subjects tested with the same technique, which may have introduced measurement bias [Evans *et al.*, 2004b, except in validation sample 2; Apicella *et al.*, 2003; Aretini *et al.*, 2003; Ozcelik *et al.*, 2003; de la Hoya *et al.*, 2002; Arver *et al.*, 2001; Vahteristo *et al.*, 2001]); and possible misclassification of variants of “unknown significance” [Frank *et al.*, 2003; Arver *et al.*, 2001].

It seems clear that although similar patterns emerge in the various models developed since 1999, specific results are still dependent upon the particular study samples and testing methods used.

6.4 VALIDATION AND/OR EXAMINATION OF EXISTING RISK MODELS

We identified eleven studies which validated or compared predictive models that assess risk of disease [Euhus *et al.*, 2002a] or risk of detecting a *BRCA* mutation (all other studies). Appendix E3 and Table 17 summarise the study designs and results, respectively. All of the studies were cross-sectional in basic design. Some of the studies compared the mutation carrier (or disease risk) probabilities derived from different models and did not actually perform molecular tests. Most of the studies summarised in Table 17 involved the comparison of carrier probabilities generated by models with *BRCA* molecular testing results. Results of these comparisons are reported in a number of ways, some authors choosing to consider as the reference either the test results or the carrier probability predicted by models.⁷⁶

In their sample of American patients meeting initial Myriad testing criteria (i.e., prior to 1998)

and tested for *BRCA1* and 2 mutations, Fries and colleagues [2002a] found that the average Couch probabilities for *BRCA1/2* mutation carriers and non-carriers did not differ significantly (14.9% versus 7.9%, respectively, with a mean probability for those positive for ‘unclassified variants’ in between, at 11.1%).⁷⁷ The Couch probability cut-off that would have generated 100% sensitivity in their sample of carriers was $\geq 3.2\%$. It should be recalled that the Couch model was designed using a sample tested for *BRCA1* only.

Euhus and colleagues [2002b] tested carrier probabilities estimated both by BRCAPRO and genetic counsellors at eight USA sites against *BRCA1/2* sequencing.⁷⁸ Median sensitivity for eight counsellors (using pedigree information alone) was high (94%) and comparable to BRCAPRO (92%) using a $>10\%$ probability cut-off. However, median specificity was a low 16% for the counsellors and 32% for BRCAPRO, thus a large proportion of non-carriers were classified incorrectly as mutation positives by both methods. When an extreme cut-off was taken ($\geq 95\%$ probability of carrying a mutation), PPV for both methods was about 75%.

A series of studies provide additional information on model and genetic test performance and their relation to various parameters [Becher and Chang-Claude, 2002; Berry *et al.*, 2002; Bansal *et al.*, 2000; Chang-Claude *et al.*, 1999]. Chang-Claude and colleagues [1999] applied multi-centre European data (various inclusion criteria; mixed methods to test for *BRCA1* mutations) to examine an adapted Claus model that uses a linkage software program incorporating entire pedigree information in order to generate carrier probabilities. The adapted Claus model relies on published estimates of mutation prevalence and of age-specific penetrance, derived either from Easton *et al.*, [1993] or from Narod *et al.*,

76. Estimates of test validity derived in two studies (Becher and Chang-Claude [2002]; Chang-Claude *et al.*, [1999]) are not included in the clinical validity section of this report because of the atypical design for estimating sensitivity and the reliance on predicted—rather than documented—carrier status.

77. Unclassified variants were prevalent in each group defined by testing criteria, at $>17\%$ [Fries *et al.*, 2002a].

78. It should be noted that the Euhus *et al.* [2002b] investigation was one of the few we reviewed for Table 17 (along with Fries *et al.* [2002a] and Stuppia *et al.* [2003]) that specified the number of families considered who were not part of their final study sample.

[1995]. Chang-Claude and colleagues compared prevalence of *BRCA1* mutations among people with very high predicted carrier probabilities for different estimates of penetrance. They then used a $\geq 90\%$ model carrier probability as a reference and estimated a 50% clinical sensitivity for the mixture of 6 different testing methods among families containing both breast and ovarian cancer. For these 239 families, the Narod parameters produced carrier probabilities that correlated better with the mutation proportions.

In further work with the same sample and adapted Claus model, Becher and Chang-Claude [2002] generated a clinical sensitivity point estimate of 57% for mutation testing in breast-ovarian families (29% for breast cancer only families) through iterative analysis,⁷⁹ recognising, however, that their sample had only been fully tested for *BRCA1* mutations. They also present interesting analysis in which they

considered the gain in information about lifetime breast cancer risk if the genetic test is negative for different levels of prior carrier probability and genetic test sensitivity. They showed little gain in information (defined as decrease in cancer risk) when prior probability (estimated by the model) is high ($>70\%$) and test sensitivity is low ($\leq 50\%$); in this cases, the negative test result is likely a false negative. Bansal and colleagues [2000] estimated PPV and NPV for the Frank *et al.*, [1998] model under the assumptions of 85% clinical sensitivity and $>99.95\%$ specificity for *BRCA1/2* sequencing. They showed that the model is most informative for prior probabilities of 10-50% (with a steady decrease in NPV for probabilities $>50\%$). This model performance result, generated with Bayesian analysis, relies on the test sensitivity and specificity assumptions: the authors assume an absence of false positive results, since only “functionally relevant” mutations were considered positives.

79. Becher and Chang-Claude assumed 100% molecular test specificity.

Table 17.
Validation or examination of existing risk models (since 1999): study results

REFERENCE	SAMPLE SIZE / GENE(S) TESTED / MODEL(S)	RESULTS OF COMPARISONS OR CARRIER PROBABILITIES	MODEL OR TEST PERFORMANCE RESULTS
Chang-Claude <i>et al.</i>, [1999]	618 / <i>BRCA1</i> / adapted Claus	For those with >95% model carrier prob: (2 figures given*) 34 & 26% tested + (Br ca only families); 48 & 49% (for BrOv) For those with <40% model carrier prob: 4 & 5% tested + (Br ca only); 25 & 16% (BrOv)	Assuming model carrier prob $\geq 90\%$ to be the 'gold standard', sens estimate for molecular tests =50% (in BrOv families)
Bansal <i>et al.</i>, [2000]	(238) / <i>BRCA1/2</i> / Frank 1998		For model carrier prob of 10-50%: PPV $\geq 95\%$, NPV $\geq 87\%$ For prob >50%: PPV=100% but NPV steadily decreases For prob=5%: PPV=90%, NPV=99%
Becher and Chang-Claude [2002]	(618) / <i>BRCA1</i> / adapted Claus	Model carrier prob 0.4-99.9%; mean=54%; median=57%	Sens estimate for molecular tests (note: only <i>BRCA1</i> tested) =45.1% (95% CI: 39-51) for all families =57% (95% CI:49-65) for BrOv ca families =29% (95% CI: 18-39) for Br ca only families
Berry <i>et al.</i>, [2002]	301 (42% AJ) / <i>BRCA1/2</i> / BRCAPRO	For those who tested +: mean model carrier prob=79% For those who tested -: mean model carrier prob=39% For all 6 male cases who tested +: >50% model carrier prob At low carrier prob, more + tests seen than predicted by model	Assuming BRCAPRO=genotype, sens estimate for molecular tests =85% (95% CI: 79-90) for all families =83% (95% CI: 73-91) for AJ families =88% (95% CI: 78-94) for non-AJ families
Euhus <i>et al.</i>, [2002a] Exception: risk of disease	213 / <i>BRCA1/2</i> / Gail, Claus, BRCAPRO		Gail model the "best" (=highest) risk estimate 87% of the time; for BRCAPRO: 24%; for Claus, 17%
Euhus <i>et al.</i>, [2002b]	148 (10% AJ) / <i>BRCA1/2</i> / BRCAPRO		For >10% carrier prob: 94% sens for counsellors (median), 92% for model ; 16% spec for counsellors (median), 32% for model For $\leq 10\%$ prob: 71-92% NPV for counsellors, 84% for model For $\geq 95\%$ prob: 75% PPV for counsellors (median), 74% for model Area under ROC curve=67% for counsellors (median), 71% for model

* figures given using Easton or Narod model parameters, respectively
prob=probability; Br=breast; ca=cancer; Ov=ovarian; sens=sensitivity; PPV, NPV=positive, negative predictive values, respectively; spec=specificity; hx=history; dx=diagnosis

Table 17 (continued).

REFERENCE	SAMPLE SIZE / GENE(S) TESTED / MODEL(S)	RESULTS OF COMPARISONS OR CARRIER PROBABILITIES	MODEL OR TEST PERFORMANCE RESULTS
Fries <i>et al.</i>, [2002a]	90 / <i>BRCA1/2</i> / Couch	For those who tested +: mean carrier prob=14.9% For those who tested -: mean carrier prob=7.9%	% who tested + was highest for: • males with Br ca (50%; n=4) • early onset <u>and</u> bilateral/multiple 1° ca (33%; n=4) • early onset ca + family hx of ≥ 2 cases <u>or</u> 1 early dx (15%; n=20) • case + family hx of ≥ 2 cases <u>and</u> 1 early dx (25%; n=4) For those with early onset ca: 11% tested +
Shannon <i>et al.</i>, [2002]	50 (12% AJ) / <i>BRCA1/2</i> / Couch, Frank 1998, BRCAPRO	Mean model carrier prob: 25.9% for Couch; 42.7% for Frank; 30.1% for BRCAPRO 2 early onset cases (dx <age 35) scored < 10% on all models	Among 11 patients with prob $\geq 10\%$ on any model: 82% identified by Couch; 54% by Frank (1998); 64% by BRCAPRO % agreement between Couch and Frank (1998)=67%; BRCAPRO & Couch =45%; BRCAPRO & Frank (1998)=27%
Stuppia <i>et al.</i>, [2003]	68 / <i>BRCA1/2</i> / BRCAPRO	Mean carrier prob 40% (min 12.7%, max 93.5%) For n=19 with model carrier prob > 10%: 7 tested + (36.8%) For n=49 with carrier prob < 10%, 1 tested + for <i>BRCA2</i> (had prostate ca and 1 relative with Br ca)	For model carrier prob > 10%: Sens*=87.5%, Spec*=80%, PPV*=36.8%, NPV*=98% In 6/7 carriers with model prob > 10%, gene (<i>BRCA1</i> or 2) correctly identified by model
de la Hoya <i>et al.</i>, [2003]	109 / <i>BRCA1/2</i> / Couch, Frank 2002, de la Hoya, Vahteristo	Mean carrier probs for all models higher for carriers than for non- carriers (p<0.001), but probs around or > 10% for non-carriers In general, all models underestimated mutation prevalence Models performed better in absence of <i>BRCA2</i> mutations (except de la Hoya)	Model sens of 100% corresponded to probability cut-offs of 2 to 11% (spec: 25-33%, PPV: 41-43.5%) Statistically optimal cut-offs (sens): Couch>0.12 (59.5%); Frank (2002)>0.34 (67.6); de la Hoya>0.23 (78.4); Vahteristo>0.15 (73) Areas under ROC curves not significantly different across models (varied from 0.77 to 0.82)

* calculated by reviewer when either not reported or incorrectly reported in article

prob=probability; dx=diagnosis; ca=cancer; Br=breast; sens=sensitivity; spec=specificity; NPV, PPV=negative, positive predictive values, respectively; mod=moderate

Table 17 (continued).

REFERENCE	SAMPLE SIZE / GENE(S) TESTED / MODEL(S)	RESULTS OF COMPARISONS OR CARRIER PROBABILITIES	MODEL OR TEST PERFORMANCE RESULTS
Marroni <i>et al.</i>, [2004]	428 / <i>BRCA1/2</i> / Couch, Shattuck- Eidens, Frank 2002, de la Hoya, Vahteristo, Claus, BRCAPRO, adapted BRCAPRO	All <i>BRCA1/2</i> models greatly underestimated carrier prob for families at lowest risk ($\leq 10\%$ prob): O/E ratios=1.8-6.4 For mod risk group (10-40% prob): Mendelian models and de la Hoya performed better (O/E=0.8-1.1); for high risk group ($>40\%$ prob), all models overestimated except Frank (O/E=1) Models distinguishing between <i>BRCA1</i> and 2 (BRCAPRO and adapted BRCAPRO) overestimated # of <i>BRCA1</i> mutations & underestimated # of <i>BRCA2</i> mutations (n=568)	For model carrier prob $>10\%$ (<i>BRCA1/2</i> models): Sens of 67-84%, Spec of 35-62% (best combined results for BRCAPRO & adapted BRCAPRO: 80, 56% & 84, 51%, respectively) <i>BRCA1</i> models (Couch, Shattuck-Eidens) had fairly similar results Areas under ROC curves for <i>BRCA1/2</i> models: 0.76 for BRCAPRO and adapted BRCAPRO 0.72 for Vahteristo and Frank (2002) 0.65 for de la Hoya 0.61 for Claus

* calculated by reviewer when either not reported or incorrectly reported in article
prob=probability; dx=diagnosis; ca=cancer; Br=breast; sens=sensitivity; spec=specificity; NPV, PPV=negative, positive predictive values, respectively; mod=moderate

Berry and colleagues [2002] present a “validation” (authors’ term) of BRCAPRO in a sample of families from 6 USA high risk clinics that used diverse testing criteria. The testing techniques included full *BRCA1/2* sequencing for non-Ashkenazi Jewish persons and specific testing for 3 founder mutations in Ashkenazi Jewish families. The average BRCAPRO carrier probabilities were 79% for those testing positive for mutations and 39% for non-carriers (these were significantly different, at $p < 0.001$), and showed good general correlation with *BRCA1/2* mutation status. Although these figures speak to the potential for discrimination between carriers and non-carriers on the basis of BRCAPRO, no performance figures are generated. Instead, the authors go on to consider BRCAPRO to represent the genotype and present an estimated sensitivity for the mutation testing of 85.4%. This is not the standard way to validate BRCAPRO as a ‘diagnostic’ tool and assumes that the prevalence and penetrance parameters in the BRCAPRO model are appropriate. The latter point is addressed by the authors by lowering the values of penetrance and examining the average carrier probabilities, which decreased; this resulted in an increase in genetic test sensitivity. Again, however, this is not a convincing demonstration of the validity of BRCAPRO given the use of the model as the ‘gold standard’.

Stuppia *et al.* [2003] report on the agreement between model carrier probability as predicted by BRCAPRO and results of molecular testing in both *BRCA1* and 2. Their sample was composed of individuals from high risk Italian families or “sporadic” cases (with early onset cancer or male breast cancer) who received genetic counselling in a two-year period. Performance figures for a greater than 10% carrier probability cut-off can be calculated from their data as 87.5% sensitivity, 80% specificity, 36.8% positive predictive value, and 98% negative predictive value. These results, derived from a relatively small-scale study ($n=68$), are among the most favourable reported to date for the BRCAPRO model.

Two studies in Table 17 involved comparisons between different statistical models without

reference to genetic testing results (Euhus *et al.* [2002a] and Shannon *et al.* [2002], estimating disease and mutation risk, respectively). In their comparison of a modified Gail model versus BRCAPRO and Claus for the estimation of breast cancer risk, Euhus and colleagues [2002a] used the criterion of the *highest* risk estimate to indicate the most appropriate model (in an American breast cancer clinic-based sample). As this was the Gail model 87% of the time, they conclude that the Gail was the most generally applicable of the three, but suggest combining the three models in practice. The informativeness of such an investigation is debatable without any other way to validate these estimates. Also, in this study and that by Shannon *et al.* [2002], the information on cancer history and ages at diagnosis in family members was not independently confirmed.

Shannon and colleagues [2002] used a hospital-based sample of American incident breast cancer cases (consecutive patients, unselected for family history and age at diagnosis) to look at the agreement between the Couch, Frank 1998, and BRCAPRO models in the identification of persons with $\geq 10\%$ carrier probability. Thirty-four percent of the patients had one relative with breast or ovarian cancer and 56% had no family history. Agreement between each pair of models was disappointing, varying from 27 to 67%, and none of the 11 persons who scored above 10% carrier probability on at least one model would have been identified by all three models.⁸⁰ The authors warn that “women with borderline low to moderate risk may not be assessed accurately by any of these risk models” (p. 310), and suggest always using Couch plus at least one another model in clinical practice. Their study design does not allow evaluation of which of the models actually performed best since no mutation testing was performed.

Finally, two studies compared the performance of several models predicting carrier probabilities to genetic testing results. De la Hoya and colleagues [2003] compared models that were empirically derived on the basis of high risk

80. However, at least 10 of these 11 persons would have been identified if any two models had been used.

samples in different countries, including the Couch (Table 14), Vahteristo (Table 16) and de la Hoya (Table 16) models, or on the basis of testing at the Myriad Genetics laboratory (Frank tables from 2002, Table 16). Applying these models to 109 high risk families attending oncogenetic clinics, the authors found that all tended to underestimate *BRCA1/2* mutation prevalence and that all but the de la Hoya model performed better for families carrying *BRCA1* rather than *BRCA2* mutations. No statistically significant differences in the areas under the ROC curves were observed for the models. The authors conclude that statistically optimal probability cut-offs may not be clinically relevant if a high sensitivity is paramount: these thresholds varied from 0.09 to 0.34 for associated sensitivities of 86.5 to 67.6%, respectively. From this perspective, appropriate carrier probability cut-offs may need to be selected at a different level for each model rather than relying on a 10% probability threshold throughout.

Eight models were investigated by Marroni *et al.* [2004], including those empirically derived (Couch, Shattuck-Eidens, Frank 2002 tables, de la Hoya and Vahteristo, Tables 14 and 16) and those based on Mendelian rules of transmission and published penetrance and prevalence data (Claus and BRCAPRO, Tables 13 and 14). An additional model was also tested, derived from BRCAPRO and refined with respect to the penetrance estimates for individuals with bilateral tumours or both breast and ovarian tumours (referred to as the ‘adapted BRCAPRO model’). Since the empirical models estimate the probability of detecting a mutation whereas the Mendelian models evaluate carrier probabilities, the study authors adjusted the probability values calculated by the latter to take the clinical sensitivity of the molecular tests used into account.⁸¹ Among 568 families having undergone testing⁸² at five cancer genetic clinics, the eight models could be used in

428 families for whom both *BRCA* genes had been tested with a variety of techniques. Models were assessed in terms of calibration (observed versus expected number of mutations [O/E ratio]), goodness of fit, and performance (sensitivity, specificity, and ROC curves).

Among the models accounting for both *BRCA 1* and 2 in the Marroni study, the sensitivity and specificity estimates at the 10% carrier probability cut-off, as well as the areas under the ROC curves, suggest that BRCAPRO and its adapted version demonstrate the best discriminatory power. Consideration of both calibration and goodness of fit data suggests that even for models with an overall number of predicted mutations close to the observed number, these results could be explained by compensation between family strata. Stratified analyses show that all models underestimated the number of mutations in families estimated to be at low ($\leq 10\%$) risk. BRCAPRO and its adapted version overestimate the number of *BRCA1* mutations (particularly in breast cancer only families) and underestimate the number of *BRCA2* mutations (in breast cancer only and breast and ovarian cancer families). Overall, the study authors consider Mendelian models to be superior to empirical ones, partly because they cover a larger range of familial configurations. Further research including sub-group analyses could be useful to orient the adjustment of the Mendelian models’ parameters. Areas needing improvement are the discrimination between the two genes which could allow for more cost-effective testing in sequence and the discrimination among families considered at low risk, a potentially large group among counselees for whom mutation frequency is currently underestimated.

Large scale, methodologically sound validation of some risk models (i.e., models by Evans, de la Hoya, Vahteristo, Frank [2002] and their colleagues) has recently been undertaken, but these efforts need to be pursued further as there is no consensus as to which is(are) the best. The NICE technology appraisal report of 2004 stated: “validation of [*BRCA*] risk assessment models is urgently needed” (p. 144) [NICE, 2004]. As it now stands, these statistical models

81. A clinical sensitivity of 70% was assumed since PTT-SSCP was most often used in the five centres; values of 60 and 80% were also tested.

82. Various eligibility criteria were used, but most often the families contained multiple breast and ovarian cancer cases or early onset cancer.

should only be used as *supplements* to the genetic counsellor or clinical geneticist's own judgement of risk based on experience. These models are thus not appropriate for use by practitioners without such specialised training and experience, with the exception of a referral tool specifically designed for primary care providers (i.e., the FHAT [Gilpin *et al.*, 2000]). Thorough knowledge of each model's characteristics and limitations is necessary to determine its applicability to given patterns of familial aggregation of cancer, and reliance on only one model for testing decisions should be avoided [Domchek *et al.*, 2003]. These recommendations seem to reflect current practice in clinical cancer genetics in Canada.⁸³

6.5 NEW TRENDS IN MODELLING RESIDUAL FAMILIAL RISK

Since 2002, a regain in interest in the prediction of cancer risk has been witnessed. Most previous modelling efforts have focussed on high risk families, both at the development and the validation stages. The resulting models, therefore, are limited in their ability to discriminate among *a priori* high risk families that fall around the 10% cut-off for mutation risk, as pointed out by Evans *et al.* [2004b], and may also not be appropriate for moderate risk families.

Alterations in *BRCA1* and *BRCA2* presumably explain only 16% of the familial clustering of breast cancer [Peto *et al.*, 1999] and 55-60% of that for ovarian cancer [Chappuis and Foulkes, 2002] (D. Easton).⁸⁴ A meta-analysis of 52 epidemiological studies has shown that among women with breast cancer, only 1% had two affected first-degree relatives and 12% had one affected first-degree relative [CGHFBC, 2001]. Prevalence of *BRCA1/2* mutations is less well

documented in moderate risk families than in high-risk families, but is likely to be much lower. Furthermore, research aimed at discovering another breast cancer susceptibility gene has been disappointing, despite intense efforts, and it thus seems unlikely that the residual familial clustering is related to a single, dominantly inherited, high penetrance gene. If a polygenic inheritance is involved, the relevance and cost-effectiveness of adopting a systematic mutation testing strategy will be more difficult to demonstrate. The emergence of new models to predict cancer risk, on the basis of family history and personal risk factors, is consistent with an approach aimed at orienting management for moderate risk families directly, rather than setting testing indications. In parallel, the management of families at moderately increased risk has recently received attention, with outlines of specific referral and surveillance strategies included in the most recent guidelines (see chapter 7). The discussion below provides a brief overview of these most recent models.

Antoniou and colleagues [2002] used sophisticated segregation analyses to explore the inheritance patterns that best explained residual familial aggregation, once *BRCA 1* and *2* were taken into account. Their analysis was based on two UK samples: a population-based series of 1,484 breast cancer cases (diagnosed before age 55) from the Anglian Cancer Registry, and 156 high risk families with at least two breast cancer cases (one diagnosed before age 50). Molecular CSGE analysis revealed that 62 families carried *BRCA1/2* mutations. The best fitting model included, besides *BRCA 1* and *2*, a polygenic effect which could correspond to the combined multiplicative effect of several common low penetrance genes acting both on *BRCA1/2* carriers and non-carriers. This model, known as BOADICEA (Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm), can be used to estimate *BRCA1* and *BRCA2* carrier probabilities separately as well as cancer risk, taking family history into account.

In subsequent research, Antoniou *et al.* [2004] estimated the predicted carrier probabilities using BOADICEA and BRCAPRO for women with breast cancer at different ages, according to

83. W. Foulkes (McGill University), C. Gilpin (Children's Hospital of Eastern Ontario), and K. Panabaker (BC Cancer Agency), personal communication, September 4, 15, and 23, 2003, respectively.

84. Evaluating the contribution of *BRCA1*, *BRCA2* and *BRCA3*: modelling and modifying risks; oral presentation at the Joint conference on inherited susceptibility to breast and ovarian cancer: Second annual INHERIT BRCA meeting and first national hereditary cancer task force, Quebec City, November 24-26th, 2002.

different scenarios (0, 1 or 2 affected first degree relatives). They compared the age-specific familial relative risks of breast cancer (for those with one affected first degree relative) predicted by the BOADICEA, BRCAPRO and Claus models to empirical data from the Collaborative Group on Hormonal Factors in Breast Cancer [2001]. The authors concluded that the age-specific contribution of *BRCA1/2* to breast cancer is generally in line with population data, with respect to both relative risks and carrier probabilities: for the latter, *BRCA2* estimates were close to the values observed by Peto *et al.* [1999]⁸⁵ although there was underestimation for *BRCA1*. This type of investigation of the BOADICEA predictions has proven useful in guiding the authors towards areas of potential refinement, but does not constitute a validation of the model on an independent sample. A preliminary report (a published abstract) presents a comparison of predicted carrier probabilities by the BOADICEA, BRCAPRO, “Myriad I” [Shattuck-Eidens *et al.*, 1997], “Myriad II” [Frank *et al.*, 1998], and Couch models to results of molecular analyses in 121 high risk families. Findings suggest that the BOADICEA model demonstrates better ROC curves among non-Ashkenazi families, but a detailed account of this study is not yet available [Barcenas *et al.*, 2003].

Jonker and colleagues [2003] relied on a Bayesian approach to model familial clustering of breast cancer. They assumed that a hypothetical third *BRCA* gene, either recessive or dominant, could be used to represent all of the residual familial aggregation from a statistical—but not necessarily biological—point of view. For *BRCA1* and *BRCA2*, published mutation prevalence and penetrance values were used,⁸⁶ whereas parameter estimates were derived for the hypothetical *BRCA3* gene as a function of the relative risks for breast cancer for women with a history of breast cancer in first degree relatives reported by the Collaborative Group on

Hormonal Factors in Breast Cancer [CGHFBC, 2001]. To compare the models with a recessive versus dominant third *BRCA* gene, lifetime cancer risks were estimated for an unaffected relative from 196 consecutive Dutch breast/ovarian cancer families seeking genetic counselling [van Asperen *et al.*, 2004; Jonker *et al.*, 2003]. According to Jonker and colleagues, no substantial differences in risk prediction using the two models were found for most families. In further work, these cancer risks—along with those estimated by the Claus and BRCAPRO models—were compared to risks derived from tables based on the Claus model, using scatter plots, percentage agreement per risk category, and Spearman rank correlation coefficients [van Asperen *et al.*, 2004]. Only moderate agreement with the tables was found. Finally, van Asperen and colleagues [2004] developed a linear regression formula to estimate lifetime cancer risk on the basis of the Jonker model, incorporating as independent variables the risks yielded by the Claus tables, presence of ovarian cancer, presence of bilateral breast cancer, and more than two affected relatives with breast cancer. The purpose of the formula, which serves to ‘extend’ the Claus tables, is to provide a simple tool that can be applied in clinical practice to estimate an unaffected woman’s cancer risk based on family information. This tool has yet to be validated.

Tyrer *et al.* [2004] took the modelling exercise yet a step further, by integrating both familial and personal risk factors into their Bayesian model. Penetrance and prevalence for *BRCA1/2* mutations were taken from the literature,⁸⁷ and the survivor function in carriers and non-carriers was derived so as to be coherent with UK national incidence data. A hypothetical low penetrance dominant gene was included in the model as a surrogate for the residual familial aggregation, assuming a proportional hazards model. Empirical data on a cohort of daughters of women affected with breast cancer were used to estimate model parameters, in particular the prevalence of mutations in the hypothetical gene

85. Similar to the BOADICEA development samples, Peto *et al.*, [1999] screened coding sequences of both *BRCA1* and 2 in UK samples with CSGE.

86. Ford *et al.*, [1995] and Peto *et al.*, [1999] for mutation prevalence; Ford *et al.*, [1998] and Easton *et al.*, [1995] for penetrance.

87. Peto *et al.*, [1999] for mutation prevalence; Ford *et al.*, [1998] and Narod *et al.*, [1995] for penetrance regarding breast and ovarian cancer, respectively.

and the associated relative risk. Finally, risks related to personal factors, such as age at menarche, age at menopause, age at first childbirth, parity, height, body mass index, atypical hyperplasia and lobular carcinoma *in situ* are assumed to be multiplicative and are used to adjust the absolute risk based on family history. The model, available as a computer program, provides individualised estimation of *BRCA1* and *BRCA2* carrier probabilities separately, as well as 10-year and lifetime risk of breast cancer [Cuzick, 2003]. Amir and colleagues [2003] assessed the goodness of fit and the discriminatory value of several models predicting breast cancer risk, using a cohort of 1,933 women from the Family History Clinic in Manchester, UK. These women were followed in a mammography screening program for a mean of 6.39 years (range: 0.28-15 years) and 52 of them developed breast cancer. Both in terms of the expected to observed number of cancer cases and the area under the ROC curve, the Tyrer model seemed to show better results than the Gail, Claus and BRCAPRO models (the latter using Ford parameter estimates), which tend to underestimate cancer risk in this moderate to high risk population, but confidence intervals were large and overlapping.

In summary, these models can be used for individualised predictions either exclusively to predict cancer risk or to predict *BRCA* mutation probability as a first step towards the computation of cancer risk. A new trend is the additional calculation of risk for the next 5 or 10 years instead of only lifetime risk, which could be more meaningful for immediate management decisions. Some models can be seen as extensions of the original Claus model for estimating cancer risk, extensions that consider more detailed features of family history. One combines familial and personal risk factors, such as hormonal and clinical factors [Tyrer *et al.*, 2004].

A number of studies have compared the cancer risks predicted by these recent models to the earlier Gail, Claus, and BRCAPRO models. These studies tend to show quite a dispersion of results, but do not as such constitute validation studies. In terms of individualised risk

assessment, only one study with a mean follow-up of approximately 6 years reports on cancer risk predictions [Amir *et al.*, 2003] and one preliminary report examines the validity of pre-test carrier probabilities [Barcenas *et al.*, 2003]. No study has yet compared the recent models to each other. Several theoretical advantages have been put forward, both in the clinical setting—to provide individualised risk predictions based on comprehensive personal and familial data for moderate risk families and high risk families with inconclusive testing results—and in the research setting, to improve the comparability of groups in studies of surveillance or prophylactic management approaches. In practice, the applicability of these models to moderate and/or high risk groups remains to be demonstrated.

6.6 CONCLUSION

- Given the complexity of the data on prevalence and penetrance of *BRCA* mutations, statistical modelling was seen as a means of simplifying risk assessment and of identifying those families that are most likely to carry *BRCA* mutations. The earlier statistical models were designed to predict risk of breast cancer and did not integrate results of molecular testing. Subsequently, several models have been developed to estimate the probability of a *BRCA1/2* mutation explaining the familial aggregation of cancer. These models are either based on published data on population mutation prevalence, age-specific and cumulative penetrance and cancer incidence, or are derived from the analysis of samples of high risk families fulfilling particular eligibility criteria and for whom molecular testing was performed.
- Some models are limited to *BRCA1* only, whereas others consider *BRCA1* and *BRCA2*. The range of risk factors integrated in these models is variable (AJ ancestry or male breast cancer cases may or may not be considered, for example) and such factors are not defined in a consistent way (for early onset of cancer, for instance). The complete family structure is not systematically considered. Most models, therefore, do not

apply to all possible familial constellations of risk factors.

- Some common predictors of *BRCA* mutation status have been identified through multivariate analysis. Among these, the presence of ovarian cancer and the proband's age at diagnosis are prominent, while history of cancer in first degree relatives is found mainly in models developed in AJ samples, and bilateral breast cancer in the family mainly in models developed in non-AJ samples. It seems clear that although similar patterns emerge in the various models, specific results are still dependent upon the particular study samples, the testing methods and the precise definitions of variables used.
- A number of authors have compared the carrier probabilities generated by one or several models with *BRCA* mutation testing results. Others have compared the predictions of several models to each other. On the whole, these models are reported to have fairly high sensitivity and negative predictive value, but disappointing specificity and positive predictive value. The approaches used to compare the performance of these models are varied and sometimes questionable. In addition, most comparative studies have been performed on samples of exclusively high risk families, so that the ability to discriminate between carrier and non-carrier families among borderline low to moderate risk families may not have been assessed accurately.
- Only a few of the models developed since 1999 have undergone validation with samples independent of those with which they were derived. The lack of proper validation and the numerous classification schemes of risk factors may contribute to the fact that none of these models has been unanimously adopted in clinical practice. As it now stands, these statistical models should only be used as supplements to the genetic counsellor or clinical geneticist's own judgement of risk based on experience. Efforts are under way to develop more user-friendly tools for use in risk assessment in tertiary care and as referral guidelines for primary care. The lack of consensus on appropriate models in risk assessment presents a particular challenge with respect to equity of patient care between cancer genetic centres.
- The most recent risk models were developed to account for all facets of the familial aggregation of breast cancer. In all cases, efforts have been made to be coherent with population-based incidence data. Whatever the inheritance pattern used to model the residual familial clustering, the parameters estimated for these models support the relative importance of the residual hereditary component of breast cancer as compared to *BRCA1/2*. These models could play an important role in the future, particularly for individuals in moderate risk families. However, their validation remains to be demonstrated.

7. Testing indications: guidelines

7.1 INTRODUCTION

Indications for *BRCA1/2* mutation testing in the clinical setting can be based on (1) expert opinion, (2) application of risk assessment models, and (3) review of the literature on familial and personal risk factors associated with specific levels of mutation prevalence and penetrance. We examined many guidelines in order to see which testing criteria are recommended and how the formulation of these criteria is linked to the scientific evidence. We also examined guidelines for referral to specialised services for further risk assessment and, possibly, genetic testing. We looked at guidelines produced by a variety of bodies, from professional organisations and two technology assessment groups (SBU and NICE) to a laboratory-based survey; the potential impact of such documents likely depends on the particular organisation and whether the guidelines are specifically presented as “policy statements”. We focussed on recommendations for *clinical and risk indications* (e.g., patterns of cancer in families), rather than the associated ethical, organisational, and legal issues.

Literature searching results

As described in the methods chapter, we searched for testing guidelines using bibliographic databases, review articles, and reference lists. We also examined all of the guidelines listed on the InheritBRCA website, a site maintained by an interdisciplinary and international team in oncogenetics, funded by CIHR (www.inheritbrcas.info). We selected and summarised guidelines that specifically mentioned indications for genetic testing or referral to family cancer clinics, clinical geneticists, genetic counsellors, etc. Some of these guidelines also discussed clinical management, but this aspect is not reviewed here. We included recommendations for referral to different levels of specialised care (within which a decision to test for mutations may or

may not be made) since these also give an idea of the risk levels in use for assessment of family and personal cancer history in clinical practice. Thirty-six guidelines (and 12 citations) were identified; see Appendix B3 for flow chart.

A number of different countries and regions were represented among the guidelines, including Canada, USA, the United Kingdom, Australia, Scotland, France, the Netherlands, Italy, Norway, Wales and Sweden. Half of the guidelines were published in scientific journals; the others were available on websites. We included all relevant guidelines dated since 1996, the year when the first American Society of Clinical Oncology (ASCO) guidelines were published and guidelines began to discuss clinical rather than research use of *BRCA* mutation testing. The ASCO (1996) guidelines were the most frequently cited in our literature review. Appendix F summarises the suggested testing indications, risk classification scheme or referral guidelines of the thirty-six guidelines in order from the most recent to the oldest.

7.2 CONTENT OF THE GUIDELINES

The ASCO 1996 guidelines appear to have been the first to support the use of *BRCA* genetic testing outside of research studies, as long as three conditions—which also apply to cancer genetics in general—are satisfied. [ASCO, 1996] These conditions relate to a quantitative assessment of risk based on family history, the analytical validity of the molecular tests used, and the informativeness of test results for the clinical management of persons identified to be at elevated risk of disease. Other organisations have reiterated the need to link indications for testing with the possibility of benefit from medical interventions.

All of the reviewed guidelines focus on groups considered to be at elevated risk because of personal and/or family history of cancer. The guidelines that specify clinical criteria for testing/referral include, as risk factors, a count of

the *number* of affected relatives, consider the *age at diagnosis*, and refer to the *type or characteristics of cancer* in the family (e.g., breast, ovarian, bilateral disease, male or other cancer). Most also identify Ashkenazi Jewish ancestry as a factor associated with additional risk. Some guidelines differentiate between criteria for affected versus unaffected individuals, or for persons at various levels of elevated risk. In this way, various definitions of ‘low’, ‘moderate’ and ‘high’ risk are presented by different guidelines. These categories generally refer to the particulars of the cancer history in the family (if any), and in some guidelines are quantified in terms of the level of increased risk (compared to the general population), age-specific or cumulative (lifetime) risk, or probability of carrying a known susceptibility mutation.

There is general concordance between the various guidelines as to the most important risk factors, although the specific testing criteria vary somewhat in their restrictiveness. For example, the broadest guidelines consider a woman affected with breast cancer at an early age to be eligible for genetic testing *regardless* of her family history; in this category, the age at diagnosis criterion is defined variously as

‘before age 35’ [OPCGSC, 2001], ‘before age 45’ [ACMG, 1999b; HTAC, 1998] or ‘before age 49’ [Fries *et al.*, 2002b]. In cases where there are two family members diagnosed with breast cancer (and no ovarian cancer), various cut-offs are specified for ‘early’ age at diagnosis in order to be eligible for genetic testing, including the requirement that both cases were diagnosed ‘before age 50’ [OPCGSC, 2001], or that at least one of the cancers was diagnosed ‘before age 40’ [SBU, 2000], ‘before age 45’ [ACMG, 1999b; HTAC, 1998] or ‘before age 49’ [Fries *et al.*, 2002b]. When multiple members of the family have a history of breast cancer and age at diagnosis/onset is not specified, some guidelines require at least three affected first or second degree relatives as eligibility criteria for genetic testing [Fries *et al.*, 2002b; OPCGSC, 2001; ACMG, 1999b; HTAC, 1998]. There is also variation in the age cut-offs and number of affected relatives necessary for referral criteria. These are not systematically less stringent, as might be expected, than the criteria for genetic testing; several guidelines, for example, require at least four affected relatives for referral or identification of high risk families [NICE, 2004; Lucassen *et al.*, 2001; SCG, 2001; Blamey *et al.*, 1998; SIGN, 1998; PHGU, 1998].

As an overall summary, the guidelines we examined specify seven general categories of factors which are associated with increased risk and are used in the genetic testing and referral criteria:

- (1) young (premenopausal) age at diagnosis/onset of breast cancer;
- (2) male breast cancer at any age (in some guidelines, this must be accompanied by breast or ovarian cancer in a close relative);
- (3) bilateral breast cancer (in the Ontario guidelines, with first tumour diagnosed before age 50 [OPCGSC, 2001]);
- (4) ovarian cancer at any age (generally in two relatives, or one invasive serous case);
- (5) multiple affected family members (generally breast cancer diagnosed before age 50, accompanied by one other early breast cancer in a first or second degree relative or ovarian cancer at any age; or three or more close relatives with breast or ovarian cancer at any age);
- (6) Ashkenazi Jewish history (in general, accompanied by any breast or ovarian cancer in a close relative); and
- (7) a known *BRCA1/2* mutation in the family.

The only Canadian guidelines included in this review come from the province of Ontario [OPCGSC, 2001]. A taskforce with representatives from across Canada as well as international collaborators is preparing consensus guidelines on *BRCA1/2* genetic testing.⁸⁸ A number of issues surrounding specific criteria are being discussed by this group⁸⁹ including, for example, the most appropriate age cut-off to denote early onset of breast cancer (in the absence of a family history); whether the diagnosis of breast and ovarian cancer in the same woman or bilateral breast cancer should be restricted to early onset of the first tumour; whether male breast cancer should be accompanied by another cancer in the family; the criteria for individuals with Icelandic or French Canadian ancestry; the pathology types to be considered for ovarian cancer; and whether there should be age at onset restrictions in the case of three or more affected relatives.

7.3 METHODOLOGICAL CONSIDERATIONS⁹⁰

Twenty-eight of the 36 guideline documents we reviewed state specific clinical criteria for testing or referral, by defining ‘high risk’ or ‘strong family history’ in terms of number of affected family members, for example.⁹¹ It was not always clear what source(s) of data or methods of synthesis were used in these documents: the link between the recommendations and the supporting evidence was often not clearly stated. The distribution of information about sources for the 28 documents

is summarized in Table 18 (in some cases these sources were inferred).

Although thirteen documents (46% of 28) cited specific research studies on *BRCA* mutation prevalence/penetrance and/or risk prediction models, none of the testing guidelines we examined provided specific data on the prevalence of *BRCA1/2* mutations associated with each testing/referral criterion. The recent NICE [2004] assessment and the Association of Breast Surgery (ABS) guidelines [Sauven *et al.*, 2004] were the only documents which rated the level of evidence associated with each of their referral recommendations and management guidelines, respectively. NICE provided a grade D for its referral eligibility guidelines, which indicates that these were either based on “evidence from expert committee reports or opinions and/or clinical experience of respected authorities” (p. 12), or were based on extrapolations from research study evidence, as opposed to being directly measured in studies specific to this purpose [NICE, 2004]. ABS rates each of its management recommendations, but does not assign grades to the specific referral and genetic testing criteria mentioned in the guideline. ABS assigned grade C to its recommendation regarding risk stratification into three groups according to family history, which indicates that these were either based on “evidence obtained from well-designed non-experimental descriptive studies” (p. 662) such as comparative, correlation or case studies, or extrapolated from studies of stronger (higher level evidence) design. For its recommendation that high risk women be referred to a specialised genetics clinic, ABS assigned grade D.⁹² Using these 28 documents, then, we were unable to verify the extent of linkage between the sources of the guidelines and the specific criteria, nor fully examine the extent of overlap between sources used by different guidelines.

88. This National Hereditary Cancer Task Force is built upon a partnership between members of the Interdisciplinary Health Research International Team on Breast Cancer Susceptibility (INHERIT BRCA), the Canadian Association of Provincial Cancer Agencies (CAPCA), and Health Canada. The Task Force is also planning to examine clinical management and educational issues (J. Simard, personal communication, May 30, 2005).

89. D. Horsman, BC Cancer Agency, personal communication, September 24, 2003.

90. The Appraisal of Guidelines for Research and Evaluation (AGREE) instrument was used to inform this section.

91. The guidelines that did not provide specific and detailed clinical criteria (to accompany definitions of high risk, for example), were ASCO [2003], NSC [2001], ACOG [2000], ONS [2000], SSO [Klimberg *et al.*, 1999], Stanford Program [Koenig *et al.*, 1998], ACMG [1996], and NBCC [1996].

92. defined as indicated above by NICE.

Table 18.
Source information for documents with specific testing guidelines (N=28)

SOURCES	NUMBER OF DOCUMENTS (%)
1. Literature review (stated or inferred)	11 (39.3)
2. Expert opinion / clinical practice	6 (21.4)
3. Both 1 and 2	6 (21.4)
4. Survey of laboratories	1 (3.6)
5. No sources specified	4 (14.3)

Many of the guideline documents stated numeric cut-offs to specify who should be offered genetic testing for cancer susceptibility or, at least, further risk assessment, although these were not supported by citing specific references. For example, some guidelines specified using a greater than 25% lifetime risk of breast cancer as a criterion for genetic testing eligibility or referral to specialised services (related to the *penetrance* concept), while others specified a greater than 10% prior probability of having a *BRCA* mutation (based on the *mutation prevalence* concept). The first of these represents a risk that is about 2-3 times that of lifetime breast cancer risk in the general population, depending on the background rate. The nine guideline documents that numerically specified high lifetime risk definitions used the following cut-offs: >30% lifetime risk [NICE, 2004; van Asperen *et al.*, 2001], 25-50% lifetime risk [NHMRC-NBCC, 2000; NHMRC, 1999; RACGP, 1997], 25-80% lifetime risk [CRC, 2003], ≥ 3 times the general lifetime risk [SIGN, 1998], and lifetime risk of 1 in 4 to 1 in 3 [Sauven *et al.*, 2004; Eccles *et al.*, 2000].

The >10% probability of a mutation being harboured in the family, that was also seen in some guidelines, is presumably related to the 1996 policy statement by ASCO, which appears to be the first document to formally recommend this figure. This cut-off was utilised by the

Ontario testing guidelines [OPCGSC, 2001]. In contrast, both lower and higher cut-offs for testing or referral were used by other groups: $\geq 5\%$ or $\geq 7\%$ depending on which risk model was used [Fries *et al.*, 2002b]; 10-25% for moderate risk and >25% for high risk [Lucassen *et al.*, 2001]; and $\geq 20\%$ [NICE, 2004; PHGU, 1998].⁹³ Finally, in 1998 the French National Ad Hoc Committee recommended $\geq 25\%$ as a cut-off justifying the offer of testing, and $\leq 5\%$ as a cut-off to justify not offering the test [Eisinger *et al.*, 1998]. In a 2004 update, this Committee recommends the figures of $\geq 25\%$ and $< 10\%$ respectively as thresholds, which, at present, should correspond to detection rates of *BRCA1/2* mutations of approximately 15% and 6%, respectively, according to the authors⁹⁴ [Eisinger *et al.*, 2004].

A significant change to the ASCO policy statements was recently introduced, which calls mutation carrier probability cut-offs into question. The 2003 update to the 1996 statement states that “Given the known limitations and wide variations inherent in models for estimating mutation probability in a given family or individual, and the lack of such models for many cancer predisposition syndromes, **it is neither feasible nor practical to set numerical thresholds for recommending genetic risk assessment services**” [ASCO, 2003, p. 2398]. Instead, ASCO recommends that interpretation of family history and judgement of the appropriateness of testing should rely on evaluation by a health care professional experienced in cancer genetics. This new statement has significant implication for risk assessment research, since much of the development and validation of statistical risk models has used the >10% cut-off for *BRCA* carrier probability.

93. The guidelines by the Department of Defense [Fries *et al.*, 2002b] and Oxford Regional Genetics Service (ORGS) [Lucassen *et al.*, 2001] refer to testing and referral, respectively. While the 20% cut-off is stated in the context of a referral guideline by PHGU (to identify a high risk group), NICE [2004] states this cut-off in the context of indications for genetic testing (although referral is the focus of the NICE report).

94. based on a 60% detection rate, as agreed upon by an expert panel to reflect analytical and clinical sensitivity (P. Eisinger, personal communication, August 9, 2005).

Finally, it should be noted here that in order to fully examine the conditions under which *BRCA1/2* mutation testing should be offered, one needs to also consider (1) the potential clinical benefit that may be obtained through identification of mutation carriers and their subsequent medical management (including surveillance, prophylactic intervention, and treatment), (2) the psychological impact of genetic testing; and (3) the analytical and clinical validity of the available testing methods for *BRCA1/2* mutations.

7.4 CONCLUSION

- We reviewed clinical guidelines produced by a variety of bodies on indications for performing molecular testing for *BRCA1/2* mutations or on risk classification schemes, with a view to referring families to the appropriate level of care. This review shows that most guidelines are based on expert opinion or are extrapolated from research study evidence. However, the links between the recommendations and the supporting evidence, i.e., the source and the specific levels of mutation penetrance and prevalence associated with familial and personal risk factors, were not stated.
- Genetic testing is currently recommended for high risk families only, and there is general concordance between the various guidelines as to the most important risk factors used to define high risk families. The identified risk factors are consistent with findings from the literature on penetrance and prevalence and with the common results of the risk assessment statistical models discussed in chapter 6. It should be noted that this concordance applies to broadly defined risk factors, such as early onset of breast cancer, male breast cancer, bilateral breast cancer, ovarian cancer, multiple affected family members and Ashkenazi Jewish ancestry.
- In contrast, little consensus appears to have been reached as to the precise criteria that should guide testing within each of these

broad risk factors and the combination thereof. This is not a trivial issue, particularly if some risk factors are used in isolation. If, for instance, early age at diagnosis is used as a sufficient criterion for testing (without requirements with respect to number of cancer cases in relatives for instance), the choice of age cut-off greatly influences the number of tests being requested and the ability of laboratories and the health care system to assume these activities. A similar situation arises if male breast cancer or ovarian cancer are used without additional criteria related to age at onset or family history. If such guidelines were to be considered in Quebec, the probability of detecting mutations under these conditions would have to be appropriately documented and the implications estimated, in terms of impact on services and health care organisation. A lack of consensus regarding risk criteria also poses challenges in ensuring appropriate referral from primary care to specialised services.

- The most recent guidelines have placed more emphasis on criteria for referral to tertiary genetic services or for access to genetic counselling than on precise testing indications. Likewise, the 2003 ASCO statement has backed away from the use of a numerical threshold as a criterion to recommend *BRCA1/2* mutation testing, placing greater emphasis on the role of the evaluation made by health care professionals experienced in cancer genetics in determining appropriateness of testing. Such a position may be more coherent with current practices and more realistic at the present time, given the limitations of available data and the lack of consensus around precise guidelines and statistical models. However, the updated ASCO statement has important implications for future risk assessment research. In terms of planning of clinical and laboratory services, such a change would make it more difficult to predict volume of testing. Cost implications could be significant, if testing were to extend beyond the environment of specialized centres.

8. Clinical validity

As outlined in the test performance section (section 3.2), we mainly focus below on the clinical sensitivity of exon and intron/exon screening techniques (EX/SP screening methods), by examining the proportion of hereditary breast and ovarian cancer (HBOC) families carrying point mutations and small rearrangements in *BRCA1/2*, as opposed to large genomic rearrangements and non-coding region mutations in these genes. In a subsequent section, we examine the available evidence on the clinical sensitivity of testing for common mutations in several founder populations. Finally, we briefly discuss the clinical sensitivity of a particular technique, the protein truncation test.

Several difficulties were encountered in reviewing the literature to extract these data, relating to definition of the HBOC phenotype, gaps in current knowledge regarding mutation distribution, variability of selection criteria and techniques used, and both the nature of available studies and the ways of reporting results. These issues will be reviewed first, since they influenced the methods used to retrieve publications and to extract clinical sensitivity data.

8.1 METHODOLOGICAL ISSUES IN CLINICAL VALIDITY STUDIES ON *BRCA1/2* GENETIC TESTS

We did not find any good empirical studies designed to document clinical validity for *BRCA1/2* mutation testing. Most studies on *BRCA1/2* mutations are biological research oriented, with the objective of advancing knowledge of these mutations. Results of such studies are not always directly applicable to the clinical setting due to the manner in which at-risk subjects are selected (i.e., convenience samples) and due to the use of techniques that may not be routinely available in clinical service laboratories.

The definition of the HBOC phenotype, and thus the selection of study subjects in HBOC research, is the source of many difficulties in this kind of analysis. Of interest is how well the test performs in detecting hereditary cases of breast and ovarian cancer related to mutations in the *BRCA1* and 2 genes (box D in Figure 1 of section 3.2). In practice, given the lack of specific phenotypic features to identify this group, there is no alternative but to use HBOC cases as the reference (box F in the same Figure 1). The definition of HBOC relies on family history but there is currently no consensus as to the minimal set of risk factors defining an HBOC family.⁹⁵ In effect, the definition of hereditary breast and/or ovarian cancer families differs notably across studies with regard to age at diagnosis of cancer and to the number of cancer cases. Studies based on affected individuals from families thought to be at high risk of HBOC (e.g., multiply affected families) are seen as least likely to misclassify cases,⁹⁶ although generalizability of results to hereditary cancer cases with less overt family history is compromised. For instance, both Serova *et al.* [1996] and Ford *et al.* [1998] studied families selected for linkage analysis (with high numbers of breast or ovarian cancer cases) [Ford *et al.*, 1998; Serova *et al.*, 1996]. For these families, the probability of a *BRCA* mutation being present is higher than in families seen in cancer clinics, such as those studied by Unger *et al.* [2000] and Gad *et al.* [2002] which usually have a somewhat less severe family history.

An additional step towards more stringent selection of cases is to study only families that have been shown to be genetically linked to *BRCA1/2*.⁹⁷ It should be pointed out, however,

95. Unless a mutation has been identified in the family, but this does not help in identifying a gold-standard or reference definition of phenotype that is independent of molecular testing.

96. In this case, the denominator for the clinical sensitivity computation corresponds, in Figure 1, to a subgroup of F.

97. In this situation, the denominator corresponds approximately to D in Figure 1 (section 3.2).

that depending on the lod score that is chosen as a significant cut-off point in the analysis, there is a certain probability of misclassification of phenotype (genetic versus non-genetic cases) and linkage analysis itself is dependent on assumptions regarding penetrance and prevalence of mutations. Furthermore, depending on the study design, the age distribution of study subjects⁹⁸ may influence estimates of clinical validity, since penetrance for susceptibility mutations is incomplete. Finally, not every study has reported whether the *BRCA1/2* analysis was done on an affected index case in each family. If only unaffected cases are tested, clinical sensitivity could be underestimated.

Molecular techniques have evolved over time and this evolution not only limits the comparability of studies performed at different times and in different settings, but also affects the extent of our current knowledge regarding different types of mutations [Narod and Foulkes, 2004]. The distribution of all susceptibility mutations in the *BRCA1/2* genes is currently unknown, due to the complexity of detecting mutations in these genes (related to the absence of a “hot spot” region and large gene size). Many testing laboratories only screen for point mutations and small rearrangements in the exons and the intron/exon junctions of the *BRCA1/2* genes.⁹⁹ It has been noted for some time that families thought to be at high risk for breast and/or ovarian cancer can test negative following EX/SP screening methods or can test positive for variants of unknown clinical significance. Both of these situations require additional investigations, which are not necessarily done in clinical service laboratories. Certain laboratories have been investigating *BRCA1/2* mutations that cannot be detected by EX/SP screening, but these more exhaustive searches for mutations have generally been performed in the context of research.

A further challenge in our computation of clinical sensitivity for EX/SP screening arises from the fact that a wide variety of different molecular techniques are used both for the point mutation/small rearrangement search and for the detection of large rearrangements. The resulting data are implicitly dependent on the various levels of analytical sensitivity associated with these methods and it is thus problematic to combine values across investigations. For instance, only Serova and colleagues [1996] systematically used sequencing for the EX/SP screen, considered the gold standard for analysis of point mutations/small rearrangements. A mixture of techniques was often used within the same study: in Puget *et al.* [1999a], for example, EX/SP screening was carried out by sequencing in some high risk families and by a combination of PTT with HDA/SSCP in others. It should be kept in mind that the large variability in techniques and study populations precludes a precise evaluation of the influence of such factors on the clinical sensitivity estimates.

8.2 LITERATURE SEARCH AND COMPUTATION METHODS

8.2.1 Literature searching results

We initially identified 87 reports on the prevalence of *BRCA1/2* mutations or on *BRCA* mutation testing methods that potentially contained information on the distribution of susceptibility mutations, using the systematic literature search, review articles published between January 2001 and June 2002, and reference lists of retrieved material (see Appendix B4). We also looked for information about large genomic rearrangements and non-coding mutations in articles on the performance of molecular testing for *BRCA1/2*. We included abstracts in our search. We were particularly looking for studies that used the following methods in sequence: (1) testing for *BRCA1/2* point mutations and small rearrangements with EX/SP screening; and (2) testing for large rearrangements/non-coding region mutations in those with negative results in step 1. In populations with founder effects (e.g., Ashkenazi Jewish) we looked for studies that

98. Individuals carrying a susceptibility conferring mutation but having not reached the age of expression of disease will be classified as ‘controls’.

99. These EX/SP screening methods are not able to detect certain types of mutations (e.g. large genomic rearrangements, mutations in non-coding regions).

enabled us to reconstruct the proportion of common and non-common *BRCA1/2* mutations.

As shown in Appendix B4, many reports (n=40) were excluded for the following reasons: (a) absence of testing for large rearrangements or non-coding region mutations in high risk families with negative EX/SP results (i.e., no step 2); (b) absence of testing or incomplete testing for non-common mutations in samples from founder populations; (c) absence of EX/SP results for the original sample tested; (d) no reporting of the original number of families tested for any of the types of *BRCA1/2* mutations considered, or missing results for some of the tested families;¹⁰⁰ or (e) reports were reviews, editorials, or methodological in nature. As a result of these exclusions, we extracted data from 24 studies on families at high risk of breast and/or ovarian cancer to estimate clinical sensitivity of EX/SP screening in mostly heterogeneous populations and of common mutations in populations with a founder effect. We also used 25 other articles for background information and discussion.

It should be noted that we often had to extract and combine data from two or three subsequent publications in order to reconstruct the proportion of different types of *BRCA1/2* mutations detected.¹⁰¹ Also, although we report on numbers of ‘families’ in these studies, it should be kept in mind that an affected individual from each family was tested for mutations, rather than all relatives.

8.2.2 Computation of clinical sensitivity

For a given population, a theoretical value for the clinical sensitivity of *BRCA1/2* mutation testing can be estimated as the proportion of clinically important mutations detectable by a given technique. While the numerator is easily characterised as the number of cases with a

detected *BRCA1/2* mutation, the definition of the denominator is open to different interpretations. This problem arises because, as previously mentioned, the distribution of all types of susceptibility mutations is not well documented and there is no consensus as to the definition of the HBOC phenotype (and thus no consistency in study subject selection criteria). Is it more appropriate to use a broad or a restricted definition of HBOC cases (corresponding to F or a subgroup of F in Figure 1 of section 3.2)? Should the denominator be restricted to cases in families linked to *BRCA1/2* (D in Figure 1) or is the total number of cases with a known *BRCA1/2* mutation the most appropriate choice (A+B in Figure 1)? These questions show that, in analysing study results, the classification of families who have negative results following all mutation screening strategies (involving both clinical service and research techniques) is problematic: clinically significant mutations could have been missed by the methods used, or the cases could be the result of other susceptibility genes or non-genetic, environmental factors.

A crucial step in this method of estimating clinical sensitivity is thus how to account for the possibility of misclassification of results in the analysis. Families in which all genetic tests were negative likely represent a combination of false negative subjects (in whom *BRCA1/2* mutations are actually present but were missed by the testing methods) and true negative subjects (in whom the testing was correct and disease status is not associated with *BRCA1/2*).

For our estimate of the clinical sensitivity of EX/SP screening for *BRCA1/2*, we expressed the results as a range of values: the minimum value places all the tested families in the denominator, including those who tested negative (with the assumption that they actually carry mutations); the maximum value excludes all negative families from the denominator (by

100. For example, in the study by Puget *et al.*, [1999a], 27 American and 51 French families were screened for large *BRCA1/2* rearrangements after negative EX/SP screening results; however, the results for the French families are not provided in the article.

101. For example, two families originally studied by Serova *et al.*, [1996] were further characterised for large genomic rearrangements in articles by Puget *et al.*, [1999a,b].

assuming they are truly negative).¹⁰² Test negative families likely include a mixture of families with true negative and false negative results, in a proportion depending on the study selection criteria, the analytical sensitivity of the molecular techniques used and the thoroughness of the analysis and reporting for the entire sample. For simplicity we analysed the data using the two extremes only. Figure 2 presents the strategy used for our calculations of clinical sensitivity of EX/SP screening methods, illustrating the two options for classifying test-negative families. Of these two values, the minimum value is the closest to the definition of clinical sensitivity since the denominator reflects the phenotype, whereas for the maximum value the denominator corresponds to the known mutations and thus reflects genotype distribution. However, as discussed below, the maximum values are more often reported than the minimum values in the literature and the assumptions related to each definition are rarely acknowledged. Considering each approach has its limitations (essentially, problems of phenotype definition and variability of selection criteria for the minimum value, and limited yet evolving knowledge of mutation spectrum and variability of techniques used for the maximum value), we considered it essential to present both the minimum and the maximum values for each study.

A much smaller number of large genomic rearrangements have been identified in the *BRCA2* gene than in *BRCA1* [Peelen *et al.*, 2000b; Nordling *et al.*, 1998]. Some data suggest that genomic rearrangements in the *BRCA2* gene are frequent in families with cases

of male breast cancer [Tournier *et al.*, 2004]. At the time of our literature search for the present assessment, data on large *BRCA2* rearrangements were insufficient for us to derive estimates separately for *BRCA1* and *BRCA2*. Our computation of clinical sensitivity of EX/SP screening methods in heterogeneous (non-founder) populations therefore applies only to *BRCA1* mutations. Data regarding mutations in *BRCA2* are nevertheless presented in detail for each study in Appendix G. For the clinical sensitivity of testing for common founder mutations, mutations in both *BRCA1* and *BRCA2* are considered when appropriate.

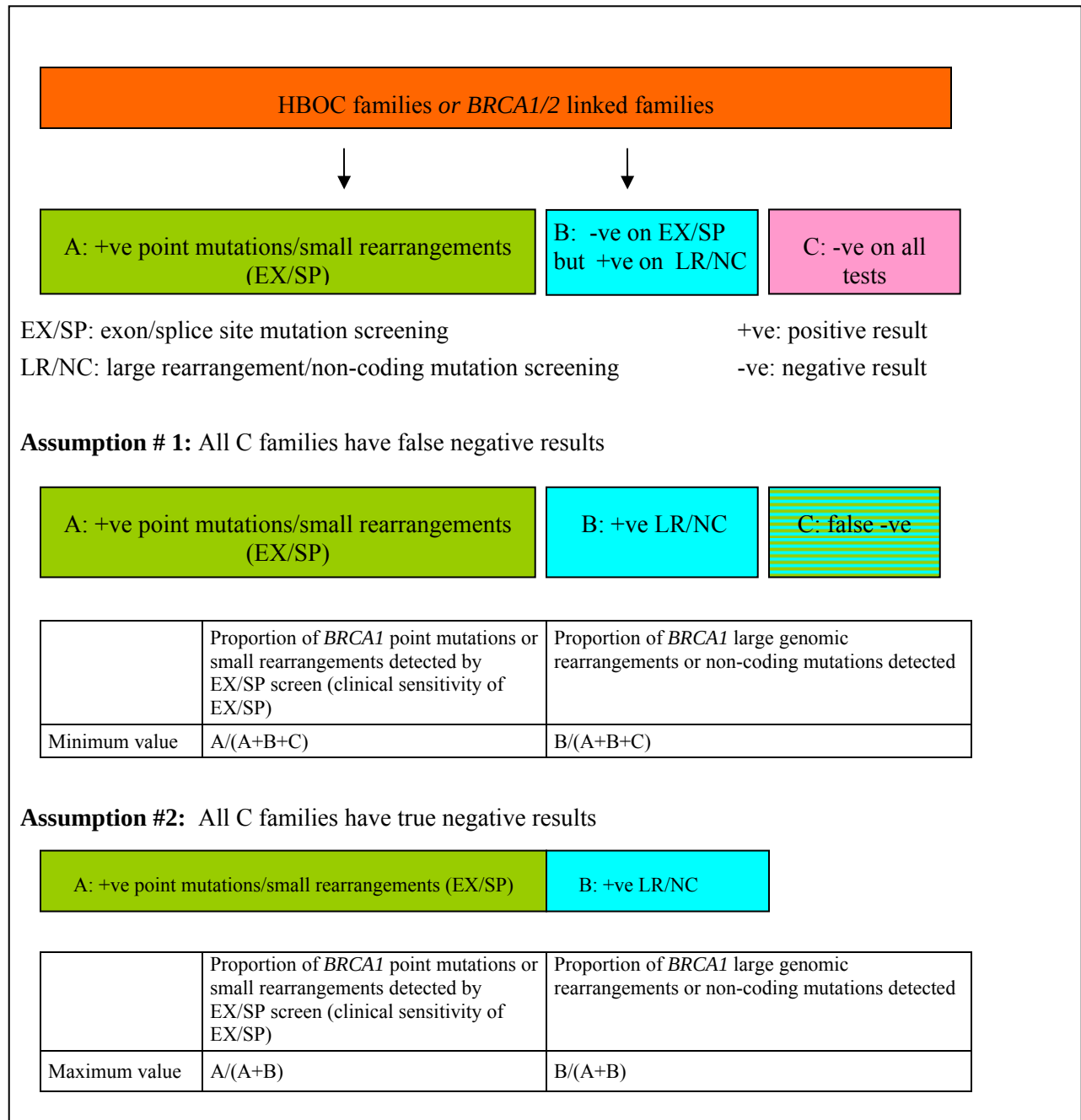
8.3 CLINICAL SENSITIVITY RESULTS AND DISCUSSION

We first present results for heterogeneous populations, following the computational method described above for each study which allowed estimation of clinical sensitivity for EX/SP screening. We then present estimated clinical sensitivity of testing for common mutations, using the distribution of common and non-common *BRCA1/2* mutations known to confer risk in different populations with a founder effect (Ashkenazi Jewish, Dutch, Finnish, Hungarian and French Canadian). Finally, we present the studies used to derive an estimate of clinical sensitivity for the protein truncation test (PTT) with respect to *BRCA* point mutations/small rearrangements that lead to truncated proteins. Details on the methodology of sample selection and mutation testing, the distribution of mutations observed and our computations are presented for each study in Appendix G.

¹⁰² Thus under the second assumption, if all the genetic testing techniques used were considered together (i.e., tests for point mutations, small rearrangements, large rearrangements, and non-coding region mutations), the maximum value for our estimate of clinical sensitivity of testing for any known kind of *BRCA1/2* mutation would be 100%. This ignores the existence of other types of *BRCA* mutations not yet elucidated.

Figure 2.

Estimation of clinical sensitivity of EX/SP screening methods, based on the distribution of *BRCA1* mutations in HBOC families (or *BRCA1/2* linked families) after EX/SP screening and analysis for large genomic rearrangements/non-coding region mutations



8.3.1 Clinical sensitivity of *BRCA1* EX/SP screening methods

Table 19 summarises the clinical sensitivity estimates for EX/SP screening of *BRCA1* for HBOC families from mostly heterogeneous populations (for example, from the USA). We include the results of two Dutch studies (from a population with founder effects) since their methodology also involved analysis for large genomic rearrangements in families with negative EX/SP screening results [Hogervorst *et al.*, 2003; Petrij-Bosch *et al.*, 1997]. As mentioned previously, methodological and population differences explain the variability of results and prevent the combination of clinical sensitivity estimates shown in Table 19. Differences are particularly striking between founder and non-founder populations. Using our point estimates, the proportion of large *BRCA1* rearrangements detected in the Dutch population [Hogervorst *et al.*, 2003; Petrij-Bosch *et al.*, 1997] where two deletions are founder mutations is generally higher than the proportion observed in heterogeneous samples, with the exception of one Italian study [Montagna *et al.*, 2003].

Table 19 shows that the difference between the minimum and maximum values for the clinical sensitivity estimates can be considerable in size, with up to a 50% difference (e.g., Hartmann *et al.*, [2004]; Gad *et al.*, [2003]; Hogervorst *et al.*, [2003]; Montagna *et al.*, [2003]; Casilli *et al.*, [2002]; Petrij-Bosch *et al.*, [1997]). This is partly related to the relative frequency of mutation carriers and non-carriers in each study. If the proportion of non-carriers is large, their inclusion in the denominator (or not) makes a considerable difference. The two studies with the smallest difference [Serova *et al.*, 1996; Ford *et al.*, 1998] have both the smallest sample sizes and the most stringent selection criteria, with high probabilities of linkage to *BRCA1*. It should be noted that the search for large rearrangements/non-coding mutations was more thorough in the study by Serova and colleagues [1996]; Ford and colleagues [1998] were not able to provide informative results for 6 of the 9 families that were negative after the EX/SP screen. Of the four families with large rearrangements in the Serova study, one had Dutch heritage and two others had British ancestry (for all of these, the backgrounds were mixed with other groups such as Irish, German, and Scottish) [Serova *et al.*, 1996].

Table 19.

Proportion of large rearrangements/non-coding region mutations detected and clinical sensitivity of EX/SP screen for *BRCA1*

REFERENCE(S)		ORIGINAL STUDY SAMPLE (ORIGINAL SET OF FAMILIES ANALYSED BOTH FOR EX/SP AND NON-EX/SP MUTATIONS)		PROPORTION OF <i>BRCA1</i> LARGE REARRANGEMENTS OR NON-CODING REGION MUTATIONS DETECTED		PROPORTION OF <i>BRCA1</i> POINT MUTATIONS / SMALL REARRANGEMENTS DETECTED (CLINICAL SENSITIVITY OF EX/SP SCREEN)		
		Selection criteria	# families	Region(s) of origin	Estimated proportions (%)†	Technique(s) used for detection	Estimated proportions (%)†	Technique(s) used for EX/SP screen
Serova <i>et al.</i> , [1996]; Puget <i>et al.</i> , [1997, 1999b]		BrOv ca families: ≥ 4 cases and ≥ 1 case of each cancer type (at least 80% probability of linkage to the <i>BRCA1</i> gene)	20	USA, France	20.0; 23.5	SSCP on cDNA, LHR (Southern blot or, in some families, PCR screen for splicing defects)	65.0; 76.5	SEQ
Petrij-Bosch <i>et al.</i> , [1997]		Br and/or Ov ca: ≥ 3 1st degree relatives Br or Ov ca, ≥ 1 before age 50 (10% <i>a priori</i> risk of being a <i>BRCA1</i> carrier according to Shattuck-Eidens <i>et al.</i> , [1995])	170	The Netherlands	8.2; 38.2	Specific detection of 3 deletions (3835bpdel, 510bpdel, EXdel22 IVS22 +5G->A)	13.3; 61.8	PTT and FLA
Ford <i>et al.</i> , [1998]		Br $\pm$ Ov ca families: ≥ 4 female Br ca before age 60 or any male Br ca $\pm$ Ov ca (high probability of linkage to <i>BRCA1</i> : lod score >1.0)	30	North America and Europe (multi-centre)	10.0; 12.5	LHR analysis or SSCP on cDNA done & informative for 3/9 available families (see Appendix G, Table G3)	70.0; 87.5	SEQ, DSDI/PTT, SSCP-HA, SSCP alone, CSGE or CDGE
Puget <i>et al.</i> , [1999a, 1999b]		Br $\pm$ Ov ca families: ≥ 2 1st degree relatives with Ov ca <u>OR</u> ≥ 3 1st degree with Br ca (with ≥ 1 Br ca before age 40) <u>OR</u> 4 with Br ca (≥ 3 of these 1st degree <u>OR</u> 2 cases of Br ca before age 40) (ascertained for linkage but no results given)	71	USA	9.8; 15.0	Quantitative Southern blot; entire gene deletion analysis; 5' UTR , 3' UTR and promoter region by HDA analysis; specific screening for exon 13 duplication	55.7; 85.0	SEQ or combination of PTT, HDA and SSCP
Unger <i>et al.</i> [2000]	Martin <i>et al.</i> *	BrOv ca families: ≥ 1 Br ca + ≥ 1 Ov ca in the same lineage	106	USA; includes Ashkenazi Jewish	5.1; 8.3	Southern blot; exon 13 duplication screening	55.6; 91.7	CSGE
	Sinilnikova <i>et al.</i> *	Br and/or Ov ca (details not reported)	48	Not reported	11.1; 14.7	Not reported	64.4; 85.3	Not reported
Gad <i>et al.</i> , [2002]; Casilli <i>et al.</i> , [2002]		Br and/or Ov ca: ≥ 3 cases (any relatives), 1 Ov ca + 2 Br or Ov ca <u>OR</u> ≥ 2 cases in 1st degree relatives, Br or Ov ca <u>OR</u> 1 Br-Ov ca in one case	120	France (multi-centre)	4.7; 11.6	Bar code on combed DNA; QMPSF	35.8; 88.4	DGGE or DHPLC

Table 19 (continued).

REFERENCE(S)	ORIGINAL STUDY SAMPLE (ORIGINAL SET OF FAMILIES ANALYSED BOTH FOR EX/SP AND NON-EX/SP MUTATIONS)			PROPORTION OF <i>BRCA1</i> LARGE REARRANGEMENTS OR NON-CODING REGION MUTATIONS DETECTED		PROPORTION OF <i>BRCA1</i> POINT MUTATIONS / SMALL REARRANGEMENTS DETECTED (CLINICAL SENSITIVITY OF EX/SP SCREEN)	
	Selection criteria	# families	Region(s) of origin	Estimated proportions (%)†	Technique(s) used for detection	Estimated proportions (%)†	Technique(s) used for EX/SP screen
Hogervorst <i>et al.</i>, [2003]	Br and/or Ov ca (details not reported)	805	The Netherlands	4.3; 27.3	MLPA; specific detection of deletions	11.3; 72.7	DGGE, DHPLC or PTT
Montagna <i>et al.</i>, [2003]	BrOv ca families: ≥ 3 Br or Ov in 1st degree relatives (or 2nd if transmitting male), any age, same parental branch, over ≥ 2 generations	37	Italy	18.8; 40.0	MLPA; Southern blot; genotyping of SNPs in the <i>BRCA1</i> gene	28.1; 60.0	SSCP+PTT and DHPLC
Hartmann <i>et al.</i>, [2004]	Br+/-Ov ca families: ≥ 2 Br ca cases with at least 2 Br before age 50; <u>OR</u> ≥ 1 case of male Br ca; <u>OR</u> ≥ 1 case of Br ca and ≥ 1 case of Ov ca	140	Southern Germany	3.3; 7.7	MLPA	39.0; 92.3	DHPLC + sequencing

Abbreviations: Br=breast cancer; Ov=ovarian cancer; SNP=single-nucleotide polymorphism

*unpublished results reported in Unger *et al.*, [2000]

† minimum and maximum values estimated as described in section 8.2.2; detailed calculations in Appendix G.

Table 20.

Our estimates versus cited values for proportion of large rearrangements or non-coding region mutations in *BRCA1*

STUDY REFERENCE (REFERENCE FOR CITED VALUES)	PROPORTION OF LARGE REARRANGEMENTS OR NON-CODING REGION MUTATIONS DETECTED IN <i>BRCA1</i>		
	OUR ESTIMATES FROM STUDY DATA, %	CITED VALUES, % (CALCULATION DETAILS; ALSO SEE APPENDIX G)	LIMITATIONS OF CITED VALUES
Serova <i>et al.</i>, [1996]; (Shattuck-Eidens <i>et al.</i>, [1997])	20.0; 23.5	5 – 15 (1/20 and 3/20 using 1 and 4 families marked with * and **, respectively, App. G, Table G-1)	Underestimates: they do not include further analysis performed on some families [Puget <i>et al.</i> 1997, 1999b] and do not consider that families with negative results after all tests could be true negatives
Petrij-Bosch <i>et al.</i>, [1997]	7.6; 36.4 ¹	36 ¹ (B'/A+B', App. G, Table G-7)	This is a maximum value: they do not consider that families with negative results after all tests could be false negatives
Ford <i>et al.</i>, [1998]; (Swensen <i>et al.</i>, [1997])	10.0; 12.5	10 – 30 ² (10%=B/A+B+C; 30%= B+C/A+B+C, App. G, Table G-3)	The 30% is likely overestimated by including all of the families with negative results after all tests (C) in the numerator (point mutations could have been missed, and some or all negative families could be true negatives)
Puget <i>et al.</i>, [1999a, b]; (Puget <i>et al.</i>, [1999a])	9.8; 15.0	15 (B/A+B, App. G, Table G-2)	This is a maximum value: they do not consider that families with negative results after all tests could be false negatives
Unger <i>et al.</i>, [2000] - unpublished data by Sinilnikova <i>et al.</i>; (Myriad technical note, 2004)	11.1 ; 14.7 ³	15 ⁴ (B/A+B, App. G, Table G-4b)	This is a maximum value: they do not consider that families with negative results after all tests could be false negatives

1. These proportions are computed for the two most common deletions, 510bp and 3815bp, rather than for three deletions as in Table G7 in Appendix G and in Table 19.

2. There is no reference for these figures in the article by Swensen *et al.*, 1997 but we believe that they arise from Ford *et al.*, 1998.

3. We presume that the 15% value attributed in the Myriad technical note to Unger *et al.*, 2000 has been rounded up from the maximum value of 14.7% estimated from the data of Sinilnikova and colleagues.

4. In the Myriad Genetic Laboratories technical note, 2004 the cited value of 15% refers to *BRCA1* and *BRCA2*; however, in the original article by Unger *et al.*, 2000 no screening was performed for large *BRCA2* genomic rearrangements.

Table 21.
Clinical sensitivity for specific testing of founder *BRCA1/2* mutations in different populations

REFERENCE(S)	ORIGINAL STUDY SAMPLE (ORIGINAL SET OF FAMILIES ANALYSED BOTH FOR COMMON AND NON-COMMON MUTATIONS)			PROPORTION OF NON-COMMON MUTATIONS DETECTED		PROPORTION OF COMMON MUTATIONS DETECTED (CLINICAL SENSITIVITY OF SPECIFIC TESTING FOR FOUNDER <i>BRCA1/2</i> MUTATIONS)	
	Country or ethnic origin	Selection criteria	# families	Estimated proportions (%)†	Technique(s) used for detection	Estimated proportions (%)†	Technique(s) used for detection
Schubert <i>et al.</i>, [1997]	Ashkenazi Jewish	≥ 4 Br or Ov ca	17	5.9 ; 11.1	SSCP for <i>BRCA1</i> ; SSCP plus PTT in exons 10-11 for <i>BRCA2</i>	47.1 ; 88.9 ¹	Not reported
Frank <i>et al.</i>, [1998]	Ashkenazi Jewish	Br ca before age 50 or Ov ca + ≥ 1 female relative (1st or 2 nd degree) with Br ca before age 50 or Ov ca at any age	47	4.3 ; 10.0	SEQ for <i>BRCA1</i> and 2	38.3 ; 90.0 ¹	SEQ for <i>BRCA1</i> and 2
Phelan <i>et al.</i>, [2002]	Ashkenazi Jewish	≥ 2 Br or Ov ca, in 1st or 2nd degree relatives	245	0.4 ; 1.2 ²	<i>BRCA1</i> : PTT for exon 11; DGGE for all other exons (no <i>BRCA2</i> analysis done)	34.7 ; 98.8 ^{1,2}	PCR multiplex with specific primers for the 3 mutations
Vehmanen <i>et al.</i>, [1997a]	Finland: Helsinki and southern regions	≥3 Br and/or Ov ca in 1st or 2nd degree relatives (however, 2 families analysed have one Br ca and one Ov ca)	100	8.6 ; 38.1	<i>BRCA1</i> : HA/SSCP/PTT of exon 11 and SEQ; <i>BRCA2</i> : HA/SSCP/PTT of exon 10-11; linkage analysis in 12 families	14.0 ; 61.9 ³ 6.5 ; 28.6 ⁴	<i>BRCA1</i> : HA/SSCP/PTT of exon 11 and SEQ; <i>BRCA2</i> : HA/SSCP/PTT of exons 10-11; linkage analysis in 12 families
Huusko <i>et al.</i>, [1998]	Finland, Oulu region	≥2 Br and/or Ov ca in 1st degree relatives or other HBOC features (onset<40y, bilateral or multiple tumours)	88	1.1 ; 9.1	<i>BRCA1</i> : CSGE /PTT of exon 1; <i>BRCA2</i> : CSGE/PTT of exons 10-11	11.4 ; 90.9 ⁵	<i>BRCA1</i> : CSGE /PTT of exon 1; <i>BRCA2</i> : CSGE/PTT of exons 10-11

1. Panel of the 3 common *BRCA1/2* mutations in the Ashkenazi Jewish population: *BRCA1* 185delAG, *BRCA1* 5382insC, *BRCA2* 6174delT.

2. Estimated values if we assume the 3 detected missense mutations (B' in Table G12, Appendix G) are not clinically significant.

3. Panel of 8 common *BRCA1/2* mutations.

4. Panel of the 4 common mutations reported by Huusko *et al.*, 1998.

5. Panel of 4 common mutations: *BRCA1* 3745delT, *BRCA1* 4216 2ntA->G, *BRCA2* 9346 2ntA->G, *BRCA2* 999del5.

Table 21 (continued).

REFERENCE(S)	ORIGINAL STUDY SAMPLE (ORIGINAL SET OF FAMILIES ANALYSED BOTH FOR COMMON AND NON-COMMON MUTATIONS)			PROPORTION OF NON-COMMON MUTATIONS DETECTED		PROPORTION OF COMMON MUTATIONS DETECTED (CLINICAL SENSITIVITY OF SPECIFIC TESTING FOR FOUNDER <i>BRCA1/2</i> MUTATIONS)	
	Country or ethnic origin	Selection criteria	# families	Estimated proportions (%)†	Technique(s) used for detection	Estimated proportions (%)†	Technique(s) used for detection
Ramus <i>et al.</i>, [1997]	Hungary	≥ 2 1 st degree relatives with Br or Ov ca diagnosed at any age (cases with a strong family history were preferentially selected)	32	3.6 ; 14.3	<i>BRCA1/2</i> : PTT or SSCP/HA	21.4 ; 85.7 ¹	<i>BRCA1/2</i> : PTT or SSCP/HA
Ladopoulos <i>et al.</i>, [2002]	Greece	≥ 2 Br ca before age 50 in 1 st or 2 nd degree relatives or other HBOC characteristics (Br ca before age 35, bilateral breast ca)	85	8.2 ; 50.0	<i>BRCA1</i> PTT exon 11, SEQ other <i>BRCA1</i> exons, <i>BRCA2</i> PTT exon 10-11; or SSCP all exons <i>BRCA1</i> , SSCP exon 10-11 <i>BRCA2</i>	8.2 ; 50.0 ²	<i>BRCA1</i> PTT exon 11, SEQ other <i>BRCA1</i> exons, <i>BRCA2</i> PTT exon 10-11; or: SSCP all exons <i>BRCA1</i> , SSCP exon 10-11 <i>BRCA2</i>
Gorski <i>et al.</i>, [2004a]	Poland	100 fam: ≥3 Br ca (no Ov ca) 100 fam: ≥3 Br ca and Ov ca	200	9.0 ; 14.0 ⁴	<i>BRCA1/2</i> : EX/SP sequencing	55.5 ; 86.0 ^{3,4}	<i>BRCA1/2</i> : EX/SP sequencing
Simard <i>et al.</i>, [2004] (J. Simard)⁵	French Canadian	≥3 Br or Ov ca cases at any age in 1 st degree relatives, OR ≥ 4 1st or 2nd degree relatives with Br ca and/or Ov ca	191 ⁶	5.2 ; 17.9	1. Specific detection of 24 <i>BRCA1/2</i> mutations by targeted sequencing followed by sequencing in all EX/SP, if negative 2. MLPA analysis of <i>BRCA1/2</i> in 134 families negative after sequencing 3. Southern blot analysis of <i>BRCA1/2</i> in a subsample of families	22 ; 75 ⁷ 24.1 ; 82.1 ⁸	1. Specific detection of 24 <i>BRCA1/2</i> mutations by targeted sequencing followed by sequencing in all EX/SP, if negative 2. MLPA analysis of <i>BRCA1/2</i> in 134 families negative after sequencing 3. Southern blot analysis of <i>BRCA1/2</i> in a sample of families

1. Panel of 2 common *BRCA1* mutations: 185delAG and 5382insC; 2. Single common *BRCA1* mutation: 5382insC; 3. Panel of 3 common *BRCA1* mutations: 5382insC, C16G, 4153delA.

4. Estimated values if we assume the detected missense mutations (B''' in Table G16, Appendix G) are not clinically significant; 5. Oral presentation "Familial breast/ovarian cancer in the French Canadian founder population", Oncogenetics: Achievements and challenges. Les 17ièmes Entretiens du Centre Jacques Cartier, Montréal, Québec, Oct 7-8, 2004 and personal communication with J. Simard (January 28, 2005); 6. Number of families wherein testing was performed on a member affected with cancer or an obligate carrier; 7. For 2 founder mutations: *BRCA1* 4446C→T and *BRCA2* 8765delAG; 8. For 4 common mutations: *BRCA1* 4446C→T, 2244insA, 2953del3+C and *BRCA2* 8765delAG.

Table 21 (continued).

REFERENCE(S)	ORIGINAL STUDY SAMPLE (ORIGINAL SET OF FAMILIES ANALYSED BOTH FOR COMMON AND NON-COMMON MUTATIONS)			PROPORTION OF NON-COMMON MUTATIONS DETECTED		PROPORTION OF COMMON MUTATIONS DETECTED (CLINICAL SENSITIVITY OF SPECIFIC TESTING FOR FOUNDER <i>BRCA1/2</i> MUTATIONS)	
	Country or ethnic origin	Selection criteria	# families	Estimated proportions (%)†	Technique(s) used for detection	Estimated proportions (%)†	Technique(s) used for detection
Oros <i>et al.</i>, [2004]	French Canadian	≥ 3 confirmed female Br ca before or at age 65, epithelial Ov ca or male Br ca in 1 st , 2 nd and 3 rd degree relatives occurring in the same branch of family	169	4.7 ; 10.8	1. Specific detection of 20 <i>BRCA1/2</i> mutations in EX/SP regions by SSCP 2. 83/95 –ve fam had a complete analysis by SEQ of EX/SP in <i>BRCA1</i> or <i>BRCA2</i> or both; or had a partial analysis by PTT in <i>BRCA1</i> exon 11 and <i>BRCA2</i> exons 10-11 or in <i>BRCA1</i> exon 11 only.	36.7 ; 83.8* 39.0 ; 89.2+ 24.8 ; 56.8“	1. Specific detection of 20 <i>BRCA1/2</i> mutations in EX/SP regions by SSCP 2. 83/95 –ve fam had a complete analysis by SEQ of EX/SP in <i>BRCA1</i> or <i>BRCA2</i> or both; or had a partial analysis by PTT in <i>BRCA1</i> exon 11 and <i>BRCA2</i> exons 10-11 or in <i>BRCA1</i> exon 11 only.

† minimum ; maximum values estimated as described in section 8.2.2; detailed calculations in Appendix G.

* 5 common mutations: *BRCA1* 4446C→T and 2953del3insC, *BRCA2* 6085G→T, 8765delAG and 3398delAAAAG.

+ 7 common mutations: *BRCA1* 4446C→T, 2953del3insC, 3875delGTCT and *BRCA2* 6503delTT, 6085G→T, 8765delAG and 3398delAAAAG.

“ For the 2 founder mutations that are common to Simard *et al.*, [2004]: *BRCA1* 4446C→T and *BRCA2* 8765delAG.

Table 22.

Distribution of *BRCA1* point mutations/small rearrangements detected and proportion predicted to lead to truncated proteins

REFERENCE	STUDY SAMPLE	TECHNIQUE(S) USED	GENE TESTED	DISTRIBUTION OF MUTATIONS	PROPORTION OF POINT MUTATIONS AND SMALL REARRANGEMENTS PREDICTED TO LEAD TO TRUNCATED PROTEINS
Shattuck-Eidens <i>et al.</i>, [1995]	372 unrelated patients (from North America and UK) with breast or ovarian cancer, mostly from high-risk families with high probability of linkage to <i>BRCA1</i>	SEQ, SSCP, SEQ/SSCP/ASO, SSCP/SEQ/HA or ASO	<i>BRCA1</i>	all identified mutations (n=63): 71% FS; 10% NS; 5% SP or R; 14% MS	86% *
Serova <i>et al.</i>, [1996]	20 high-risk French families with breast and/or ovarian cancer	SEQ	<i>BRCA1</i>	all identified mutations (n=17): 44% FS; 25% NS; 12.5% SP; 6% MS; 12.5% IR	81.5% +

Abbreviations for types of mutations: FS=frame shift; NS=nonsense; SP=splice site; MS=missense; IR=inferred regulatory; R=regulatory

* As reported by the authors and includes frameshift, nonsense, and splice site mutations.

+ As reported by the authors and includes frameshift, nonsense, and splice site or regulatory mutations.

A particular large rearrangement, which results in duplication of *BRCA1* exon 13, appears to be a founder mutation in populations of British origin [*BRCA1* Exon 13 Duplication Screening Group, 2000]. Where data were available, we estimated the detected proportion of this genomic rearrangement from studies on the distribution of mutations in HBOC families from heterogeneous populations such as in France and USA (see Appendix G, Tables G-1, G-2 and G-4a). This genomic rearrangement represents between 20 and 50% of the large rearrangements detected in these studies.¹⁰³ In the UK, this deletion seems to be found mainly in the Yorkshire and north-east Midlands areas [Evans *et al.*, 2004a]. In a series of 7570 patients tested for five recurrent *BRCA1* rearrangements by Myriad Genetics Laboratories, the exon 13 duplication represented 2.5% (26/1036) of all mutations identified [Hendrickson *et al.*, 2003]. As such, this duplication is the single most prevalent non-Ashkenazi *BRCA1* mutation in the Myriad samples [Hendrickson *et al.*, 2003]. Insufficient data are available to estimate clinical sensitivity, however.

We found various estimates in the literature of the proportion of large rearrangements and non-coding region mutations in *BRCA1* that cannot be detected by EX/SP screening. In general, authors do not provide both the minimum and maximum values, as we have done here. Table 20 compares the different figures estimated according to our methods above with figures cited in the literature, either found in the original study or in other articles. Different assumptions and ways of deriving estimates explain the observed discrepancies.

Shattuck-Eidens and colleagues [1997], referring to the study by Serova *et al.* [1996], cite an interval of values but these are underestimates, since they do not take further analysis into account, nor the possibility of true negative families. By not including in their calculations the families who remain negative after all tests

were completed, the Myriad Genetic Laboratories technical note [2004],¹⁰⁴ Puget *et al.*, [1999a], and Petrij-Bosch *et al.* [1997] implicitly assume that the test results for these families are all false negatives. Swensen *et al.* [1997] do not provide details about their values of 10-30% which appear to have been based on unpublished data at the time of citation; however, we believe these figures arise from the study of 30 families with previous linkage results by Ford and colleagues [1998]. The 10% value cited by Swensen *et al.* [1997] appears to correspond to our minimum figure (see Appendix G, Table G-3). The 30% figure, however, appears to assume that all families who tested negative by the end of the study by Ford and colleagues [1998] actually carried large genomic rearrangements or non-coding region mutations. We believe that this is likely an overestimation because some point mutations/small rearrangements may have been missed by the EX/SP screen (which used a combination of methods besides sequencing), and since at least some of these families could be true negatives.

The most frequently cited figure of clinical sensitivity is derived from a multi-centre study on 237 families published by Ford *et al.* in 1998. As the authors acknowledge, their estimate of clinical sensitivity is quite crude, since different screening techniques were used at the various centres, including sequencing and a variety of EX/SP screening methods. High-risk families were selected for inclusion in this study, and although most participating centres were located in Europe and North America, it is not entirely clear whether some of these families may have come from founder populations. The main difference, however, with our results presented in Table 19 is that Ford *et al.*'s published estimate of 63% (95% CI: 51-77%) does not represent the proportion of high risk or linked families for whom the molecular analyses revealed a *BRCA* mutation. Indeed, molecular analyses were not performed in all families. The study design is thus not typical for a molecular

103. The estimated interval for the proportion of this particular duplication varies from 5.5-9% [Serova *et al.*, 1996], 4.5-7.5% [Puget *et al.*, 1999a, b], and 1-1.7% (Martin *et al.* in Unger *et al.*, [2000]).

104. http://www.myriadtests.com/provider/doc/tech_specs_brac.pdf, consulted January 18, 2005.

test evaluation. Their computational method relies on the maximisation of likelihood functions, incorporating not only the pedigree structures with the distribution of cases and non-cases and assumptions regarding mutation prevalence and penetrance, but also the results of the molecular testing. (This raises a question about the independence between the test result and the reference standard.)

The results for a subset of 30 families, for whom molecular analyses were performed and linkage to *BRCA1* was established, can be analysed in the traditional way. These results were reported by Ford *et al.* [1998] as yielding a clinical sensitivity estimate of 70% and are presented in Table 19 and Appendix G (Table G-3). Both estimates (63% and 70%) correspond to minimum values according to our computational approach since no families were excluded from the denominator.

8.3.2 Clinical sensitivity of *BRCA1/2* mutation testing for common founder mutations

As mentioned previously, founder effects in certain populations result in an elevated frequency of one or more specific variants in *BRCA1/2* among descendants of a common group of ancestors. It is important to distinguish founder mutations from mutations that are recurrent because they arise at genetic “hot spots” but do not occur in families with a common ancestor or common haplotypes [Evans *et al.*, 2004a].

We calculated estimates of clinical sensitivity for the testing of specific *BRCA1/2* mutations from studies providing the distribution of common and non-common mutations known to confer risk in different populations with founder effects. A similar computational method as above was applied to derive minimal and maximal values. Results and key features of the original study samples are presented in Table 21, while details and calculations can be found in Appendix G.

Ashkenazi Jewish

Research in the Ashkenazi Jewish (AJ) population has shown that one individual in 40 (~2.5%) carries one of three common point mutations: *BRCA1* 185delAG, *BRCA1* 5382insC or *BRCA2* 6174delT [Iau *et al.*, 2001; Culver *et al.*, 2000]. In contrast to the large spectrum of *BRCA1/2* mutations found in heterogeneous populations, only about 30 other mutations have been found in the AJ population according to Phelan *et al.* [2002]. We estimated the clinical sensitivity of testing for the three common AJ *BRCA1/2* mutations from the distribution of founder and non-founder mutations within AJ HBOC families. The values were 47.1 and 88.9% for Schubert *et al.* [1997] and 38.3 and 90% for Frank *et al.* [1998]. Phelan and colleagues [2002] did not screen for non-founder mutations in the *BRCA2* gene, likely underestimating the overall proportion of non-founder mutations [Phelan *et al.*, 2002].

In several review articles on *BRCA1/2* genetic testing (published between January 2001 and June 2002), we found statements that the three common mutations account for about 90% of all *BRCA* mutations in the AJ population (for example, Srivastava *et al.* [2001]; Taylor [2001]), but no references were given for this figure. We believe that this proportion denotes the prevalence of the three mutations in high risk AJ families as reported by Tonin and colleagues [1996]. In this study of 28 families with at least two cases of breast cancer and at least two cases of ovarian cancer, 25 families carried one of the three common mutations (25/28 ≈ 90%) [Tonin *et al.*, 1996]. However, no screening for other types of *BRCA1/2* mutations was carried out among the three families who tested negative for the common mutations. Although this figure of 90% is very similar to our maximum value derived from the studies by Schubert *et al.* [1997] and Frank *et al.* [1998], it is based on the opposite assumption, i.e., that the remaining three families are true negatives for common mutations.

Dutch

A high proportion of large rearrangements in *BRCA1*, including two founder mutations, was first reported in the Dutch population. According to the original study by Petrij-Bosch *et al.* [1997], the clinical sensitivity for three large *BRCA1* deletions in Dutch HBOC families can be estimated as 8.2 to 38.2%. Papelard and colleagues [2000] report that screening for 35 *BRCA1* mutations using the DSDI (Detection of Small Deletions and Insertions) test allows for the detection of 75% of the Dutch and Belgian mutations, according to a mutation database (given as <http://ruly70.medfac.leidenuniv.nl>, not accessible). Among these 35 mutations, three are the large-size deletions found by Petrij-Bosch *et al.* [1997] and 10 are small insertions or deletions that recur in HBOC families of Dutch and Belgian origin [Peelen *et al.*, 1997]. It is possible that some of these mutations are recurrent rather than founder mutations. Papelard *et al.* [2000] also report on the distribution of *BRCA1* mutations in 370 carrier families identified in eight genetics clinics in the Netherlands. Unfortunately these data are presented according to the number of unique mutations rather than for the total number of mutations identified. The authors point out that the mutation spectrum for *BRCA1* is not complete since genetic screening in the different centers did not provide full coverage of the coding regions of the gene. In 2003, Hogervorst *et al.* reported on a large sample of breast and/or ovarian cancer families, in whom the estimated proportion of large *BRCA1* rearrangements varied from 4.3 to 27.3%. The two founder mutations described by Petrij-Bosch *et al.* [1997] were identified in 28 families out of the 33 carrying large rearrangements.

Finnish

We estimated the clinical sensitivity of testing for common *BRCA1/2* mutations using two studies of Finnish families at high risk for breast or ovarian cancer (Table 21). Huusko *et al.* [1998] studied families from the Oulu region (in the north of Finland) among whom five different mutations were identified; four of these were considered common and yielded clinical

sensitivity estimates of 11.4 to 91% (this particularly wide difference is due to the low proportion of mutation carriers). Vehmanen *et al.* [1997a, b] analysed *BRCA1/2* mutations in families from other regions of Finland (Helsinki region and the southern part of the country). Eight mutations were considered common among the 16 different mutations identified. We calculated several values for the clinical sensitivity of testing for common mutations in these families: (1) figures of 14 to 61.9% for the eight common mutations, and (2) figures of 6.5 to 28.6% for the four common mutations which were reported in the study by Huusko *et al.* [1998]. It is clear that specific testing for the four founder mutations considered by both studies is likely to be more sensitive in Oulu than in Helsinki and more southern regions.

It appears that the frequency of large *BRCA1/2* genomic rearrangements in the Finnish population is much lower than in American, French and Dutch populations, based on our point estimates. In fact, no large rearrangements in the *BRCA1/2* genes were detected among 82 families from a sample of 149 families testing negative after EX/SP screening [Lahti-Domenici *et al.*, 2001]. The authors of this study did not describe how the 82 families were selected so we cannot assess their representativeness.

Hungarian

In HBOC families of Hungarian origin, three *BRCA1* mutations (185delAG, 5382insC and 300T→G) have an important founder effect and are reported to account for as much as 80% of all *BRCA1* mutations identified [van der Looij *et al.*, 2000]. As shown in Table 21, we identified one study with which we could estimate the clinical sensitivity of screening for common mutations among high risk Hungarian families, based on the distribution of *BRCA1* mutations [Ramus *et al.*, 1997]. The figures of 21.4 to 85.7% apply only to the detection of two common mutations (*BRCA1* 185delAG and 5382insC).

French Canadian

Seven common *BRCA* mutations were initially identified in the French Canadian population [Tonin *et al.*, 1998]. More recently two groups have presented or published data that allow for the calculation of clinical sensitivity estimates. Simard and colleagues¹⁰⁵ performed a systematic analysis of EX/SP regions in high-risk French Canadian families and identified 4 recurrent mutations and 11 unique mutations [Simard *et al.*, 2004] (J. Simard¹⁰⁶). The two most frequent mutations, *BRCA1* C4446T and *BRCA2* 8765delAG, are founder mutations in this population. We calculated clinical sensitivity in a subset of 191/256 families wherein testing was performed on a member affected with cancer or an obligate carrier. For the two founder mutations *BRCA1* C4446T and *BRCA2* 8765delAG, the estimated clinical sensitivity values were 22 and 75% and for the four recurrent mutations, they were 24.1 and 82.1% (Table 21). False negative results are unlikely in this study because quite a systematic and extensive EX/SP mutation analysis was performed. No genomic rearrangements were detected by MLPA analysis for the *BRCA1* gene in the 135 families negative after the EX/SP screen, nor for the *BRCA2* gene in 133 of these families (no MLPA analysis in *BRCA2* was done for two families). Southern blot analysis likewise revealed no large rearrangements in a sub-sample of 61 of the families negative after the EX/SP screen (in which 63 individuals were tested). These analyses suggest there are no recurrent *BRCA1/2* genomic rearrangements in the French Canadian population.

The second group, Oros and colleagues [2004], screened for a panel of 20 *BRCA1/2*

mutations¹⁰⁷ in a cohort of high-risk French-Canadian families. Amongst *BRCA1/2* positive families, 15 distinct mutations were identified: 7 mutations were recurrent and 8 mutations were unique. Haplotype analyses suggested a founder effect for 5 mutations (*BRCA1* 4446C→T and 2953del3insC, *BRCA2* 6085G→T, 8765delAG and 3398delAAAAG). Subsequent molecular analyses of the *BRCA1/2* genes were done in some negative families but these did not reveal any new variants. However, the techniques used to detect the *BRCA1/2* mutations were not the same in all families (see Table 21). We calculated two sets of values of clinical sensitivity from these data: one set for the 5 founder *BRCA1/2* mutations, which varied from 36.7 to 83.8%; and one set for the 7 recurrent mutations, which was only slightly higher (39 and 89.2%).

The profile of founder mutations differs somewhat in the research by Simard *et al.* [2004] and Oros *et al.* [2004] although the two most frequent mutations are the same (*BRCA1* 4446C→T and *BRCA2* 8765delAG). The clinical sensitivity estimates for *BRCA1* 4446C→T and *BRCA2* 8765delAG were about 25 to 57% in the study by Oros and colleagues and were 22 to 75% in the study by Simard and colleagues. Of the 3 remaining founder mutations identified by Oros *et al.*, only one mutation, *BRCA1* 2953del3insC, was identified by Simard *et al.* These differences could be due to regional founder effects, but other explanations cannot be excluded at present. The protocols used to analyse mutations and the selection criteria differed between these studies.

Polish

In order to establish the relative contribution of founder and non-founder mutations, Gorski *et al.* [2004a] determined, by EX/SP sequencing, the spectrum of *BRCA1/2* mutations in 200 high

105. Data have been extracted from an oral presentation and a poster presentation, and completed with personal communication with J. Simard (January 28, 2005).

106. 1. Oral presentation "Familial breast/ovarian cancer in the French Canadian founder population", Oncogenetics: Achievements and challenges. *Les 17ièmes Entretiens du Centre Jacques Cartier*, Oct 7-8, 2004, Montréal, Québec, Oct 7-8, 2004. 2. J. Simard, personal communication, January 28, 2005.

107. The 20 mutations had already been identified in the French-Canadian population: *BRCA1* 360C→T, 1081G→A, 1623delTTAAA, 2072insG, 2953delGTAAinsC, 3768insA, 3875delGTCT, 4160delAG, 4184delTCAA, 4446C→T, 5221delTG; *BRCA2* 2558insA, 2816insA, 3034delAAAC, 3398delAAAAG, 3773delTT, 6085G→T, 6503delTT, 7235G→A, 8765delAG.

risk families: 100 families with three or more cases of breast cancer but no ovarian cancer, and 100 families with cases of both breast and ovarian cancer. Based on this study, the clinical sensitivity of testing for three founder mutations in *BRCA1*, 5382insC, C16G, and 4153delA, varied from 55.5 to 86.0% in the Polish population (Table 21). Screening for a larger panel including the six most common mutations would increase the maximal estimate of clinical sensitivity from 86 to 92%.

Greek

The *BRCA1* 5382insC mutation seems to be the most frequent in the Greek population. On the basis of one study on 85 high risk breast and ovarian cancer families, the clinical sensitivity of the common 5382insC mutation was estimated as 8.2 to 50% [Ladopoulos *et al.*, 2002].

8.3.3 Clinical sensitivity of the protein truncation test

The protein truncation test (PTT) permits detection of mutations when the protein is prematurely truncated (reduced in size), as a result of the introduction of a premature 'stop' codon. Point mutations/small rearrangements which lead to a premature stop codon include the nonsense and frameshift type (the latter alters the reading frame of the genetic code); splice site mutations may also lead to a premature stop codon and thus produce truncated proteins [Schubert *et al.*, 1997; Serova *et al.*, 1996]. It is possible to estimate the theoretical clinical sensitivity of PTT for the detection of *BRCA1/2* point mutations by calculating the proportion of point mutations which result in altered protein products. Many studies that have analysed *BRCA1/2* point mutations (by sequencing, for example) report the distribution of distinct mutations observed. However, for our purpose it is necessary to know the distribution of all point mutations identified.

We identified two published studies which fulfilled this criterion and the results are shown in Table 22. The clinical sensitivity estimates for

detection of *BRCA1* point mutations and small rearrangements by PTT vary from 81.5 to 86% according to these authors [Serova *et al.*, 1996; Shattuck-Eidens *et al.*, 1995]. Possible reasons for the variation include differential distribution of mutation types according to populations and types of families (levels of risk) and the use of different testing techniques such that certain types of point mutations/small rearrangements could have been preferentially detected [Serova *et al.*, 1996]. As pointed out previously, these estimates represent theoretical estimates of clinical sensitivity based on mutation distributions (equivalent to maximum values) and were not derived empirically. In practice, PTT may not detect all mutations that should, in principle, lead to truncated proteins. Concerns have in particular been expressed with regard to protein truncating mutations situated at both ends of the large exons (*BRCA1* exon 11 and *BRCA2* exon 11), which include recurrent mutations in some series [Evans *et al.*, 2003]. On the other hand, the above calculations refer to the detection by PTT of *BRCA1* point mutations/small rearrangements only (due to the type of studies used for the estimates), whereas PTT also detects some, but not all, large rearrangements that lead to truncated proteins.

8.4 CONCLUSION

- Clinical validity is a function of the target population, depending both on the ethnic group and on the familial risk factors used to select study samples. Data were extracted from the literature to estimate clinical sensitivity (1) of exon and intron/exon screening techniques (EX/SP screening) for heterogeneous (non-founder) populations, by examining the proportion of HBOC families carrying point mutations and small rearrangements in *BRCA1*, as opposed to large genomic rearrangements and non-coding region mutations; (2) of testing for common mutations in different populations with founder effects (Ashkenazi Jewish, Dutch, Finnish, Hungarian, French Canadian, Polish and Greek), using the distribution of common and non-common *BRCA1/2* mutations; and (3) of the protein truncation test (PTT).

- The reporting of clinical sensitivity based on observed distributions of mutations is specific to the molecular techniques used for mutation detection. For HBOC families from France and USA, for example, our clinical sensitivity calculations indicate that if sequencing is used for *BRCA1* mutation testing, the proportion of clinically important mutations that will be detected will likely vary from 65 to 76.5%. These data are based on a single, small-scale study. A greater range of estimates is obtained from the studies using other EX/SP screening methods.
- If point mutation/small rearrangement and large rearrangement/non-coding region screening were systematically combined, higher level of clinical sensitivity would be reached; however, exhaustive testing for large rearrangements and non-coding region mutations is not routine in all clinical settings. Myriad Genetic Laboratories currently includes specific testing for five large *BRCA1* rearrangements if a comprehensive analysis is requested.¹⁰⁸ A number of laboratories have recently switched to combining DHPLC (to detect point mutations and small rearrangements) with MLPA (to detect large rearrangements), but there were not enough data in the literature we reviewed to examine a combined clinical sensitivity.
- For PTT, the theoretical clinical sensitivity of 81.5-86% is derived from the reported proportion of point mutations/small rearrangements in *BRCA1* which can result in truncated protein products. This value does not account for the large genomic rearrangements that can yield truncated proteins.
- The distribution of *BRCA1/2* mutations can vary from one population to another and can also vary within the same population (regional differences) due to the founder effect of certain mutations. As a consequence, clinical sensitivity of *BRCA1/2* mutation testing also depends on the population being evaluated. For example, the sensitivity of EX/SP screening for *BRCA1* point mutations/small rearrangements based on our point estimates appears to be higher in the heterogeneous groups studied than in the Dutch population in which large genomic rearrangements could contribute up to 38% of all clinically important *BRCA1* mutations in high risk families.
- The clinical validation of *BRCA1/2* mutation testing continues to be incomplete since the distribution of all susceptibility mutations for breast and ovarian cancer is not well documented. As a result, some high risk families thought to be potential carriers remain negative after extensive laboratory testing. We accounted for this lack of knowledge by estimating minimum and maximum values for clinical sensitivity under certain assumptions. As mentioned previously, these estimates cannot be combined across studies due to the great variability in molecular techniques used, in the definition of what constitutes a hereditary breast-ovarian cancer family, and in the ethnic composition of the study samples. We were not able to compute estimates for the proportion of large *BRCA2* genomic rearrangements due to the scarcity of available data at the time of our analysis. Finally, the designs used in the studies we identified involved testing only affected cases of families thought to be carriers, which precludes evaluation of clinical specificity.
- Among the populations with founder effects that we analysed, the AJ and Polish stand out as having the strongest founder effects, such that analyses for a limited number of mutations would detect the majority of mutations. In contrast, in Finland and Quebec for instance, several common founder

108. According to the BRACAnalysis® technical specifications, these include the exon 13 duplication reported by Puget *et al.*, 1999b and Unger *et al.*, 2000; the exon 8 and 9 deletion reported by Unger *et al.*, 2000; and the exon 13 and exon 22 deletions reported by Petrij-Bosch *et al.*, 1997. http://www.myriadtests.com/provider/doc/tech_specs_brac.pdf, consulted January 18, 2005.

mutations have been identified, but their point estimates of prevalence are lower and their distribution could vary by region.

- Given the nature of the data currently available, it is worthwhile pursuing research on clinical validity, in order to increase the precision of clinical sensitivity estimates, for instance. Future studies should, however, use sound methodology and clear selection criteria for the population of interest.

Furthermore, data will be required from subjects that have undergone comprehensive, highly sensitive mutation detection.

Recently, efforts have been made in several countries, such as Germany [Meindl *et al.*, 2002] and Poland [Gorski *et al.*, 2004a], to gather data on a regional basis, in order to document population-specific data on the distribution of *BRCA1/2* mutations and thus produce useful data for health policy.

9. Test impact on risk evaluation and genetic counselling

In the previous chapters, current gaps in knowledge with respect to prevalence and penetrance have been discussed in relation to how they affect the development of guidelines for testing indications and the estimation of clinical validity. The latter are important data to inform the choice of the most appropriate testing strategies for specific populations. We also examined the efforts undertaken to improve individualised risk assessment, including *a priori* cancer risk assessment and pre-test carrier probabilities.

In this chapter we turn to the post-test risk assessment, which relies on penetrance data but is also related to the proper interpretation of test results and to the clinical and analytical validity of the tests. We will discuss how these issues have an impact on the genetic counselling of individuals and their families who opt for *BRCA1/2* mutation testing. First, we describe the essentials of genetic counselling for individuals who consult for the purpose of undergoing *BRCA1/2* mutation testing. Next, we explain the different results that *BRCA1/2* mutation testing can yield and look at the different consequences that these can have on the genetic counselling of the individuals tested and their families. This chapter is not based on a systematic review of the literature. Information has been collected from review papers published since 1994 and two meeting presentations. The placing of this information in context, however, has been informed by our systematic review of material presented in chapters 5 through 8.

9.1 CANCER GENETICS SERVICES

Persons diagnosed with breast (or ovarian) cancer who have a family history of cancer will need a variety of diagnostic, treatment, preventive and follow-up services. In addition, they will need counselling with regard to familial risk, their personal risks, and to the

potential impact of this information on relatives and family dynamics. Their relatives, as well as other persons concerned by their family history of breast and/or ovarian cancer, will similarly require assessment and counselling regarding these risks and the options available to them. Such services have developed either from genetic or from oncology departments, or both, and have been organised in a number of different ways. A variety of professionals are involved in these services, such as geneticists, surgeons, oncologists, gynaecologists, nurses, psychologists, and, where the profession exists, genetic counsellors. The co-ordination of these services is a key feature of quality services.

The literature on cancer genetics service organisation will be reviewed systematically for a subsequent report. The present chapter does not deal with service organisation nor with the delivery of the entire range of services listed above. This chapter deals essentially with the provision of post-test risk assessment and counselling, which is part of so-called ‘genetic counselling.’ Genetic counselling is a communication process during which individuals and members of their families are informed of the nature of the disease, the risk of recurrence, the burden that the disease poses, the risks and benefits of testing, and the significance of the test results. It also includes support services to help the family deal with the repercussions of receiving genetic information [Noorani and McGahan, 2000]. A nondirective approach and the informed consent are some of the basic tenets of genetic counselling.

Given the complexity of this field, genetic counselling services should be provided by health professionals with specific training and who are familiar with the problems associated with *BRCA1/2* mutation testing. Although professional responsibilities may vary from country to country, individuals that provide

counselling services usually have a Masters level degree in genetic counselling, or are geneticists, or both. Of note, in the province of Quebec, nurses are also involved in genetic counselling for *BRCA1/2* mutation testing. In the text that follows, the term “genetic counsellor” will be used to designate the person providing this service.

9.2 GENETIC COUNSELLING IN THE CONTEXT OF *BRCA1/2* GENETIC SUSCEPTIBILITY TESTING

There is no universally accepted practice standard for genetic counselling in the context of genetic susceptibility testing for breast or ovarian cancer [Cummings and Olopade, 1998]. However, a number of expert organizations in the field of genetics and oncology (e.g., National Society of Genetic Counsellors, American Society of Clinical Oncology) have issued recommendations on the protocol to be used for the provision of *BRCA1/2* mutation testing [Barnes-Kedar and Plon, 2002; Fries *et al.*, 2002b; Kutner, 1999]. Genetic counselling for cancer predisposition testing should include at least two sessions if testing is chosen by the patient. The first session is intended to give information about the test and explain its benefits and limitations (pre-test session). The second session should provide the test results and discuss their implications (post-test session) [Barnes-Kedar and Plon, 2002].

9.2.1 Pre-test session

During this session, the genetic counsellor should provide enough information for the individual to make an informed decision regarding *BRCA1/2* mutation testing. The essential elements that should be discussed in order to ensure informed consent are listed in Box 5 [Barnes-Kedar and Plon, 2002; Schneider, 2002; Olopade and Cummings, 2000]. In addition, the genetic counsellor should discuss, with the patient, why he/she wants to undergo *BRCA1/2* genetic testing and his/her readiness to be tested. Furthermore, familial and personal risk assessment should be provided, for which the genetic counsellor estimates the individual's cancer risk and risk of being a *BRCA1/2*

mutation carrier. For these purposes, the genetic counsellor elicits the individual's family history and draws the full pedigree. As discussed previously, statistical models can be used as an adjunct in risk assessment. It is recommended that reported cancer diagnoses in relatives be confirmed by means of pathology reports or medical records in order to establish more precise risk assessment and ensure appropriate genetic testing.

Box 5. Pre-test session

- Aim of the *BRCA1/2* tests
- Mode of transmission of the *BRCA1/2* genes
- Accuracy of the test (i.e., sensitivity and specificity)
- Possible test results and implications for the individual and her/his family
- Clinical management options and limitations
- Psychosocial impact of knowing the test results
- Potential risks of discrimination (e.g., by insurers, employers)
- Alternative options to testing

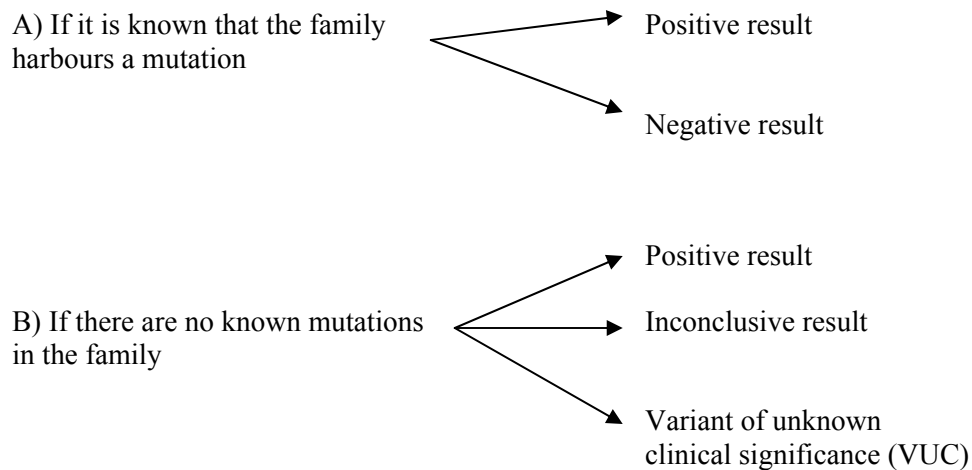
9.2.2 Post-test session

If an individual has decided to proceed with genetic testing for *BRCA1/2*, the primary purpose of this session is to inform the individual of the test results, after due verification that she/he still wishes to know the results. Other items that need to be discussed during the post-test session are listed in Box 6 [Schneider, 2002].

Box 6. Post-test session

- implications of the test results for the patient and his/her family
- management options available to the patient
- adjustment process regarding the results

Figure 3.
Possible *BRCA1/2* mutation test results



9.3 IMPACT OF GENETIC TEST RESULTS ON RISK ASSESSMENT AND GENETIC COUNSELLING

In this section, we discuss the different results that *BRCA1/2* tests can yield and their implications for the genetic counselling of at-risk individuals and their families. The implications depend primarily on whether or not a mutation has been previously identified in the family (Figure 3).

A. If it is known that the family harbours a mutation: If a deleterious *BRCA1/2* mutation has previously been identified in the family (for example, in the index case), there are two possible results in relatives who decide to be tested:

1. Positive result

- Carriers of a familial *BRCA1/2* mutation are at substantially higher risk of developing breast and ovarian cancer than the general

population.¹⁰⁹ However, because the *BRCA1/2* genes have incomplete penetrance and because of the variability in the penetrance estimates in the literature, it is difficult to provide precise estimates of the risk of breast and ovarian cancer associated with *BRCA1/2* mutations. As a result, it is appropriate to indicate a risk interval for breast and ovarian cancer [Ang and Garber, 2001; Carter, 2001; Chang-Claude, 2001]. According to section 5.2, breast cancer risk estimates at age 70 in heterogenous populations vary from 40 to 87% for carriers of a *BRCA1* or *BRCA2* mutation. For ovarian cancer, the estimated risk varies from 36 to 66% for *BRCA1* and from 10 to 27% for *BRCA2*. In the clinical setting, the extent of the family history and ethnicity can modulate the risk estimate [Carter, 2001; Hopper, 2001]. For example, in families where there are multiple cases of early onset breast cancer, estimation of risk would be situated at the higher end of the risk interval. In some

109. *BRCA1/2* mutations are also associated with increased risks of other types of cancer, but these risks are generally low in absolute terms (see section 5.2).

founder populations (e.g., Ashkenazi Jewish), risk figures that are specific to particular mutations can be provided.¹¹⁰ It is currently uncertain to what extent other genetic or environmental factors may also influence this risk.

- The different surveillance options and prophylactic management for breast and ovarian cancer in *BRCA1/2* mutation carriers should be discussed.
- The first-degree relatives of the carrier have a 50% chance of having inherited the mutation and can be tested, if they so desire.

2. Negative result

- Individuals who test negative are not considered carriers of the familial mutation and have a risk comparable to the general population for developing breast or ovarian cancer.
- Their offspring are not at higher risk for being *BRCA1/2* mutation carriers than the general population and do not need to consider genetic testing for the mutation present in their family.

In contrast to the situation described next in case B, the clinical sensitivity of the test is not relevant to the interpretation of a positive or negative test result when a mutation is known to segregate in the family. The analytical sensitivity of the test is, however, relevant in all circumstances, and the possibility of a technical error in carrying out the test or in the interpretation of the test result has to be explicitly discussed before testing and when providing test results.

B. If there is no known mutation in the family: If a person is the first in the family to consult for the purpose of undergoing *BRCA1/2* mutation testing, it is suggested that the molecular analysis be performed initially for a family member with breast or ovarian cancer (index

case), if possible. The analysis of the *BRCA1/2* genes in the index case can yield three possible results:

1. Positive result

- The mutation very likely explains the cancer cases in the family, and the implications for genetic counselling are the same as if the family was known to harbour a mutation.

2. Inconclusive result

No mutation was identified by the standard *BRCA1/2* mutation screening techniques.

- This result can decrease the probability that the individual has a mutation in a known gene but cannot completely rule out this possibility, because of the inherent limitations of *BRCA1/2* mutation testing which translate into suboptimal clinical sensitivity. Indeed a *BRCA1/2* mutation may have been missed by the standard molecular techniques or the family may carry a mutation in another breast cancer susceptibility gene.¹¹¹ The difficulties in gathering reliable clinical sensitivity data have been discussed previously.
- The lack of precise data in part explains why precise post-test probabilities are more difficult to provide to families in this situation. Instead, emphasis is put on the inconclusive nature of the result. To estimate the posterior or post-test probability of carrying a mutation, one needs to take into account not only the clinical sensitivity but also the prior probability. As for other molecular tests, the impact on post-test probability of a test result depends on the prior probability. A negative test result has less impact on post-test probability when the pre-test probability is high. In practice, this means that in multiple-case families (with a

110. W. Foulkes, personal communication, September 4, 2003.

111. As discussed in Sections 5.1 and 8, mutation detection using the standard approaches (screening exons and splice junctions or EX/SP) is not optimal, especially when use is not made of sequencing or a combination of several PCR-based techniques. Screening for *BRCA1/2* mutations not detectable by EX/SP screening, such as genomic rearrangements, are beginning to be integrated in clinical practice. Investigations for the purpose of searching for other *BRCA* genes are usually performed in a research setting, which can take time.

very high prior probability), negative results should not alter the clinical management recommendations [Schneider, 2002].

- It is also possible that the index case's cancer is sporadic, especially if age of onset was above 50 years [Barnes-Kedar and Plon, 2002; Schneider, 2002]. To check if this could be the case, another affected relative with an earlier age of onset can be tested, if available [Schneider, 2002; Peshkin *et al.*, 2001].
- This result is not informative for other family members, and offering genetic testing to relatives is not useful at this stage until more information can be obtained, by testing another affected person in the family, for example. In populations which carry a high prevalence of *BRCA1/2* mutations such as the Ashkenazi Jewish, the spouse's family history of cancer can be checked to improve risk assessment for the offspring [Allweis *et al.*, 2004].

In summary, interpretation of a negative result in the initial person tested in a family depends on the particular circumstances of the person tested (affected with cancer or not) and his/her family history; however, the result is generally considered inconclusive [Barnes-Kedar and Plon, 2002].

2. A variant of unknown clinical significance (VUC)¹¹²

This type of mutation can be a polymorphism of no clinical significance, or a deleterious *BRCA1/2* mutation that is not yet recognized as such [Deffenbaugh *et al.*, 2002].

- The results are not informative for the tested individual, since they do not change the *a priori* estimated cancer risk nor the recommendations relating to cancer screening [Schneider, 2002] and prophylactic methods, such as surgery [Peshkin *et al.*, 2001].

112. These are usually missense mutations that substitutes one amino acid for another. See Section 5.1.5 for more information.

- Without a functional assay to determine the implication of the genetic change, the interpretation of the variant is dependent upon clinical observation,¹¹³ and it can sometimes take months or years before having a clear interpretation [Schneider, 2002]. Presently, studies are being done at the research level to classify many reported VUCs [Abkevich *et al.*, 2004; Goldgar *et al.*, 2004; Mirkovic *et al.*, 2004; Spurdle *et al.*, 2004; Deffenbaugh *et al.*, 2002]. Some groups are attempting to develop a model to classify VUCs which integrates different epidemiologic data, and that could be used in the clinical setting [Goldgar *et al.*, 2004] (S. Tavtigian).¹¹⁴ Considering the difficulties VUCs present for clinicians and genetic counsellors, such research efforts are extremely valuable [Hodgson *et al.*, 2004].
- The results are not informative for the family, and it is not useful to proceed with testing in other family members until the situation is clarified.

9.4 CONCLUSION

- Different results can be obtained after testing for *BRCA1/2* mutations and these have different implications for the genetic counselling of individuals and their families.
- The results of *BRCA1/2* genetic testing are clearer when a deleterious mutation has previously been identified in the family. However, the cancer risk estimate in *BRCA1/2* mutation carriers still involves a great deal of uncertainty and it is recommended to provide a risk interval. The extent of the family history can modulate the

113. A variant is more likely to represent a deleterious mutation if the alteration segregates with the disease in the family; if it has not been observed in women who also have a clearly identified deleterious *BRCA1/2* mutation; and if the substituted amino acid is located in a region that is important for protein function and is preserved in other animal species, such as the mouse [Barnes-Kedar and Plon, 2002; Deffenbaugh *et al.*, 2002; Peshkin *et al.*, 2001].

114. "Classification of missense variants in high-risk cancer susceptibility genes"; oral presentation at the Oncogenetics: Achievements and challenges. Les 17ièmes Entretiens du Centre Jacques Cartier, Montréal, Québec, Oct 7-8, 2004.

risk estimate; it is currently uncertain to what extent other genetic or environmental factors may also influence this risk.

- If a mutation has not been previously identified in the family, interpreting certain results (inconclusive results or the detection of a variant of unknown clinical significance) is a complex task for the genetic counsellor.
- Inconclusive *BRCA1/2* test results may create false reassurance for patients or fail to reduce the individual's anxiety.
- The interpretation of the test result and of its implications in terms of familial risk and

personal management options are not straightforward, in view of the complexity and degree of uncertainty associated with some of these results. Interpretation of test results and estimation of post-test probabilities of cancer development need to consider the pedigree information, the analytical and clinical validity of the technique(s) used, and the penetrance and nature of the detected mutation. Services need to be in place to support patients and families adequately given the amount and complexity of the information that needs to be conveyed. Qualified personnel and ample time have to be dedicated to providing this information and to allow full opportunity for questions and clarifications.

10. Conclusion

HBOC represents the higher end of the spectrum of breast cancer risk related to family history. In the literature, as in clinical practice, the characterization of HBOC remains ill defined. The use of *BRCA1/2* mutation testing has gradually evolved from a restricted use in selected HBOC families to a broader use in families with less severe family history. As one moves from the highest risk to the moderate risk categories the estimated frequency of *BRCA1/2* mutations decreases, from a high point estimate of 84% of families being linked to these genes, to prevalence estimates anywhere between 12.4 and 56.0% in families seen in cancer genetics clinics. According to population-based studies, *BRCA1/2* mutations only explain 16-17% of clustering of breast cancer in families, compared to 55-60% for ovarian cancer. In breast cancer cases unselected for family history and age at onset, mutation prevalence varies from 1.1 to 2.6% for *BRCA1*, depending on the study, and is estimated in one series as 1.1% for *BRCA2*. In ovarian cancer series, mutation prevalence estimates vary from 1.9 to 9.6% for *BRCA1* and from 0.9 to 4.1% for *BRCA2*. In breast cancer cases unselected for family history only, prevalence estimates vary for *BRCA1* from 0.7 to 6.0% and, for *BRCA2*, from 1.3 to 3.9%. A substantial proportion of familial breast cancer cases, in particular, has not yet been linked to any known cancer susceptibility gene and is likely related to other genes with low penetrance, environmental factors, or a combination of both.

Since the discovery of the *BRCA1* and *BRCA2* genes, considerable progress has been made in the understanding of their role in the complex cellular pathways that lead to carcinogenesis, in the description of the spectrum of mutations found in a variety of populations (defined in terms of ethnic origin and other risk factors), and in the assessment of the risk of developing cancer for *BRCA1/2* mutation carriers (penetrance). Unfortunately, research efforts in this very complex field have not been coordinated at an international level. As a result,

combining and making sense of the evidence is particularly challenging. Major problems relate to the variability in the study population selection criteria (related to the lack of a consensual definition of HBOC), in the molecular testing protocols, and in the quality of the study designs and reporting of data. This variability is particularly striking in the literature on prevalence, penetrance and clinical validity. The gaps in current knowledge have implications regarding decision-making for individuals and families, for health care providers and for policy makers.

In 1996, the American Society of Clinical Oncology (ASCO) published guidelines for molecular testing in cancer genetics in general, stating that three conditions have to be fulfilled [ASCO, 1996]. These relate to evidence of

- assessment of risk based on family history,
- the analytical validity of the molecular tests used, and
- the informativeness of test results for the clinical management of persons identified to be at elevated risk of disease.

To address the first condition, which pertains to prior or pre-test risk assessment, existing guidelines for testing indications and statistical risk assessment models were reviewed. Data on mutation prevalence and penetrance were also reviewed to examine the concordance between recommendations for clinical use of tests and the data that should underlie these recommendations. The third condition was also addressed in part, namely the informativeness of test results for post-test risk assessment. The evidence relating to analytical validity, impact on clinical management and effectiveness of clinical management options did not fall within the purview of this assessment.

Most testing guidelines are based on expert opinion or are extrapolated from research study evidence. However, the links between the recommendations and the supporting evidence, i.e., the source and the specific levels of mutation penetrance and prevalence associated with familial and personal risk factors, were not stated. Genetic testing is currently recommended for high risk families only and there is general concordance between the various guidelines as to the most important risk factors used to define high risk families. The identified risk factors are consistent with findings from the literature on penetrance and prevalence. However, this concordance applies to broadly defined risk factors, such as early onset of breast cancer, male breast cancer, bilateral breast cancer, ovarian cancer, multiple affected family members and Ashkenazi Jewish ancestry.

In contrast, little consensus has been reached as to the precise criteria, or the combination thereof, that should guide testing within each of these broad risk factors (such as the age defining 'early onset of breast cancer'). This is not a trivial issue, particularly if some risk factors are used in isolation. Age cut-off definitions, for example, have multiple effects: as shown in chapters 5 and 6, lowering age at diagnosis as an indicator for genetic testing results in a higher *BRCA1/2* mutation prevalence, and thus a higher probability of detecting a mutation among the group of persons potentially eligible. However, fewer persons will meet this testing criterion (since breast cancer diagnosed before age 35 is more rare than before age 49, for example). At the same time, a lower age cut-off will tend to yield a higher clinical sensitivity for any given testing protocol.

Statistical risk assessment models have been designed either to predict risk of breast cancer or to estimate the probability of a *BRCA1/2* mutation explaining the familial aggregation of cancer. The range of risk factors integrated in these models is variable and they are not defined in a consistent way. Although some common predictors of *BRCA* mutation status have been identified in the various models, and although these are by and large in line with prevalence and penetrance data, specific results are still

dependent upon the particular study samples, the testing methods and the precise definitions of variables used. On the whole, these models are reported to have fairly high sensitivity and negative predictive value, but disappointing specificity and positive predictive value. However, the approaches used to compare their performance are sometimes questionable. In addition, most comparative studies have been performed on samples of exclusively high risk families, so that the ability to discriminate between carrier and non-carrier families among borderline low to moderate risk families may not have been assessed accurately. The lack of proper validation and the fact that most models do not apply to all possible familial constellations may contribute to the fact that none of these models has been unanimously adopted in clinical practice. Indeed, these statistical models are currently used as supplements to the genetic counsellor or clinical geneticist's own judgement of risk.

Some of the most recent risk models show that efforts are being made to develop more user-friendly tools for use in risk assessment in tertiary care and as referral guidelines for primary care. Likewise, the most recent guidelines on testing indications have placed more emphasis on criteria for referral to tertiary genetic services or for access to genetic counselling than on precise testing indications, therefore relying more heavily on the role of health care professionals experienced in cancer genetics in determining appropriateness of testing. Such a position may be more coherent with current practices and more realistic at the present time, given the limitations of available prevalence and penetrance data and statistical risk assessment models, and the lack of consensus around precise testing indications. However, it has implications for health care planning and future data collection.

The informativeness of test results in terms of revising the individual's and family's risk assessment primarily depends on the test's analytical and clinical validity. The former refers to the technical performance of the test and this literature has not been reviewed for the purposes of this document. Clinical validity is a function

of the target population, depending both on the ethnic group and on the familial risk factors used to select study samples. In populations with strong founder effects, such as the Ashkenazi Jewish, Icelandic and Polish populations, the testing protocol can be restricted to the search for the common founder mutations. Such testing strategies achieve comparable or even higher clinical sensitivity than the screening of all coding regions in heterogeneous populations. The literature on clinical sensitivity is particularly problematic, because of the variability of the molecular techniques and study selection criteria used and because of the inconsistent testing and/or reporting of results for entire study samples. Only one eligible study systematically used sequencing as a reference for all families. Whereas published data tend to report that testing for point mutations or small rearrangements in coding regions could reveal as many as 70 to 95% of all mutations in heterogeneous populations, and as many as 90% in founder populations such as the Ashkenazi Jewish, a rigorous re-analysis of these data taking the study limitations into account suggests that these figures need to be interpreted with caution.

As far as current practices are concerned, the majority of families considered eligible for testing in cancer genetics clinics (typically families with two or three affected individuals) are not found to carry a *BRCA1/2* mutation. In this situation, the test result is not considered negative but inconclusive, and the residual risk of cancer remains higher than that of the general population. Providing precise post-test probabilities to these families is, however, difficult because good data on negative predictive value are lacking. Another inconclusive test result which represents a challenging task for geneticists and genetic counsellors and does not substantially alleviate a family's anxiety is the discovery of a variant of unknown clinical significance. This situation is relatively frequent, possibly almost as frequent as a positive test finding, but may be resolved over time as new knowledge about these variants accrues. In practice, for the very high risk families, the pre-test (prior) risk will not be

substantially modified by an inconclusive test result.

Testing primarily benefits families in which a *BRCA1/2* mutation has been discovered. For unaffected relatives that undergo testing and are found not to carry the mutation present in the family, breast cancer risk drops from a high prior probability to a post-test risk comparable to that in the general population. This 'demedicalisation' has a major impact on clinical management for such an individual and her/his offspring. Unaffected relatives in whom a mutation is identified, on the other hand, have a substantially higher cancer risk than that of the general population. In general, counsellors will provide a risk interval, to be consistent with published penetrance estimates which have evolved over time as population selection criteria have become less stringent. In heterogeneous populations, breast cancer risk estimates at age 70 vary from 40 to 87% for carriers of a *BRCA1* or *BRCA2* mutation, the higher figure corresponding to families with multiple affected members. For ovarian cancer, risk estimates at age 70 vary from 36 to 66% for *BRCA1* and from 10 to 27% for *BRCA2*. The extent of the family history can modulate the risk estimate. In terms of individual prognosis, precise predictions will likely continue to be difficult to make due to the complex nature of breast cancer. Besides refining risk assessment, a positive test result in an unaffected relative also has an impact on clinical management, be it in terms of surveillance, prevention or prophylactic measures. The effectiveness of these interventions does not fall within the purview of this assessment. In addition, individuals with breast or ovarian cancer in whom a *BRCA1/2* mutation is identified are at increased risk of developing a second cancer. The impact of such a finding on clinical management was not reviewed for the purpose of this document.

Given the complexity of the information that needs to be conveyed before and after testing, and the degree of uncertainty associated with some test results, specialised services need to be in place to adequately support patients and families. Qualified personnel and ample time

have to be dedicated to providing this information and to allow an opportunity for questions and clarifications.

In short, this review has mainly focused on risk assessment based on family history and on the informativeness of test results in relation to the refinement of this risk assessment. As underscored in the ASCO guidelines, the analytical validity of the molecular tests used and the broader informativeness of test results for clinical management are also important factors. Indeed, a comprehensive evaluation of the benefits and risks of molecular testing needs to incorporate a thorough review of the psychosocial implications of testing and of the impact of testing on clinical management. The present monograph, therefore, does not provide a complete picture of the evidence and issues that need to be considered in order to formulate recommendations on the clinical use of *BRCA1/2* molecular tests. The conclusions of recent and ongoing reviews¹¹⁵ that address analytical validity of tests, ethical and psychosocial issues, and impact on clinical management will be taken into account into a follow-up to the present monograph.

In addition, the current document has primarily focused on the literature related to testing within HBOC families. If broader testing indications were envisioned in the future, further analyses would be required to examine clinical validity data relevant for different target populations, for instance. Flexible testing indications and evolving ideas about who would benefit from genetic testing render it more difficult to estimate needs for tests and services, and to foresee costs and resource implications for the

health care system. Setting testing indications involves a trade-off between the proportion of detected *BRCA1/2* carrier families¹¹⁶ and the yield of the testing procedures. These considerations show that unresolved issues and shortcomings in the evidence base have implications for service planning.

Identified gaps in current knowledge also have implications for future research. The NIH Task force on Genetic testing [Holtzman and Watson, 1997] recommended that analytical validity be documented prior to the introduction of molecular tests into clinical practice and that, if data on clinical validity and clinical utility were incomplete at that time, the collection of data regarding these parameters would have to be pursued on an ongoing basis. The current state of knowledge on clinical validity of *BRCA1/2* mutation testing is such that the above recommendation should be followed. Future studies should, however, use sound methodology and clear selection criteria for the population of interest. In particular, efforts should be made to gather data on a regional basis in countries where founder effects are known or suspected, in order to document population-specific distributions of *BRCA1/2* mutations and clinical sensitivity estimates. This evidence would assist in defining optimal testing strategies.

Policy relevant issues will be tackled in more depth in a subsequent AETMIS report¹¹⁷ currently in preparation, which will include a review of organisational modalities of cancer genetics services in different jurisdictions, a review of the impact of models of care delivery on psychosocial consequences of testing, and a comparative cost analysis for different organisational modalities.

115. Foundation for Blood Research (FBR). Family history and *BRCA1/2* testing for identifying women at risk for inherited breast/ovarian cancer (preliminary report) (www.cdc.gov/genomics/gtesting/ACCE/FBR.htm); CCOHTA (Canadian Coordinating Office for Health Technology Assessment). Systematic review of *BRCA1* and *BRCA2* genetic testing for breast and ovarian cancer susceptibility (in preparation); US Preventive Services Task Force/Agency for Healthcare Research and Quality (USPSTF/AHRQ). Genetic risk assessment and *BRCA* mutation testing for breast and ovarian cancer susceptibility. Evidence Synthesis no. 37, 2005 (www.ahrq.gov/clinic/uspstf/uspbrgen.htm).

116. Indeed, a population-based study showed that approximately 36% of breast cancer patients carrying *BRCA1/2* mutations did not have a sufficient family history to qualify for testing.

117. AETMIS. Cancer genetic services: Economic and organisational issues (in preparation).

Appendix A

MOLECULAR TECHNIQUES COMMONLY USED TO DETECT *BRCA1/2* MUTATIONS

Color bar coding of gene on combed DNA. Technique that allows a panoramic view of genes and detects large genomic rearrangements. It combines the “stretching” of the DNA molecule (combing) with the detection of mutations by fluorescence in situ hybridization (FISH). [Gad *et al.*, 2001]

Conformational sensitive gel electrophoresis (CSGE): This method is based on heteroduplex formation between wild type and mutant DNA but differs from other heteroduplex analysis in that the PCR-amplified DNA fragments are denatured in mildly denaturing solvents. Heteroduplex fragments are detected through gel electrophoresis. [Edwards *et al.*, 2001; Blesa and Hernandez-Yago, 2000]

Constant denaturant gel electrophoresis (CDGE) and denaturing gradient gel electrophoresis (DGGE): These two methods are based on the melting characteristics of the double-stranded DNA on gel electrophoresis with constant (CDGE) or gradient (DGGE) denaturing conditions (the two strands begin to melt at the denaturant concentration equal to its melting temperature). Point mutations that alter the melting characteristics can be detected but the techniques require careful design of the PCR primers to ensure that the region of interest will denature first. [Hosking *et al.*, 1998]

Denaturing High Performance Liquid Chromatography (DHPLC): This method is based on the differential retention of homo- and heteroduplex DNA fragments (see HDA method, below). Homo- and heteroduplex DNA fragments are separated on reversed-phase chromatography supports under partial denaturation. Samples showing a different pattern than known homozygous wild-type samples or samples with multiple peaks on DHPLC analysis are assumed to contain sequence variants. [Andrulis *et al.*, 2002; Xiao and Oefner, 2001]

Direct sequence analysis (DSA): For this method, an enzymatic process (Sanger method) or chemical degradation process (Maxam-Gilbert method) is used to determine the exact nucleotide sequence of the DNA fragment. Fragments differing in size by a single nucleotide can be resolved by gel electrophoresis (forming a ladder of bands). Alternatively, the process can be automated, the resulting sequence being read and analysed by a computer. [Andrulis *et al.*, 2002]

Heteroduplex analysis (HDA): This method is based on the differential retention of homo- and heteroduplex DNA fragments. Heteroduplexes can be formed between wild type and mutant DNA during the PCR reaction, after denaturation and slowly cooling, allowing hybridization of the DNA fragments. Heteroduplexes are thought to migrate more slowly than homoduplexes and can be resolved in different gel matrices (using gel electrophoresis or DHPLC). [Andrulis *et al.*, 2002; Iau *et al.*, 2001; Hosking *et al.*, 1998]

Loss of heterozygosity at RNA level (LHR): This method indirectly detects regulatory mutations. LHR is based on the analysis of RNA heterozygosity at one of a number of intragenic polymorphisms

via the cDNA molecule (produced with RT-PCR). RNA loss is inferred from the preferential presence of a sequence corresponding to the normal allele. [Serova *et al.*, 1996]

Multiplex Ligation-dependent Probe Amplification (MLPA): This method relies on sequence-specific probe hybridization to DNA, followed by amplification of the hybridized probes by PCR with universal primers, and semi-quantitative analysis of the resulting PCR products. [Sellner and Taylor, 2004]

Protein Truncation Test (PTT): This method is based on detection of truncated (shortened) proteins. The template is a double-strand DNA fragment synthesised by PCR or RNA amplified by RT-PCR. Protein synthesis is achieved from the PCR template using a coupled transcription-translation *in vitro* system and radio-labelled protein products. Protein products are analysed by gel electrophoresis. [Andrulis *et al.*, 2002; Iau *et al.*, 2001]

Quantitative -fluorescent PCR (QF-PCR): In this method, quantitative information is obtained from a “competitive” PCR approach, in which a control target of known concentration is co-amplified with the unknown target. The concentration of the test sample is inferred by comparison with the control. In semiquantitative-PCR, the concentration of the unknown target is obtained as a ratio to the control. Each target sequence is labelled with a fluorescent probe. [Sellner and Taylor, 2004]

Single-strand conformation analysis/polymorphism (SSCA/SSCP): This method examines the altered structure of a single DNA strand. The PCR-amplified DNA fragments are denatured, and the single strands of DNA are separated through non-denaturing gel electrophoresis. A single base alteration is detected when the folding of the single strand changes sufficiently to alter its electrophoretic mobility. [Andrulis *et al.*, 2002; Hosking *et al.*, 1998]

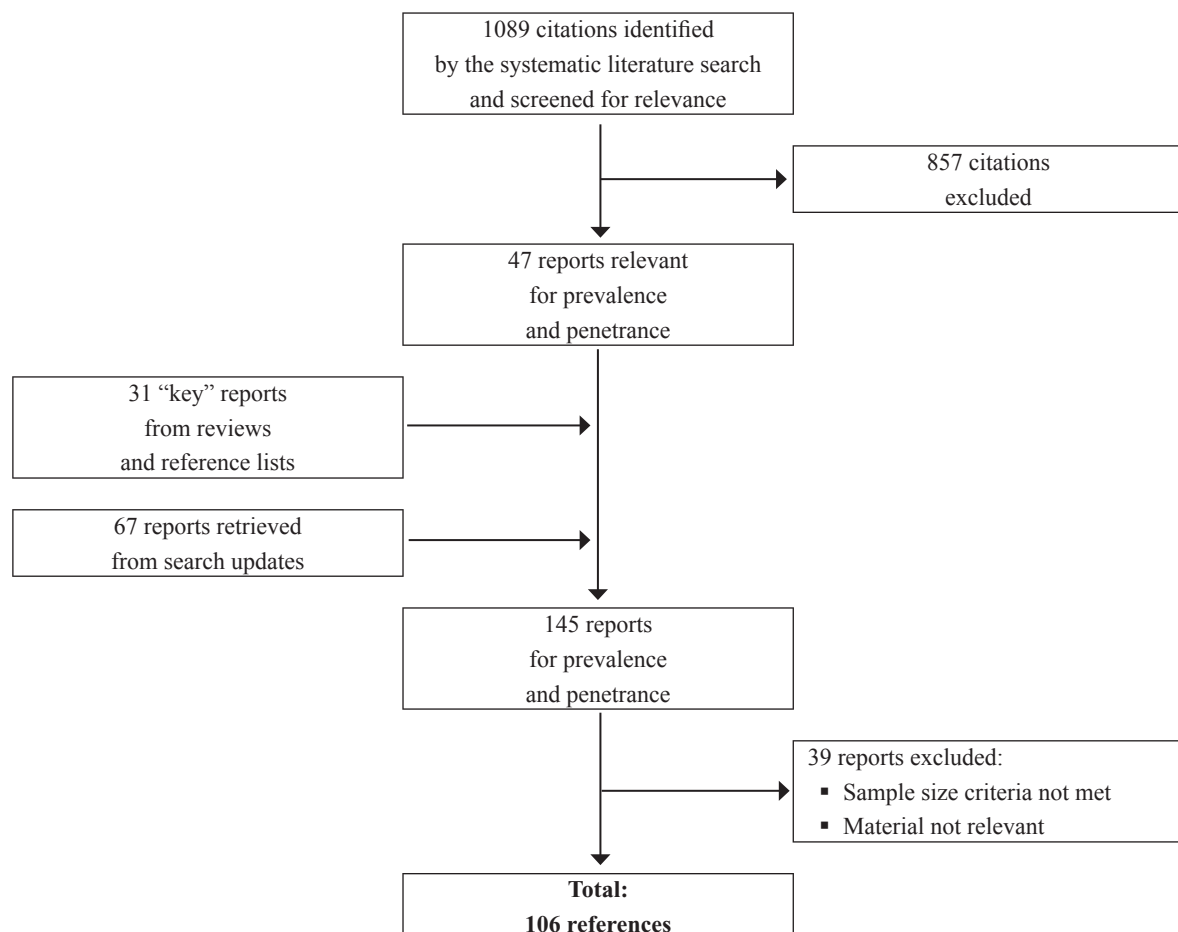
Southern blot: In this method, genomic DNA is cut in fragments by restriction enzymes and the fragments are separated on the basis of size by agarose gel electrophoresis. Specific labelled DNA probes are used to hybridize and detect fragments of interest. [Thompson *et al.*, 1991]

Appendix B

RESULTS OF THE LITERATURE SEARCH STRATEGY

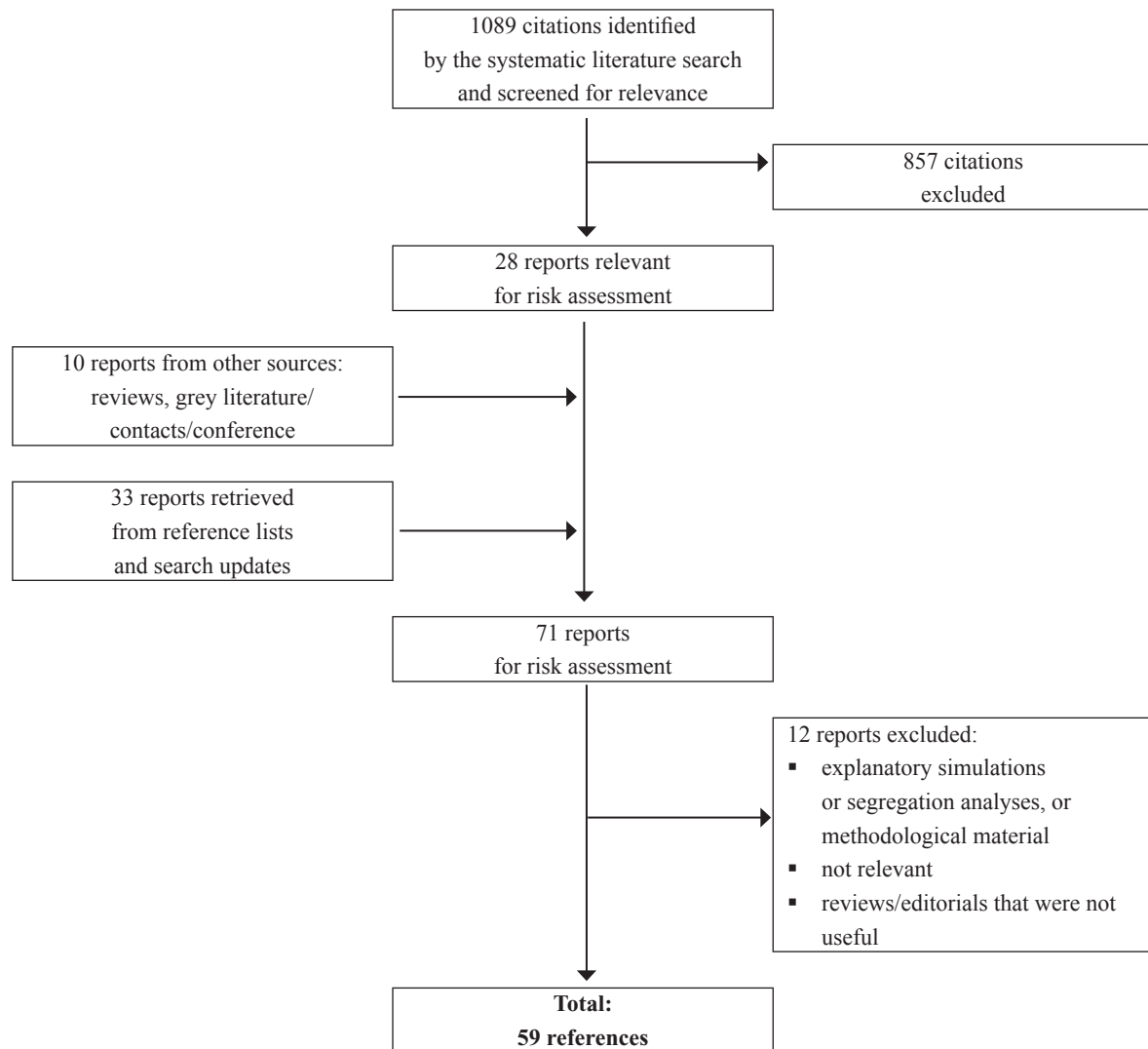
APPENDIX B1

FLOW CHART OF SELECTED MATERIAL FOR PREVALENCE AND PENETRANCE



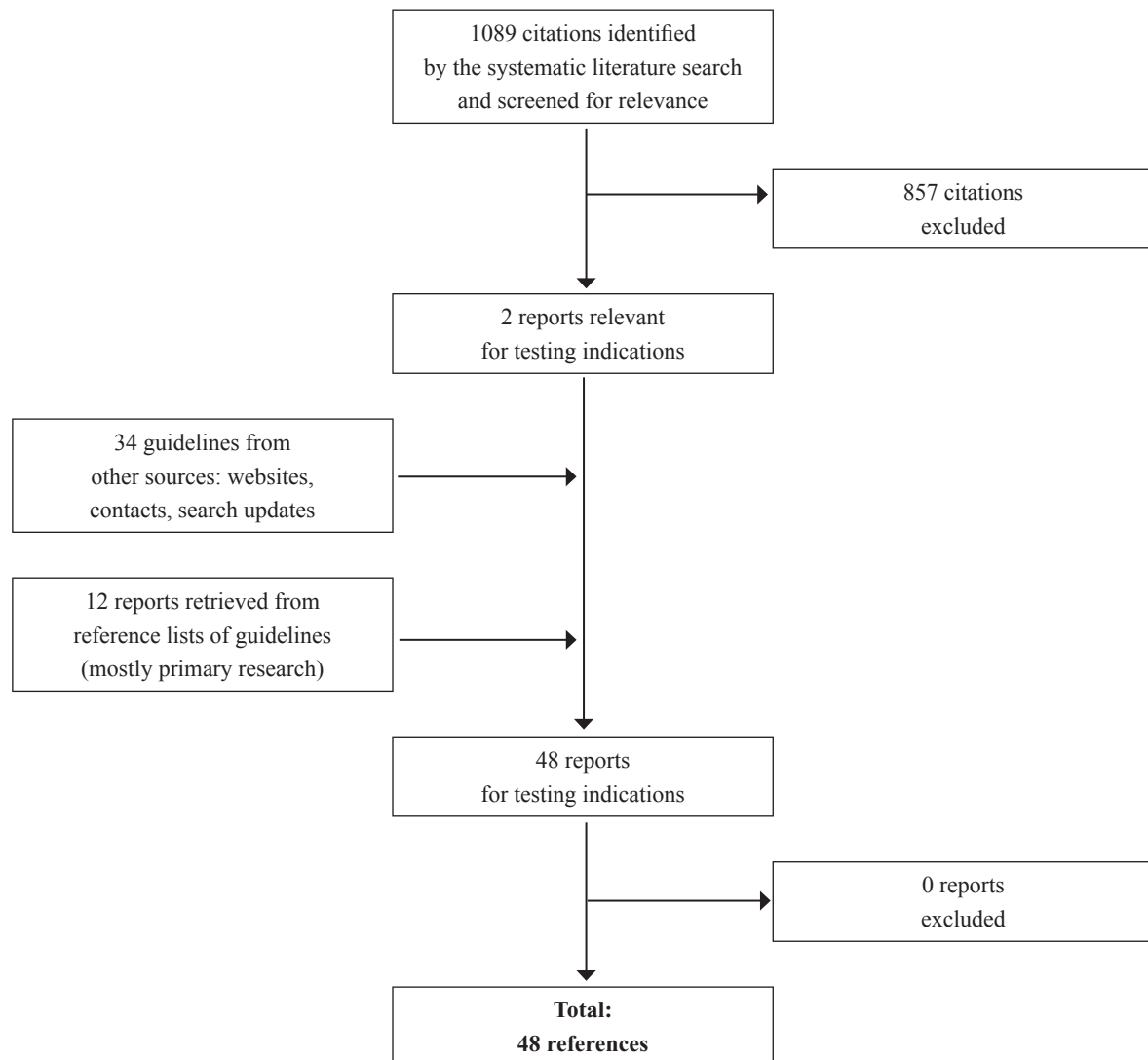
APPENDIX B2

FLOW CHART OF SELECTED MATERIAL FOR RISK ASSESSMENT

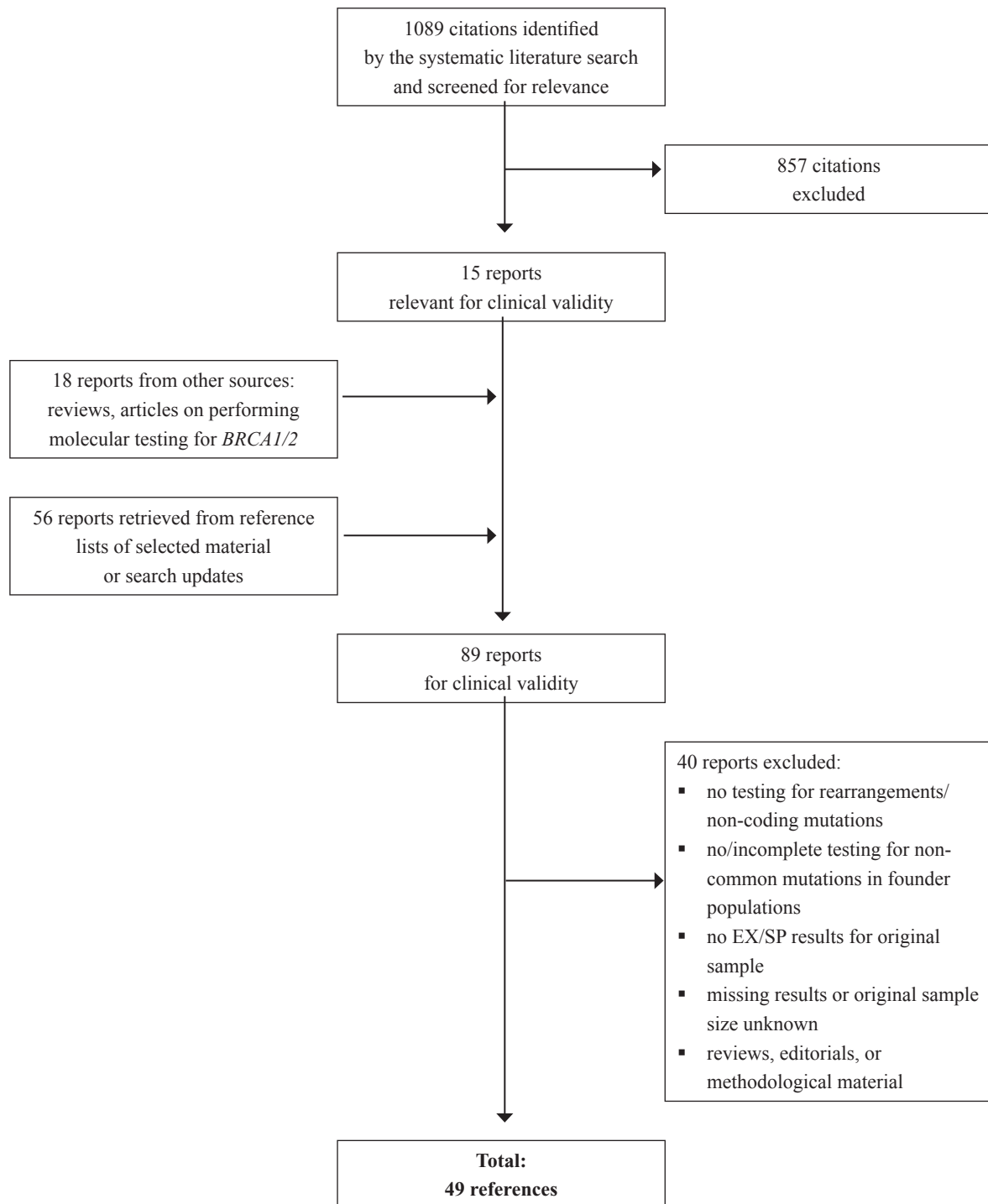


APPENDIX B3

FLOW CHART OF SELECTED MATERIAL FOR TESTING INDICATIONS



APPENDIX B4
FLOW CHART OF SELECTED MATERIAL FOR CLINICAL VALIDITY



Appendix C:

PREVALENCE OF *BRCA1/2* MUTATIONS: STUDY DESIGN AND SUBGROUP ANALYSIS

Table C1.

Prevalence of *BRCA1/2* mutations in individuals selected based on family history (study design and subgroup analyses of linkage and clinic studies, if any)

Note: cells are left blank in Table C1 if there are no applicable results reported by study concerned

REFERENCE	SAMPLE CHARACTERISTICS		
	Mode of ascertainment	Inclusion criteria	Method(s) used to detect mutations
Simard <i>et al.</i>, [1994]	Br and Br/Ov ca Caucasian fam referred to a hereditary cancer clinic for genetic counselling & risk assessment at a university hospital, Montreal, Canada (16 previously studied for linkage)	≥ 3 cases of Br ca and/or Ov ca, except 1 fam with Ov ca only all but 2 fam had early onset Br ca	<i>BRCA1</i> by SEQ except exons 6 and 7 (partially SEQ) and exon 4 (not sequenced)
Shattuch-Eidens <i>et al.</i>, [1995]	samples from patients with Br or Ov ca, mostly from high risk fam (9 laboratories, North America & UK)	heterogeneous (e.g. early onset cases, fam with 2-3 cases)	<i>BRCA1</i> : SEQ or SSCP or SEQ/SSCP/ASO or SSCP/SEQ/HA
Durocher <i>et al.</i>, [1996]	Br/Ov ca fam referred to a hereditary cancer clinic for genetic counselling, risk assessment or mutation analysis at a university hospital in Montreal, Canada	≥ 3 Br ca or Ov ca, dx at any age, or 1 Br ca, dx<40 + 1 Ov ca	<i>BRCA1</i> by SEQ except exon 4 (8 fam); specific detection of <i>BRCA1</i> 5382insC (2 fam); <i>BRCA1</i> by DDF (13 fam); specific detection of <i>BRCA1</i> 185delAG (1 fam)
Phelan <i>et al.</i>, [1996]	Br ca families referred to clinical genetics centres in Montreal and Toronto, Canada	≥ 3 Br ca, ≥ 1 dx<50, no Ov ca	EX/SP screen of <i>BRCA2</i> by SSCA (linkage analysis in 1 fam)
Tonin <i>et al.</i>, [1996]	AJ fam with Br ca for whom a family member presented for testing (in research protocols) at 10 cancer genetics centres in North America	≥ 2 Br ca, ≥ 1 dx ≤ 50 , $\pm$ Ov ca (no male Br ca in fam)	3 <i>BRCA1/2</i> AJ founder mutations screened by DS, SSCA, HDA, ASP or ASO, depending on the centre * <i>BRCA1</i> 185delAG and 5382insC; ** <i>BRCA2</i> 6174delT

PREVALENCE, % (95% CI OR # CASES WITH MUTATION/TOTAL CASES)											
Fam hx BrOv ca		Fam hx Ov ca only		Fam hx Br ca only		Br and Ov ca in a single individual		Presence of male Br ca		Presence of bilateral Br ca	
<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>
44.0 (11/25) Br + Ov ca											
16.7 (1/6) ≥2 Br + ≥2 Ov ca											
					8.9 (4/45) female Br ca only				100 (4/4) male + female Br ca		
68.3* (56/82) ≥2 Br ca + ≥1 Ov ca	4.9** (4/82)			25.4* (35/138)	3.6** (5/138) ≥2 Br ca, 1 dx<50						

Table C1 (continued).

Note: cells are left blank in Table C1 if there are no applicable results reported by study concerned

REFERENCE	SAMPLE CHARACTERISTICS		
	Mode of ascertainment	Inclusion criteria	Method(s) used to detect mutations
Couch <i>et al.</i>, [1997]	women referred to Br ca clinics because of a familial risk factor; samples collected from 1993-95 (USA)	female Br ca + familial risk factor (not specified)	<i>BRCA1</i> by CSGE
Gayther <i>et al.</i>, [1997]	families with multiple cases of Br and/or Ov ca (UK and Ireland)	≥ 3 Br ca or ≥ 2 1 st degree relatives with epithelial Ov ca + DNA available from ≥ 1 affected person	EX/SP screen <i>BRCA1</i> ; if negative, EX/SP screen <i>BRCA2</i> : SSCA/HDA and PTT exon 11 or SSCA/HDA or PTT all exons from RNA (Ov ca fam); SSCP-HAD (Br ca fam)
Stoppa-Lyonnet <i>et al.</i>, [1997]	women at familial ca clinic at Institut Curie (Paris, France), registered between January 1991 and July 1995 all except 3 index cases selected for testing had $\geq 70\%$ prior probability of a <i>BRCA1</i> mutation (BCLC model)	≥ 2 1 st degree cases (≥ 1 Br ca <41 or Ov ca at any age); ≥ 3 1 st or 2 nd degree cases, same lineage (Br or Ov, any age)	<i>BRCA1</i> by DGGE using blood from a case or putative obligate carrier
Vehmanen <i>et al.</i>, [1997a,b]	Br and Br/Ov ca fam predominantly ascertained from Br ca patients with dx <40 or bilat ca (1985-93), or unselected for age at dx (November 10, 1993-April 30, 1995), treated at a hospital oncology department or from other hospitals in Southern Finland	≥ 3 Br or Ov ca in 1 st or 2 nd degree relatives or 2 fam: 1 Br ca + 1 Ov ca	<i>BRCA1/2</i> by HA/SSCP (exons 2-10, 12-24 and exons 2-9, 12-27, respectively); <i>BRCA1</i> exon 11 by PTT; <i>BRCA2</i> exons 10-11 by PTT; EX/SP screen of <i>BRCA1</i> by SEQ in 70 fam; <i>BRCA2</i> exons 2-9, 12-27 by SEQ in 3 -ve fam; haplotype analysis in 12 fam -ve after EX/SP
Ford <i>et al.</i>, [1998]	Br ca fam that were typed by the BCLC group for linkage to <i>BRCA1</i> (multi centre, Europe and North America)	≥ 4 cases, female Br ca dx <60 or male Br ca (any age) $\pm$ Ov ca	linkage analysis for <i>BRCA1/2</i> (n=237 & 177 fam, respectively); <i>BRCA1</i> testing by SEQ, CDGE, CSGE, PTT, DSDI/PTT, SSCP-HA or SSCP (n=180 fam)
Frank <i>et al.</i>, [1998]	probands identified in 12 different familial ca clinics using uniform criteria (USA) one site used a tumour registry to identify women	probands with Br ca dx <50 or Ov ca (any age) + ≥ 1 1 st or 2 nd degree female relatives with either	<i>BRCA1/2</i> by SEQ

PREVALENCE, % (95% CI OR # CASES WITH MUTATION/TOTAL CASES) OR PROPORTION OF LINKED FAMILIES, % (95% CI) FOR FORD ET AL. [1998]											
Fam hx BrOv ca		Fam hx Ov ca only		Fam hx Br ca only		Br and Ov ca in a single individual		Presence of male Br ca		Presence of bilateral Br ca	
<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>
42.2 (19/45) ≥1 Br ca + ≥1 Ov ca				6.4 (8/124) ≥1 Br ca		66.7 (10/15) ≥1 ind with Br and Ov ca				17.5 (10/57) ≥1 bilat Br ca	
40.4 (23/57) ≥1 Ov ca + ≥1 Br ca				14.6 (15/103) ≥2 Br ca, no Ov ca							
25.9 (7/27) 22.2 (6/27) ≥2 cases (Br + Ov ca)				4.1 (3/73) 6.8 (5/73) ≥3 Br ca							
81 (68-91) 14 (5-26) ≥4, Br + Ov, female cases only (n=94)				26 (13-42) 32 (17-50) ≥4, Br ca <60, no male Br ca (n=117)				16 (2-48) 76 (43-97) ≥4, male + female Br ca <60, no Ov (n=26)			
										39.5 (17/43) 11.6 (5/43) ≥1 bilat Br ca	

Table C1 (continued).

Note: cells are left blank in Table C1 if there are no applicable results reported by study concerned

REFERENCE	SAMPLE CHARACTERISTICS		
	Mode of ascertainment	Inclusion criteria	Method(s) used to detect mutations
Tonin <i>et al.</i>, [1998]	fam recruited in 4 cancer centres in Montreal and Toronto, Canada	≥3 cases, Br ca dx<65 or epithelial Ov ca or male Br ca (2 were 1 st , 2 nd or 3 rd degree relatives of the index case)	1. screen for 8 common French Canadian <i>BRCA1/2</i> mutations by PCR-SSCP and PTT (exon 11 <i>BRCA1</i> , exons 10-11 <i>BRCA2</i>); 2. in 14 fam -ve after #1: “complete genomic screen” of <i>BRCA1/2</i> (no other details)
Gayther <i>et al.</i>, [1999]	families with at least 2 Ov ca cases confirmed by medical records identified through a familial Ov ca registry in United Kingdom	≥2 1 st or 2 nd degree relatives with epithelial Ov ca ± Br ca dx<60	1. PTT exon 11 <i>BRCA1</i> and exon 10-11 <i>BRCA2</i> 2. SSCA/HA other exons in <i>BRCA1/2</i> + boundaries of exon 11 <i>BRCA1</i> & exons 10-11 <i>BRCA2</i> exon 13 duplication screen (for fam without coding mutations)
Arver <i>et al.</i>, [2001]	Br or Br/Ov ca fam referred for oncogenetic counselling between September 1997 and March 2000 in Stockholm, Sweden	eligibility criteria for <i>BRCA1/2</i> testing in the clinical setting of Stockholm region, 1997-2000 ¹	<i>BRCA1/2</i> by PTT/SSCP and DHPLC (1 st 100 samples); <i>BRCA1/2</i> by DS (next 58 samples); 3 <i>BRCA1/2</i> AJ founder mutations (2 samples)
Martin <i>et al.</i>, [2001]	fam recruited from 2 Br ca high risk evaluation clinics (USA; Michigan 1993-94 and Pennsylvania, 1994-98)	≥1 Br ca + ≥1 Ov ca (male Br ca included if female Br ca in same lineage)	<i>BRCA1/2</i> by CSGE, HAD or DS

1. Six testing criteria: a) ≥2 affected 1st or 2nd degree relatives, ≥1 Br ca + 1 Ov ca, or 1 single female with Br and Ov ca; b) ≥3 1st or 2nd degree relatives with Br ca, ≥1 dx<51; c) 2 1st or 2nd degree relatives with Br ca, ≥1 dx<41; d) 1 case Br ca, dx<36; e) 2 1st or 2nd degree relatives with Ov ca; f) 1 case of Ov ca, dx≤51.

PREVALENCE, % (95% CI OR # CASES WITH MUTATION/TOTAL CASES)											
Fam hx BrOv ca		Fam hx Ov ca only		Fam hx Br ca only		Br and Ov ca in a single individual		Presence of male Br ca		Presence of bilateral Br ca	
<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>
44.9 (22/49)	10.2 (5/49)			4.2 (2/48)	25.0 (12/48)						
≥3 cases (Br + Ov ca)				≥3 Br ca (could be male), no Ov ca							
51.6 (32/62)	9.7 (6/62)	16.0 (8/50)	4.0 (2/50)								
≥2 Ov ca + 1 Br ca		2 Ov ca									
33.9 (19/56)	0 (0/56)	0 (0/12)	0 (0/12)	6.5 (6/92)	2.2 (2/92)						
≥1 Br ca + ≥1 Ov ca in 1 st or 2 nd degree relatives; 1 individual with Br and Ov ca		≥2 Ov ca; or 1 Ov ca, dx≤51		≥3 Br ca, ≥1 dx<51 or 2 Br ca, ≥1 dx<41, or 1 Br ca<36 (fam may contain male Br ca)							
				11.1 (1/9)	0						
				1 Br ca<36							
45.0 (45/100)	11.0 (11/100)					60.5 (23/38)	13.2 (5/38)				
≥1 Br ca + ≥1 Ov ca						≥1 ind with Br and Ov ca					

Table C1 (continued).

Note: cells are left blank in Table C1 if there are no applicable results reported by study concerned

REFERENCE	SAMPLE CHARACTERISTICS		
	Mode of ascertainment	Inclusion criteria	Method(s) used to detect mutations
Verhoog <i>et al.</i>, [2001]	fam with clustering of Br or Ov ca or a single early onset case of Br ca referred to 2 cancer genetics clinics for counselling and had mutation analysis between January 1994 and January 1999 (the Netherlands)	eligibility criteria for <i>BRCA1/2</i> testing in the clinical setting ²	1. in all fam: <i>BRCA1</i> exon 11 and <i>BRCA2</i> exons 10 and 11 by PTT; <i>BRCA1</i> exon 2 by SSCP; 2 <i>BRCA1</i> founder mutations by ASO; 2 <i>BRCA1</i> founder genomic rearrange-ments by specific PCR analysis 2. for 106 fam: <i>BRCA1</i> remaining exons by SSCP; for 23 fam: complete <i>BRCA2</i> gene by PTT RT-PCR
Meindl <i>et al.</i>, [2002]	individuals with fam hx of ca referred to 11 centres of the German Consortium for HBOC in a 4-year period about 2000 fam received counselling in that time	tested families classified in 6 groups ³	6 centres: <i>BRCA1/2</i> by SEQ 2 centres: <i>BRCA1/2</i> by SEQ except PTT of exon 11 (<i>BRCA1</i>) and exons 10-11 (<i>BRCA2</i>) 3 centres: <i>BRCA1/2</i> by SSCP except PTT of exon 11 (<i>BRCA1</i>) and exons 10-11 (<i>BRCA2</i>) 989 fam tested for <i>BRCA1</i> ; 777 for <i>BRCA2</i> presented figures inferred from reported rounded percentages
Nedelcu <i>et al.</i>, [2002]	women with primary Br or Ov ca in clinical (high risk fam) or research (unselected patients) studies at a health sciences centre in Toronto, Canada at least 1 parent with Italian ancestry	no additional information on the high risk fam or whether age at dx of ca was a criterion in patients unselected for fam hx	variety of methods (not reported) for <i>BRCA1</i> exons 2, 5 11, 16, and 20 (+ for exon 13 duplication) and <i>BRCA2</i> exons 10 and 11(this screening said to cover the majority of mutations previously described for Italian patients); then DS

2. Minimal criteria for testing: a) 1 female Br ca dx<40; b) 1 female with Br and Ov ca; c) 2 1st or 2nd degree relatives with Br ca, 1 dx<45; d) 2 affected 1st or 2nd degree relatives, one with Br ca dx<50 and one with Ov ca; e) 2 1st or 2nd degree relatives with Ov ca; f) 3 1st or 2nd degree relatives with Ov or Br ca; only paternally related 2nd degree relatives were taken into account in c-f.

3. 989 fam classified into 6 groups: a) ≥2 Br ca, ≥2 dx<50; b) ≥1 male Br ca; c) ≥1 Br ca + ≥1 Ov ca; d) ≥2 Br ca, ≥1 dx <50; e) ≥2 Br ca, dx>50; f) 1 female Br ca, dx<35.

PREVALENCE, % (95% CI OR # CASES WITH MUTATION/TOTAL CASES)											
Fam hx BrOv ca		Fam hx Ov ca only		Fam hx Br ca only		Br and Ov ca in a single individual		Presence of male Br ca		Presence of bilateral Br ca	
<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>
46.4 (64/138)	5.8 (8/138)	27.3 (3/11)	9.1 (1/11)	9.1 (31/339)	3.5 (12/339)			33.3 (4/12)	8.3 (1/12)		
≥1 Ov ca and ≥1 Br ca, 1 dx<50; OR case with both Br and Ov ca + ≥1 Br ca in fam		≥2 Ov ca (any age)		≥2 Br ca, no Ov ca				≥1 male Br ca			
				0 (0/29)	0 (0/29)						
				1 Br ca<40							
42 (105/250)	10 (25/250)			15 (104/692)	8.1 (56/692)			2.1 (1/47)	23.4 (11/47)		
≥1 Br ca + ≥1 Ov ca (at any age)				≥2 Br ca, ≥2 dx<50; or ≥2 Br ca, ≥1 dx<50; or ≥2 Br ca, dx>50; or ≥1 Br ca<35 no male Br ca				≥1 male Br ca			
				8.9 (4/45)	4.4 (2/45)						
				≥1 Br ca <35							

Table C1 (continued).

Note: cells are left blank in Table C1 if there are no applicable results reported by study concerned

REFERENCE	SAMPLE CHARACTERISTICS		
	Mode of ascertainment	Inclusion criteria	Method(s) used to detect mutations
Phelan <i>et al.</i>, [2002]	AJ fam with Br and Ov ca referred to 2 hospitals in Toronto, Canada for risk assessment & genetic evaluation	≥2 Br ca and/or Ov ca, any age, in 1 st or 2 nd degree relatives	3 <i>BRCA1/2</i> AJ founder mutations by multiplex PCR + gel electrophoresis
Shih <i>et al.</i>, [2002]	individuals referred to 2 Br ca risk evaluation clinics (USA; Michigan 1993-95 and Pennsylvania, 1995)	proband with Br or Ov ca + ≥1 Br or Ov ca in fam	<i>BRCA1/2</i> by CSGE
Velasco Sampedro <i>et al.</i>, [2002]	men and women with Br and/or Ov ca identified by oncologists, gynaecologists, and surgeons, from hospitals in Castilla y Leon (Spain) considered at moderate to high risk	classified according to study referral criteria ⁴ (for 11/153 fam, unknown hx)	<i>BRCA1/2</i> by CSGE
Diez <i>et al.</i>, [2003]	heterogeneous sample of fam + patients, 7 centers in Spain: ascertained for research, directed screening of case series of female or male breast cancer, or attendance at cancer genetics clinics	research study criteria ⁵	<i>BRCA1/2</i> EX/SP by combination of SSCP, PPT, DGGE, CSGE and DS (techniques varied between and within participating centres)
Menkiszak <i>et al.</i>, [2003]	women with invasive Ov ca + fam hx of Ov or early onset Br ca, referred to 1 of 16 cancer genetics centers in Poland in 1999-2001	invasive Ov ca + ≥1 1 st or 2 nd degree relatives with Ov ca (any age) or Br ca dx≤50	<i>BRCA1</i> 4153delA and 5328insC by multiplex specific PCR assay; <i>BRCA1</i> C61G by restriction enzyme digestion in exon 5
Rostagno <i>et al.</i>, [2003]	consecutive families with histologically confirmed Br ca, seen at oncogenetic department of an anti-cancer center (southeast France), January 1992-December 2000	inclusion criteria for department ⁶	<i>BRCA1</i> by DGGE

4. Referral criteria for the study: a) 1 female Br ca dx<40; b) 2 females with Br ca, dx<50; c) ≥3 females with Br ca (dx at any age); d) 1 Br + 1 Ov ca in same family (or 1 patient with both); e) 1 male Br ca with or without fam hx; f) unknown fam hx.

5. a) ≥1 Br ca, ≥1 dx<50 + ≥1 Ov ca; b) ≥2 Br ca, ≥1 dx<50; c) ≥1 female Br or Ov ca + ≥1 male Br ca (for groups a, b, and c, Br ca cases included affected 1st and 2nd degree relatives); d) 1 female Br ca or 1 female with Br and Ov ca; e) 1 male Br ca

6. a) ≥1 Br ca, dx<30; b) 2 1st degree relatives with Br ca, ≥1 dx≤40; c) 3 1st degree relatives with Br ca, ≥1 dx≤40; d) ≥2 Br ca + ≥1 Ov ca; e) ≥2 Ov ca.

PREVALENCE, % (95% CI OR # CASES WITH MUTATION/TOTAL CASES)											
Fam hx BrOv ca		Fam hx Ov ca only		Fam hx Br ca only		Br and Ov ca in a single individual		Presence of male Br ca		Presence of bilateral Br ca	
<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>
						76.2 (16/21) in proband		0 (0/5) in proband		50.0 (6/12) in proband	
45.4 (20/44) ≥1 Br ca + ≥1 Ov ca	4.5 (2/44)					75.0 (12/16) ≥1 ind with Br and Ov ca	0 (0/16)	12.5 (1/8) ≥1 male Br ca	37.5 (3/8)		
16.7 (1/6) 1 Br + 1 Ov ca (could be 1 patient); group d	16.7 (1/6)			8.9 (4/45) ≥3 Br ca (any age) group c 4.5 (3/66) 1 Br ca<40 group a 0/21 2 Br ca<50 group b	13.3 (6/45)			0 (0/4) 1 male Br ca group e	0 (0/4)		
35.4 (34/96) 52.1 (41.6-62.4) group a	16.7 (16/96)			7.9 (23/292) 15.4 (11.3-19.6) group b 1.5 (3/195) 2.6 (0.8-5.9) group d	7.5 (22/292)	0 (0/7) 14.3 (0.4-57.9)	14.3 (1/7)	0 59.1 (36.4-79.3) group c 0 (0/19) group e	59.1 (13/22)		
32.1 (9/28) Br and Ov ca				12.3 (10/81) Br ca						10.0 (1/10) in proband	

Table C1 (continued).

REFERENCE	SAMPLE CHARACTERISTICS		
	Mode of ascertainment	Inclusion criteria	Method(s) used to detect mutations
Cipollini <i>et al.</i>, [2004]	fam tested in 9 units of the Italian Consortium of HBOC	no details provided	no details provided, except that DS was used at one site
Claes <i>et al.</i>, [2004]	Br and/or Ov ca-prone families visiting medical genetics centre at a university hospital (Belgium)	HBOC ⁷ or FBOC ⁷ (also ascertained patients with ca at young age or of particular types, but no fam hx; results not presented)	<i>BRCA1</i> exon 11 and <i>BRCA2</i> exons 10-11 by PTT (first 85 fam; if -ve: <i>BRCA1</i> EX/SP by HA); <i>BRCA1/2</i> exons 11 by PTT and EX/SP by DGGE (next 203 fam); <i>BRCA1/2</i> exons 11 by SEQ and EX/SP by DGGE (last 62 fam) all fam: 4 recurrent <i>BRCA1</i> genomic rearrangements by PCR; if -ve, MLPA for rearrangements
Oros <i>et al.</i>, [2004]	81 fam recruited in health clinics (part of the Montreal sample of Tonin <i>et al.</i> [1998]) and 88 fam mostly recruited since 1994 in 4 hospital cancer clinics in Montreal, Canada reported grandparental French Canadian ancestry	≥3 confirmed female Br ca dx≤65, male Br ca or epithelial Ov ca in 1 st , 2 nd and 3 rd degree relatives on same branch of family	1. 20 specific <i>BRCA1/2</i> mutations by SSCP; 2. in 83 fam -ve after 1: SEQ in <i>BRCA1</i> or <i>BRCA2</i> or both, or partial analysis by PTT in <i>BRCA1</i> exon 11 and <i>BRCA2</i> exons 10-11
Simard <i>et al.</i>, [2004]	fam recruited in different regions of Quebec (no other details)	≥3 Br and/or Ov ca dx at any age in 1 st degree relatives, or ≥4 1 st or 2 nd degree relatives with Br and/or Ov ca	1. 24 specific <i>BRCA1/2</i> mutations by SEQ; 2. DS of all exons if -ve; 3. MLPA of <i>BRCA1</i> (for 135 fam -ve after EX/SP), MLPA of <i>BRCA2</i> (for 133 -ve fam) and Southern blot of <i>BRCA1/2</i> (for 61 -ve fam)

7. HBOC: ≥3 1st degree relatives with Br and/or Ov ca (or 2nd degree relatives in cases of paternal inheritance) in ≥2 successive generations and ≥1 dx <50; FBOC: ≥2 1st degree relatives with Br and/or Ov ca dx at young age (or 2nd degree relatives, in cases of paternal inheritance) but not meeting HBOC criteria.

nd: not determined; fam: family; hx: history; Br: breast; Ov: ovarian; ca: cancer; -ve: negative; bilat: bilateral; dx: diagnosis

PREVALENCE, % (95% CI OR # CASES WITH MUTATION/TOTAL CASES)											
Fam hx BrOv ca		Fam hx Ov ca only		Fam hx Br ca only		Br and Ov ca in a single individual		Presence of male Br ca		Presence of bilateral Br ca	
<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRCA1</i>	<i>BRCA2</i>
HBOC: 46.4 (13/28) FBOC: 23.1 (9/39) Total: 32.8	HBOC: 28.6 (8/28) FBOC: 0 (0/39) Total: 11.9			HBOC: 17.5 (11/63) FBOC: 8.1 (13/161) Total: 10.7	HBOC: 6.3 (4/63) FBOC: 8.1 (13/161) Total: 7.6			0 (0/12) ≥1 male Br ca in family	41.7 (5/12)		
40.0 (30/75) HBOC fam	18.7 (14/75)			6.4 (6/94) HBC fam	25.5 (24/94)			22.2 (2/9) mixture of HBC and HBOC fam	55.6 (5/9)		

Table C2.

Prevalence of *BRCA1/2* mutations in individuals selected based on family history (statistical analyses)

REFERENCE	ASSOCIATIONS WITH <i>BRCA1/2</i> MUTATION STATUS
Tonin <i>et al.</i>, [1996]	average # Br ca cases per family was the same in <i>BRCA1</i> or 2 carriers and non-carriers (no testing done) presence of Ov ca strongly associated with <i>BRCA1</i> carrier status ($p < 0.001$)
Couch <i>et al.</i>, [1997]	# Br ca cases and presence of bilateral Br ca not significantly associated with <i>BRCA1</i> carrier status <i>multivariate analysis:</i> age at dx < 55 years for Br ca significantly associated with <i>BRCA1</i> carrier status ($p = 0.004$) presence of Ov ca significantly associated with <i>BRCA1</i> carrier status ($p < 0.001$) Br and Ov in a single individual significantly associated with <i>BRCA1</i> carrier status ($p < 0.001$)
Gayther <i>et al.</i>, [1999]	# Br ca cases significantly associated with <i>BRCA1</i> and <i>BRCA2</i> carrier status in BrOv ca families ($p < 0.001$ and $p = 0.05$, respectively) # Ov ca cases significantly associated with <i>BRCA1</i> carrier status ($p < 0.001$), but not with <i>BRCA2</i>
Arver <i>et al.</i>, [2001]	# Ov ca cases significantly associated with <i>BRCA1</i> carrier status in BrOv ca families ($p = 0.006$) # Br ca cases not significantly associated with <i>BRCA1</i> carrier status in Br ca only nor BrOv ca families age at dx of Br ca significantly associated with <i>BRCA1</i> carrier status in Br ca only families ($p = 0.004$), but not in BrOv ca families
Martin <i>et al.</i>, [2001]	<i>multivariate analysis:</i> average age at dx of Br ca significantly associated with <i>BRCA1</i> or 2 carrier status in BrOv ca families ($p < 0.002$) Br and Ov in a single individual significantly associated with <i>BRCA1</i> or 2 carrier status in BrOv ca families ($p < 0.001$)
Shih <i>et al.</i>, [2002]	average age at dx of Br ca significantly associated with <i>BRCA1</i> or 2 carrier status ($p < 0.001$) proportion of families with ≥ 1 Ov ca significantly associated with <i>BRCA1</i> carrier status ($p = 0.001$) proportion of families with ≥ 1 male Br ca significantly associated with <i>BRCA2</i> carrier status ($p = 0.001$) proportion of females in family with Br ca was the same in <i>BRCA1/2</i> carriers and non-carriers

Br: breast; Ov: ovarian; ca: cancer; dx: diagnosis; fam hx: family history

Table C3.

Prevalence of *BRCA1/2* mutations in breast cancer cases (study design and subgroup analyses, if any)

REFERENCE	SAMPLE CHARACTERISTICS				PREVALENCE IN SUBGROUPS, % (95% CI OR # CASES WITH MUTATION / TOTAL # CASES)			STATISTICAL ANALYSES (SELECTED FINDINGS)
	Mode of ascertainment	Setting	Sample size (# eligible)	Method(s) used to detect mutations	Presence of Ov ca (fam hx BrOv; fam hx Ov only; Br and Ov in a single individual)		Age at diagnosis (age dx)	
					BRCA1	BRCA2		
Newman <i>et al.</i> , [1998]	women aged 20-74 with incident, primary, invasive Br ca dx, identified and interviewed between May 1993 and June 1996 at 26 hospitals in the Carolina Breast Cancer Study; oversampled younger & African-American women	population based	211 (1156)	BRCA1: 1.EX-SP screen and 5', 3' non-coding regions by multiplex SSCP; 2. ASO for 8 common European mutations; 3. PTT of exon 11	33.3* (1/3) 22.8† (0.5-45)		1.4* (0-4.1)	weighted mean age at dx of BRCA1 carriers was 51.5, of non-carriers was 55.7 (no testing reported)
	CBCS is a population based case-control study of women with Br ca from 24 counties in central and eastern North Carolina, USA			≥4 cases of Br and Ov ca *SE=0, one individual †any relative with Ov ca			age dx<50 *White women only	
Hopper <i>et al.</i> , [1999]	incident cases of 1 st primary invasive Br ca dx<40 (histologically confirmed), identified through cancer registries of Victoria and New South Wales, Australia, 1992-95 (notification of ca in registry is compulsory)	population based	388 (640)	BRCA1 by PTT (exons 2, 11 and 20), in BRCA2 by PTT (exons 10 and 11)				
Peto <i>et al.</i> , [1999]	Caucasian women with Br ca drawn from 2 sequential case-control studies: 1. regional cancer registries throughout Britain, age dx<36, registered 1982-85 2. cancer registries in South Thames, Oxford, Yorkshire (UK), age dx 36-45, registered 1988-89 lag between interviews in 1982-89 & blood drawn in 1992	population based	617 (1399)	BRCA1/2 by CSGE	45.0* (5/11) 10.9** (6/55) *fam hx: ≥ 2 1 st or 2 nd degree relatives with Br ca<60 or Ov ca **fam hx: ≥ 1 1 st degree relative with Br ca<60 or Ov ca		3.5 (9/254) age dx<36	BRCA1 mutation carriers tend to be dx at younger age than non-carriers (p=0.04); no significant difference for BRCA2 mutation carriers

Note: cells are left blank in Table C3 if there are no applicable results reported by study concerned

Table C3 (continued).

REFERENCE	SAMPLE CHARACTERISTICS				PREVALENCE IN SUBGROUPS, % (95% CI OR # CASES WITH MUTATION / TOTAL # CASES)			STATISTICAL ANALYSES (SELECTED FINDINGS)
	Mode of ascertainment	Setting	Sample size (# eligible)	Method(s) used to detect mutations	Presence of Ov ca (fam hx BrOv; fam hx Ov only; Br and Ov in a single individual)			
					BRCA1	BRCA2	Age at diagnosis (age dx)	
ABC Study Group [2000]	women with Br ca dx<55, January 1, 1991 - June 30, 1996, identified in the Anglian Cancer Registry (UK) and alive on July 1, 1996 GPs indicated whether patients were unfit to take part	population based	1435 (2036)	BRCA1/2 by multiplex HDA	5.1 (3/70 adjusted†)* 39.2 (2/6 adjusted†)** *fam hx: 2 1 st or 2 nd degree relatives with Br or Ov ca **fam hx: 3 1 st or 2 nd degree relatives with Br or Ov ca †adjusted for 15% PCR failure rate (i.e. 85% detection rate)	BRCA1 4.1† (0.5-14.1) age dx <35 †adjusted for 15% PCR failure rate (i.e. 85% detection rate)	BRCA2 8.3† (2.3-19.8)	
Anton-Culver et al., [2000]	all adult female Br ca dx during 1 year (as of March 1, 1994) ascertained through cancer registry of Cancer Surveillance Program (CSP) of Orange County, California, USA cases identified within 6 months of dx	population based	673 (1671)	7 BRCA1 mutations by ASO (common mutations in different populations)	20.0 (3/15) Ov ca only	2.0* (4/196) 4.9** (2/41) *age dx<50 **age dx<40		mean age of onset for BRCA1 mutation carriers (54 ± 14.5) versus non-carriers (58.4 ± 13) not significantly different
Malone et al., [2000]	all Br ca identified through cancer registry of Cancer Surveillance System via 2 case-control studies in Washington state, USA: 1. dx January 1, 1983 - April 30, 1990: White cases born after 1944 (lag between times of interview and drawing blood) 2. dx May 1, 1990 - December 31, 1992: cases dx<45, all races; same 3 counties as #1 criteria for molecular testing: dx<35 (n=203) or dx<45 + FDR with Br ca (n=225)	population based	386 (not reported)	BRCA1/2 by SSCP	24.2 (8/33)* 14.3 (1/7)** *BrOv ca **Ov ca only	(0/33)* (0/7)**	5.9 (3.1-10.1) 3.4 (1.4-7.0) age dx<35 unselected for fam hx	BRCA1/2 mutation prevalence for families with bilateral Br ca (9.4%) similar to that for those without (9.5%)

Note: cells are left blank in Table C3 if there are no applicable results reported by study concerned

Table C3 (continued).

REFERENCE	SAMPLE CHARACTERISTICS				PREVALENCE IN SUBGROUPS, % (95% CI OR # CASES WITH MUTATION / TOTAL # CASES)			STATISTICAL ANALYSES
	Mode of ascertainment	Setting	Sample size (# eligible)	Method(s) used to detect mutations	Presence of Ov ca (fam hx BrOv; fam hx Ov ca only; Br and Ov in a single individual)		Age at diagnosis (age dx dx)	
					BRCA1	BRCA2		
Wolpert <i>et al.</i> , [2000]	all living male Br ca identified at regional cancer centre, Toronto, Canada between April 1996 and November 1998	hospital based	14 (19)	BRCA1 exon 11 by PTT; BRCA2 all exons by SSCP; 3 AJ BRCA1/2 founder mutations in AJ patient	50 (1/2) Br + Ov in fam			
Dite <i>et al.</i> , [2003]	adult women with histologically-confirmed 1st primary Br ca dx<40 living in metropolitan areas of Melbourne and Sydney, Australia, identified through cancer registries: January 1, 1992 - September 30, 1999 (Melbourne); January 1, 1993 - December 31, 1998 (Sydney) before January 1, 1996, only English-speaking women	population based	788 (1208)	BRCA1 exon 11 + BRCA2 exons 10, 11, + 27 by PTT; For 72 of 85 with FDR or SDR with Br/Ov ca: EX/SP screen of BRCA1/2 by SEQ (except exons above); For 91 randomly selected cases (47 with ≥1 FDR or SDR with Br ca): screen of BRCA1 coding region by SEQ; For n=641, BRCA1 exon 13 duplication test				
Suter <i>et al.</i> , [2004]	incident Br ca cases in a nested case control study of >266,000 current and former female textile workers in 519 factories in Shanghai, China (recruited for a clinical trial on Br self-examination), October 1989 - October 1991 selected a higher proportion of cases dx<45	population based	645 (1801)	EX/SP screen of BRCA1 and BRCA2 by SSCP	0.4 (0.01-2.2) 1.6 (0.4-4.0) age dx<45			
Whittemore <i>et al.</i> , [2004]	White non-Hispanic women with invasive Br ca, age dx 20-64, dx January 1, 1995 - September 30, 1998, identified in Northern California component of a Br ca family registry 2-stage sampling; oversampled those with characteristics suggestive of inherited ca	population based	525 (2811)	BRCA1 by two dimensional gene screening	7.6 (3.5-15.9) age dx<35			

fam hx: family history; Br: breast; Ov: ovarian; ca: cancer; dx: diagnosis; SE: standard error; FDR: first degree relative

Note: cells are left blank in Table C3 if there are no applicable results reported by study concerned

Table C4.
Prevalence of *BRCA1/2* mutations in ovarian cancer cases (study design and subgroup analyses, if any)

REFERENCE	SAMPLE CHARACTERISTICS				PREVALENCE IN SUBGROUPS DEFINED BY AGE AT DIAGNOSIS, % (95% CI OR # CASES WITH MUTATION / TOTAL # CASES)	
	Mode of ascertainment	Setting	Sample size (# eligible, if reported)	Method(s) used to detect mutations	<i>BRCA1</i>	<i>BRCA2</i>
Takahashi <i>et al.</i>, [1995, 1996]¹	primary-site epithelial Ov ca tumour tissue obtained at a university hospital and an Ov tissue bank (Pennsylvania and Ohio, USA)	hospital based and tumour tissue bank	115 for <i>BRCA1</i> analysis, 130 for <i>BRCA2</i> analysis	<i>BRCA1/2</i> : EX/SP screening of genomic DNA by SSCP (tumour tissue) non-tumour tissue also collected to confirm mutations as germline		
Stratton <i>et al.</i>, [1997]	consecutive epithelial Ov ca cases (invasive + borderline) dx <70 and treated July 1993 - September 1995 at Royal Marsden Hospital (UK), identified at time of dx (if incident) or during follow-up (if prevalent)	hospital based	374 (482)	<i>BRCA1</i> by PCR-MHA		
Berchuck <i>et al.</i>, [1998]	epithelial Ov ca tumour tissue from women who had surgery at a university hospital, North Carolina (USA) in 1991-96	hospital based	103	<i>BRCA1</i> by DS (tumour tissue) in most cases peripheral blood also collected to confirm mutations as germline	36.8* (7/19) 9.5** (8/84) *age dx≤50 **age dx>50	
Rubin <i>et al.</i>, [1998]¹	primary-site epithelial Ov ca tumour tissue from consecutive patients treated at a university hospital in 1994-96 (Pennsylvania, USA)	hospital based	116	<i>BRCA1/2</i> : EX/SP screening by SSCP (tumour tissue) non-tumour tissue also collected to confirm mutations as germline		
Janezic <i>et al.</i>, [1999]²	consecutive Ov ca cases dx March 1, 1994 - February 28, 1995 and recruited to participate within 2 months of dx through the Cancer Surveillance Program of Orange County (California, USA)	population based	107 (~130)	<i>BRCA1</i> RNA (cDNA) screened for mutations by RNase mismatch cleavage assay		

1. These studies appear to have cases in common (according to sample characteristics).

2. These studies have cases in common.

dx: diagnosis; Ov: ovarian; ca: cancer; fam: family; hx: history

Table C4 (continued).

REFERENCE	SAMPLE CHARACTERISTICS				PREVALENCE IN SUBGROUPS DEFINED BY AGE AT DIAGNOSIS, % (95% CI OR # CASES WITH MUTATION / TOTAL # CASES)	
	Mode of ascertainment	Setting	Sample size (# eligible, if reported)	Method(s) used to detect mutations	<i>BRCA1</i>	<i>BRCA2</i>
Anton-Culver <i>et al.</i>, [2000]²	consecutive adult Ov ca cases identified within 4 weeks of dx, dx in a 2-year period, beginning March 1, 1994, and ascertained through the Cancer Surveillance Program of Orange County (California, USA)	population based	120 (342)	for 107: <i>BRCA1</i> RNA (cDNA) screened by RNase mismatch cleavage assay for 13 cases: 7 <i>BRCA1</i> mutations by ASO (common mutations in different populations)	6.5* (3/46) 1.4** (1/74)	
Risch <i>et al.</i>, [2001]	incident epithelial Ov ca cases (invasive + borderline) dx January 1995 -December 1996 (aged 20-79), identified in Ontario ca registry (Canada)	population based	515 invasive; 134 borderline	screen of 11 common <i>BRCA1/2</i> mutations by PCR-multiplex; <i>BRCA1</i> exon 11 and <i>BRCA2</i> exons 10 and 11 by PTT; in -ve: <i>BRCA1</i> screen by DGGE	*age dx <50 **age dx ≥50	2.2* (5/232) 3.8** (16/417)
Malander <i>et al.</i>, [2004]	women with invasive epithelial Ov ca dx June 1998 - June 2000, identified in ca registry in southern Sweden (registry estimated to include 98% of all cases diagnosed)	population based	161 (292)	<i>BRCA1</i> exon 11 + <i>BRCA2</i> exons 10 and 11 by PTT; <i>BRCA1</i> exon 7 and 10 + <i>BRCA2</i> exon 3 by DS; all other exons by DHPLC		*age dx ≤50 **age dx >50
Whittemore <i>et al.</i>, [2004]	White non-Hispanic women dx with invasive epithelial Ov or epithelial Ov tumours of low malignant potential at age 20-64, March 1 1997 - July 31, 2001, identified in a Ov ca fam registry recruited within 1 month of dx	population based	290 (465)	<i>BRCA1</i> by SSCP, PTT of exon 11	8.3* (1.2-41.3) 18.9** (12.5-27.5) *age dx <35 years; **age dx 35-59	

1. These studies appear to have cases in common (according to sample characteristics).

2. These studies have cases in common.

dx: diagnosis; Ov: ovarian; ca: cancer; fam: family; hx: history

Table C5.
Prevalence of *BRCA1/2* mutations in populations with founder effects (study design)

REFERENCE	SAMPLE CHARACTERISTICS					METHOD(S) USED TO DETECT MUTATIONS
	Mode of ascertainment	Setting	Ethnicity/region	Sample size		
Roa <i>et al.</i>, [1996]	AJ individuals, not selected on the basis of Br or Ov ca, who previously participated in carrier screening for common mutations in genetic diseases sampling design not reported	population based (community volunteers)	AJ / Israel and USA	185delAG: 3108 individuals; 5386insC: 3116 individuals 6174delT: 3085 individuals	3 <i>BRCA1/2</i> AJ founder mutations by semi-automated ASO	
Abeliovich <i>et al.</i>, [1997]	unrelated Jewish women with Br and/or Ov ca referred from a Jerusalem hospital's out-patient oncology clinic and oncogenetic counselling clinic (patients from the latter had fam hx of ca or other risk factors)	clinic based	199 AJ and 44 non-AJ / Israel	243 cases (n=206 of these not selected for age at dx nor fam hx)	3 <i>BRCA1/2</i> AJ founder mutations by multiplex PCR	
Karp <i>et al.</i>, [1997]	women dx with invasive Br ca before age 65 (majority from 1990-95) identified from a tumour registry database at the Jewish General Hospital in Montreal	hospital based	AJ / Canada (self-reported)	149 cases (tumours)	<i>BRCA1</i> 185delAG and 5382insC by AS-PCR (149 tumours); <i>BRCA2</i> 6174delT by SSCP (141 tumours)	
Struewing <i>et al.</i>, [1997]	AJ men and women >20 years recruited from the Washington, DC area (USA) through advertising at 15 sites over a 9-week period	population based (community volunteers)	AJ / USA	5318 individuals	<i>BRCA1</i> 185delAG by ASO; <i>BRCA1</i> 5386insC and <i>BRCA2</i> 6174delT by AS-PCR	
Fodor <i>et al.</i>, [1998]	consecutive series of AJ women with intraductal or infiltrating Br ca who had a biopsy, excision or mastectomy at a New York City cancer centre, 1986-1995	hospital based	AJ / USA (self-reported)	268 cases	3 <i>BRCA1/2</i> AJ founder mutations by ASO	
Robson <i>et al.</i>, [1999]	AJ patients who received Br-conserving treatment at a cancer center for early-stage invasive Br ca and dx from January 1, 1980 to December 3, 1990, identified from a database	hospital based (tumor registry)	AJ / USA (self-reported)	305 cases	3 <i>BRCA1/2</i> AJ founder mutations by allele specific PCR	
Warner <i>et al.</i>, [1999]	all living AJ women with invasive Br ca dx before May 1, 1998 at any of 6 oncology centres in Toronto or Montreal; data collection started in November 1996	hospital based	AJ / Canada	412 cases	3 <i>BRCA1/2</i> AJ founder mutations (<i>BRCA1</i> 185delAG, 5382insC and <i>BRCA2</i> 6174delT) by HA, DS or AS-PCR	

Table C5 (continued).

REFERENCE	SAMPLE CHARACTERISTICS					METHOD(S) USED TO DETECT MUTATIONS
	Mode of ascertainment	Setting	Ethnicity/region	Sample size		
Mostehi <i>et al.</i>, [2000]	1. living AJ women with epithelial Ov ca recruited from 11 medical centres, North America and Israel 2. all incident Ov ca cases of AJ origin identified through the Ontario cancer registry (Canada), dx 1995-6	1. hospital based 2. population based	AJ / North America and Israel	208 cases	<i>BRCA1</i> 185delAG by HA; <i>BRCA1</i> 5382insC by SSCP; <i>BRCA2</i> 6174delT by PTT; <i>BRCA1</i> exon 11 and <i>BRCA2</i> exons 10 and 11 by PTT	
Bahar <i>et al.</i>, [2001]	60% of sample is from individuals who participated in community-based carrier screening for Tay Sachs, aged 15-18 years (no other details provided)	population based	AJ / Australia (self-reported)	1202 individuals	3 <i>BRCA1/2</i> AJ founder mutations by a variant of amplification refractory mutation system PCR	
Menczer <i>et al.</i>, [2003] and Hirsh-Yechezkel <i>et al.</i>, [2003]	incident cases of histologically confirmed Ov or peritoneal ca, dx in Israeli Jewish women March 1, 1994 - June 30, 1999, and identified within an Israel-wide study from all departments of gynaecology also included women who had bilateral oophorectomy for benign or prophylactic reasons; patients who received neoadjuvant chemotherapy excluded	population based	AJ and other Jewish / Israel	68 primary peritoneal ca 896 epithelial Ov ca 40 non-epithelial Ov ca	<i>BRCA1</i> 185delAG and 5382insC and <i>BRCA2</i> 6174delT by multiplex PCR	
Shiri-Sverdlov <i>et al.</i>, [2001]	1. Jewish non-AJ women with confirmed epithelial Ov ca dx in any one of the 22 medical centres in Israel since April 1994 2. Jewish Iraqi persons from various departments and outpatient clinics of the Sheba Medical Center in Israel	1. hospital based 2. hospital based	non-Ashkenazi Jewish / Israel	1. 81 cases 2. 289 individuals	<i>BRCA1</i> Tyr978X by modified restriction enzyme digest using PCR	
Fishman <i>et al.</i>, [2000]	consecutive Jewish Israeli women with epithelial Ov ca dx March 1, 1994 - December 31, 1997, identified within an Israel-wide case-control study	hospital based	AJ and other Jewish / Israel	1240 Ov ca	3 <i>BRCA1/2</i> Jewish founder mutations by modified restriction enzyme assay, ASO and DS	

Table C5 (continued).

REFERENCE	SAMPLE CHARACTERISTICS					METHOD(S) USED TO DETECT MUTATIONS
	Mode of ascertainment	Setting	Ethnicity/region	Sample size		
Johannesdottir <i>et al.</i> , [1996]	1. female Br ca: all available DNA samples from tumour tissue from patients dx 1989-94 2. Ov ca: DNA from consecutive patients in a hospital-based tumour bank controls: randomly selected DNA samples from participants in a national diet survey (n=499)	hospital based	Icelandic	459 Br ca 38 Ov ca	<i>BRCA2</i> 999del5 by PCR and denaturing PAGE (mutation confirmed as germline using normal tissue DNA whenever possible)	
Thorlacius <i>et al.</i> , [1997]	invasive female Br ca, 1/3 of sample dx 1981-83 and 2/3 dx 1989-94, and all male Br ca dx 1955-95 samples obtained from hospitals and University of Iceland pathology department	female Br ca: hospital based; male Br ca: population based	Icelandic	632 female Br ca 30 male Br ca	<i>BRCA2</i> 999del5 by PCR and denaturing polyacrylamide gel electrophoresis	
Rafnar <i>et al.</i> , [2004]	women diagnosed with Ov from 1991 to 2000, identified in Icelandic ca registry (blood samples or paraffin-embedded tissue, if deceased)	population based	Icelandic	253 cases	<i>BRCA1</i> G5193A by PCR and DHPLC; <i>BRCA2</i> 999del5 by PCR and size fractionation	
Syrjakoski <i>et al.</i> , [2000]	consecutive, newly dx Br ca patients treated in oncology departments of the Helsinki University Central Hospital (April 1997 - March 1998) and the Tampere University Hospital (January 1997 - May 1999)	hospital based	Finnish	1035 cases	11 recurrent <i>BRCA1/2</i> mutations (84% of all <i>BRCA1/2</i> mutations detected in HBOC fam) and 8 <i>BRCA1/2</i> mutations unique to Finnish fam, by ASO or RFLP	
Dorum <i>et al.</i> , [1999a]	consecutive Ov ca patients from a defined geographic area (southern Norway) and admitted to a Norwegian hospital in Oslo, May 1993 - January 1996	hospital based	Norwegian	615 cases	<i>BRCA1</i> 1675delA and 1135insA by multiplex PCR-based fragment analysis	
Tonin <i>et al.</i> , [1999]	women with histological dx of Ov ca, identified through gynecological oncology clinics of 2 large Montreal teaching hospitals in 1995-96	hospital based	French Canadian	113 cases	8 recurrent <i>BRCA1/2</i> mutations by SSCP	
Chappuis <i>et al.</i> , [2001]	all incident 1st primary invasive Br ca in females dx July-1996-April 1998 (dx<80) identified from records at a hospital department of pathology, Montreal	hospital based	French Canadian	127 cases	7 recurrent <i>BRCA1/2</i> mutations by SSCP	

Table C5 (continued).

REFERENCE	SAMPLE CHARACTERISTICS				METHOD(S) USED TO DETECT MUTATIONS
	Mode of ascertainment	Setting	Ethnicity/region	Sample size	
Tonin <i>et al.</i>, [2001]	women with 1st primary invasive Br ca dx≤40 and recruited at Br ca clinics in 2 Montreal hospitals and from a Breast cancer survey (1993-2000)	clinic based and population-based survey	French Canadian	61 cases	6 recurrent <i>BRCA1/2</i> mutations by SSCP
Van der Looij <i>et al.</i>, [2000]	consecutive patients with invasive Br ca or epithelial Ov ca ascertained at the National Institute of Oncology in Budapest, Hungary in 1996-98	hospital based	Hungarian	500 Br ca; 90 Ov ca	HDA/SSCP for <i>BRCA1</i> 185delAG, 5382insC, <i>BRCA2</i> 6174delT, 9326insA and SSCP alone for <i>BRCA1</i> 300 T->G
Papelard <i>et al.</i>, [2000]	all primary invasive Br ca patients who were surgically treated at Leiden Academic Hospital (the Netherlands), January 1984-November 1996	hospital based	Dutch	642 cases	Detection of small deletions and insertions method for 35 <i>BRCA1</i> mutations including 3 large deletions by PCR-sequencing
Backe <i>et al.</i>, [1999]	consecutive patients with primary Br ca (unselected for fam hx of Br and Ov ca) from 2 population-based cohorts of Br ca patients, collected between January 1996 and April 1998	population based	German	800 cases	<i>BRCA1</i> 5382insC by mismatch PCR
Menkiszak <i>et al.</i>, [2003]	consecutive patients newly dx January 1999 - December 2001 with invasive Ov ca at 1 of 2 institutions in Szczecin, Poland	hospital based	Polish	364 cases	<i>BRCA1</i> 5328insC + 4153delA by multiplex specific PCR assay; <i>BRCA1</i> C61G by restriction enzyme digestion in exon 5
Gorski <i>et al.</i>, [2004]	1. consecutive patients with Br ca not selected for age at dx (21-80 years) nor fam hx, identified in 8 hospitals throughout Poland, dx 1999-2002 2. patients with Ov ca identified in 2 hospitals, Szczecin 3. controls: 1000 newborn children from throughout Poland and 1000 adults unaffected with ca from family physician practices in Szczecin	1. hospital based 2. hospital based 3. population based	Polish	1. 2012 cases 2. 364 cases 3. 2000 individuals	3 <i>BRCA1</i> Polish founder mutations (methods not specified)

AJ: Ashkenazi Jewish; dx: diagnosis; fam hx: family history; Ov: ovarian; Br: breast; ca: cancer

Appendix D

Table D.
Estimation of breast (Br) and ovarian (Ov) cancer risks in *BRCA1/2* carriers (study design)

REFERENCE / ETHNICITY OR REGION(S)	SAMPLE CHARACTERISTICS				
	Mode of ascertainment	Setting	Inclusion criteria	Sample size ¹	Gene(s) analysed and method(s)
Ford <i>et al.</i>, [1994] / Western Europe and North America	multiple case families collected by research groups (BCLC)	multi centre	≥4 cases, Br ca dx<60 or Ov ca (any age); 90% posterior probability of linkage to <i>BRCA1</i>	33 fam	<i>BRCA1</i> by linkage or pedigree analysis (obligate carriers)
Easton <i>et al.</i>, [1995] / Western Europe and North America	multiple case families collected by research groups (BCLC)	multi centre	≥4 cases, Br ca dx<60 or Ov ca (any age); 90% posterior probability of linkage to <i>BRCA1</i>	33 fam	<i>BRCA1</i> by linkage analysis
Ford <i>et al.</i>, [1998] / Western Europe and North America	multiple case families collected by research groups (BCLC)	multi centre	≥4 cases, Br ca dx <60 or Ov ca (any age) for PN estimation, fam contain <i>BRCA2</i> mutation and D13S267 typing information	32 fam	<i>BRCA2</i> by linkage and mutation analysis; D13S267 marker typing
BCLC [1999] / Europe and North America	multiple case families (heterogeneous definitions) collected by research groups (BCLC)	multi centre	≥2 cases, Br ca dx<60 or Ov ca (any age) (in some centres, more restrictive), ≥1 person carrying a pathologic <i>BRCA2</i> mutation or clear evidence of linkage to <i>BRCA2</i> (LOD score >1)	FDR of 173 carrier cases	<i>BRCA2</i> by mutation analysis (method not specified)
Hopper <i>et al.</i>, [1999] / Australia	incident 1 st primary invasive Br ca histologically-confirmed and dx 1992-95, identified through cancer registries of Victoria and New South Wales	population based	Br ca dx<40	relatives of 18 carrier cases	<i>BRCA1</i> exons 2, 11 + 20 and <i>BRCA2</i> exons 10 + 11 by PTT

1. Sample used to estimate penetrance figures presented in chapter 5.

CALCULATION OF PENETRANCE		QUALITY EVALUATION
Method	Assumption(s)	
lifetime incidence of 2 nd cancer (contralateral Br or Ov ca) in FDR with Br ca who are carriers; excluded Br ca within 3 years of first; censored at date of prophylactic mastectomy or oophorectomy, if any	individuals with Br ca were carriers unless marker typing showed them not to be (if so, excluded) or they had Br ca dx>60 and their carrier status was unknown	some ca dx independently confirmed with records; distinction not made in analysis
indirect analysis by maximization of Lod score over different penetrance functions	ascertainment of each fam made on the basis of # of cancers and age at dx but not type of ca; no heterogeneity between fam	some ca dx independently confirmed with records; also examined allelic heterogeneity
indirect analysis by maximization of Lod score over different penetrance functions (using age-specific incidence rates of Br or Ov ca and gp data); excluded 2 nd primaries and those known not to carry a <i>BRCA2</i> mutation or the linked haplotype	all <i>BRCA2</i> mutations confer the same risks; male Br ca cases likely to be carriers	PN estimates used smaller sample of 32 (out of 237) fam
lifetime incidence of 2 nd cancer (contralateral Br or Ov ca) in FDR with Br ca who are carriers; follow-up commenced at 1 st Br ca or 1960 (whichever later); synchronous bil ca & Ov ca prior to Br ca excluded; censored at date of prophylactic mastectomy or oophorectomy, if any	women dx ≥ 60 for 1 st Br ca and no Ov ca considered unaffected; tested non-carrier cases excluded	not reported if dx of 2 nd Br ca or Ov ca confirmed with records; unclear if analysis included only known carriers (by typing) or also included 'probable carriers'
based on incidence of Br ca in FDRs and SDRs of carrier index cases, by: 1. maximum likelihood (modified segregation analysis) 2. repeated random sampling and Kaplan Meier method excluded non-carrier relatives, those not genetically related to carrier index cases + those of unknown ca status	for 2: all relatives with Br ca were carriers (thus, no phenocopies); carrier status determined by molecular analysis in some relatives, estimated probabilistically in others; reported non-cases assumed unaffected	sought confirmation of all ca dx with records; 2 methods used to calculate PN yielded similar results

Table D (continued).

REFERENCE / ETHNICITY OR REGION(S)	SAMPLE CHARACTERISTICS				
	Mode of ascertainment	Setting	Inclusion criteria	Sample size ¹	Gene(s) analysed and method(s)
ABC Study Group [2000] / UK	female patients dx with Br ca (January 1, 1991 - June 30, 1996) identified in a cancer registry and alive on July 1, 1996	population based	Br ca dx<55, not selected for fam hx	FDR of 24 carrier index cases	<i>BRCA1/2</i> EX/SP screen by MHA
Antoniou <i>et al.</i>, [2000] / UK	women with epithelial Ov ca seen at a London hospital July 1993-September 1995 (mixture of incident and prevalent cases)	hospital based	epithelial Ov ca dx<70 (invasive or borderline), not selected for fam hx	FDR + SDR of 12 carrier index cases	<i>BRCA1</i> EX/SP screen by PCR-MHA
Sutcliffe <i>et al.</i>, [2000] / UK	fam at high risk of Ov ca identified in a national ovarian cancer registry beginning January 1991; follow-up questionnaire about every 2 years total length of follow-up: 2800 person-years for Ov ca risk, 3053 person years for Br ca risk	population based	≥2 confirmed epithelial Ov ca in FDR	54 carrier families for Ov ca risk, 55 for Br ca	<i>BRCA1/2</i> EX/SP screening by combination of PTT (<i>BRCA1</i> exon 11; <i>BRCA2</i> exons 10 + 11) and SSCA/ HA (<i>BRCA1</i> exons 2, 3, 5-10, 12-24; <i>BRCA2</i> exons 2-9, 12-27)
Risch <i>et al.</i>, [2001] / Canada	primary epithelial Ov ca (including borderline) dx January 1995 - December 1996, identified in cancer registry in Ontario	population based	primary epithelial Ov ca, not selected for age at dx nor fam hx (649 cases)	451 FDRs of 60 carrier index cases; 4,378 FDRs of 589 non-carrier index cases	1. screen for 11 common <i>BRCA1/2</i> mutations by PCR-multiplex 2. <i>BRCA1</i> exon 11 + <i>BRCA2</i> exons 10 and 11 by PTT 3. in -ve cases: <i>BRCA1</i> screen by DGGE

1. Sample used to estimate penetrance figures presented in chapter 5.

CALCULATION OF PENETRANCE		QUALITY EVALUATION
Method	Assumption(s)	
based on hx of ca in all FDR and mutation analysis in index case; maximum likelihood of pedigree given phenotypic and genotypic information to estimate RR for Br and Ov ca in carriers versus gp for Br ca risk, done in 3 age groups: 20-39, 40-49, 60-79; Ov risk kept constant over age	non-carriers develop ca at gp incidence rate; it is not clear if all FDR of carrier cases were assumed to be carriers	selection of prevalent cases could have an impact on PN; no pathological confirmation of ca dx in relatives; large CI for PN estimates
based on hx of ca in FDR and SDR of carrier index cases; maximum likelihood of pedigree given phenotype and genotype information to estimate RR for Br and Ov ca in carriers versus gp censored at age of any oophorectomy	non-carriers develop ca at gp incidence rate (England and Wales, 1973–77); probability of developing Br ca independent of Ov ca given genotype at any age; <i>BRCA1</i> gene frequency of 0.001; best fitting model had monotonic RR for Br ca, varying RR for Ov ca	according to original article (Stratton <i>et al.</i> [1997]), cancer dx not confirmed in all relatives; large CI for PN estimates (small # of fam with mutations)
prospective cohort study lifetime incidence of Ov and Br ca in unaffected FDR and SDR of carriers or in affected relatives at risk of developing a 2nd primary ca; calculated RR comparing observed + expected incidence (using rates in England and Wales) and converted to absolute risks using national incidence + all-cause mortality data women at risk of developing Ov ca / Br ca censored at 1st occurrence of Ov, peritoneal or fallopian tube ca / Br ca, or confirmed bil oophorectomy / confirmed/reported bil mastectomy (or death, age 85, or last follow-up)	non-carriers develop ca at gp incidence rate (for England and Wales)	proportion of unaffected relatives that were carriers is not clear, thus estimated risk is not a direct measure of PN all Ov ca dx independently confirmed by records, physician note, or histology only included women with complete follow-up information, so unaffected may be more likely to have been excluded; absolute risk in non-carriers slightly higher than for carriers
1. RR of ca in FDR, according to mutation status of proband, using proportional-hazards regression (ca risk for relatives of non-carrier cases used as baseline) 2. cumulative incidence of ca among relatives from survival function calculated to age 80 years 3. PN for carriers estimated with kin-cohort method	family member at risk until either a dx of Br or Ov ca, death, or age at the time fam hx was recorded	no pathological confirmation of ca in relatives, but PN calculated only in FDR which minimizes recall problems; may underrepresent Ov ca cases with poor prognosis

Table D (continued).

REFERENCE / ETHNICITY OR REGION(S)	SAMPLE CHARACTERISTICS				
	Mode of ascertainment	Setting	Inclusion criteria	Sample size ¹	Gene(s) analysed and method(s)
Brose <i>et al.</i>, [2002] / USA	<i>BRCA1</i> mutation positive Br/Ov ca families seeking Br ca risk counselling at 2 academic cancer risk evaluation clinics: University of Michigan (January 1991-July 1994) and University of Pennsylvania (August 1994-Dec 2001)	clinic based	<i>BRCA1</i> carriers (excluded those with missing information, n=159)	483 carrier relatives of 147 carriers	analysis in coding sequence (exons 1-24) of <i>BRCA1</i> for deleterious mutations (n=316; method not described) or status presumed by pedigree analysis (n=167)
Antoniou <i>et al.</i>, [2003] / multi centre (Canada, Australia, UK, USA, Finland, Hungary, Hong Kong, Iceland, Italy, Poland, Sweden, Israel)	22 studies identified by literature search using Medline and personal contact through BCLC recruitment methods varied widely between studies	hospital and population based	1. <i>BRCA1</i> and/or <i>BRCA2</i> mutation testing of female or male Br ca or invasive epithelial Ov ca cases 2. sampling + mutation analysis independent of fam hx (selection could be by age at dx or ethnicity) 3. enumeration of at least all FDR of identified carriers, with known ages at dx of Br and/or Ov ca and ages at last observation 4. carriers had “pathogenic” mutations	280 FDRs of <i>BRCA1</i> mutation carriers, 218 FDRs of <i>BRCA2</i> mutation carriers	<i>BRCA1</i> and/or <i>BRCA2</i> screening of part or entire coding sequence, or specific <i>BRCA1/2</i> founder mutations, depending on study
Struewing <i>et al.</i>, [1997] / AJ	volunteer AJ men and women >20 years recruited through advertising at 15 sites over a 9-week period in Washington, DC	community based	individuals of AJ origin, not selected for personal nor fam hx of ca (some were ca survivors)	306 FDRs of carrier probands; 13,018 FDRs of non-carrier probands	3 <i>BRCA1/2</i> common AJ mutations by ASO or AS-PCR
Fodor <i>et al.</i>, [1998] / AJ	cases: consecutive series of 298 self-identified AJ women with intraductal or infiltrating Br ca who had a biopsy, excision, or mastectomy at a New York City cancer centre, 1986-1995 controls: AJ men and women referred for prenatal carrier testing in New York metropolitan area	hospital based	cases: not selected for age at dx nor fam hx of ca; amplifiable DNA extracted from archived tissue controls: no personal hx of Br ca	268 cases, 1715 controls	3 <i>BRCA1/2</i> common AJ mutations by ASO

1. Sample used to estimate penetrance figures presented in chapter 5.

CALCULATION OF PENETRANCE		QUALITY EVALUATION
Method	Assumption(s)	
cumulative age-adjusted ca risk in carrier relatives using lifetime data: in each 10-year age interval, proportion of ind dx with ca over total # of ind at risk in that interval this decade method used to account for low # of older ind in the study	some affected ind considered unaffected because age at dx of ca unknown (may have lead to underestimation of PN) mutation carriers identified by direct mutation analysis or presumed carriers based on pedigree	ind without information on current age or age at death excluded; unaffected may be more likely to have been excluded due to incomplete data (excluded sample had a lower PN estimate for Br ca); did not consider prophylactic oophorectomy (may decrease risk); included presumed + tested carriers, not all affecteds, to minimise selection bias
meta-analysis maximum likelihood method, using modified segregation analyses and based on hx of ca in FDR of carriers information on mutation status in relatives incorporated when available; females born before 1890 excluded; ind with no age info or no year of birth censored at age 0 relatives followed from age 20 and censored at age at first ca dx, at death, at last follow-up, or age 70, whichever came first	main analyses based on fitting fixed age-specific incidence rates for carriers; incidences in non-carriers were country- and birth cohort-specific carrier status of most relatives were assigned probalistically	large CIs: low mutation frequency in unselected case series; confirmation of ca dx in relatives was not possible in all studies; results likely to be generally applicable to protein truncating but not missence mutations; mixture of founder and heterogeneous ethnicities; authors tested for heterogeneity of risk by region (in 5 groups), not by centre
comparison of ca hx between female FDR of <i>BRCA1/2</i> carriers versus female FDR of non-carriers; calculated risk of disease among relatives using Kaplan-Meier method self-reports of ca not considered in risk calculations	all FDR of <i>BRCA1/2</i> carrier cases with ca were also carriers	ca dx in relatives depends on recall in probands (no pathological confirmation); no genotyping of FDR; possible over-sampling of persons with fam hx of Br ca and other ca
case-control : risk for a carrier (R_c), calculated with the formula $R_c = C_{BC} \times R_G / F$ where C_{BC} is the proportion of mutation carriers among the patients with Br ca, R_G is the risk of Br ca in the gp (0.125), and F is the carrier frequency in the AJ control sample	lifetime risk of Br ca in AJ non-carriers similar to those of USA as a whole (0.125 by age 85)	further study needed for PN of the 5382insC mutation due to low sample frequency

Table D (continued).

REFERENCE / ETHNICITY OR REGION(S)	SAMPLE CHARACTERISTICS				
	Mode of ascertainment	Setting	Inclusion criteria	Sample size ¹	Gene(s) analysed and method(s)
Warner <i>et al.</i>, [1999] / AJ	cases: all living self-reported AJ women with invasive Br ca dx from November 1996 to May 1998, followed at any of 6 oncology centres in Toronto or Montreal (prevalent cases) controls: 380 healthy Jewish female workers (paid + unpaid), no ca hx, at 1 Canadian & 4 US hospitals & community members, Canada; control patients: 360 non-Jewish women with Br ca from outpatient clinics at several participating hospitals	hospital based	not selected for age at dx nor fam hx of ca controls: no ca hx control patients: Br ca, non-Jewish	128 and 1112 FDRs of carrier & non-carrier cases, 1154 FDRs of controls, 1109 FDRs of control patients	3 <i>BRCA1/2</i> common AJ mutations by HA, direct sequencing or AS-PCR 48 carrier and 364 non-carrier cases
Moslehi <i>et al.</i>, [2000] / AJ	cases: 1. living AJ women with epithelial Ov ca, recruited from gynecology and oncology departments of 11 medical centres in North America and Israel 2. all incident Ov ca dx 1995-96 from an Ontario cancer registry for 1, 2: median time, dx to recruitment=2.2 years controls: Jewish female workers (paid + unpaid) at collaborating hospitals & Jewish female community members in Toronto	hospital based and population based	no selection for age at dx nor fam hx of ca controls: no hx of Br or Ov ca	253 FDRs of 86 carrier index cases, 1130 FDRs of 386 controls	3 <i>BRCA1/2</i> common AJ mutations by HA, SSCP or PTT also tested for non-common mutations by PTT (<i>BRCA1</i> exon 11, <i>BRCA2</i> exons 10 + 11)
Satagopan <i>et al.</i>, [2001] / AJ	archival pathological tissue from 3 incident Br ca series in AJ females: 1. dx 1980-90 at a New York City cancer centre (and received Br-conservation therapy) 2. 1 st primary invasive Br ca dx 1986-95 at the Jewish General Hospital, Montreal (no dx>65) 3. see Fodor <i>et al.</i> , [1998] above controls: unaffected volunteer AJ women recruited in Washington DC area [Struewing <i>et al.</i> , 1997]	cases: hospital based controls: community based	3 case series: incident primary Br ca, self-identified AJ origin (not selected for age at dx nor fam hx) controls: no Br or Ov ca	782 (total, in 3 series), 3434 controls	3 <i>BRCA1/2</i> AJ founder mutations cases: technique depends on series (details in other articles); DNA from tumour tissue analysed in all series controls: direct mutation analysis of blood DNA by ASO or AS-PCR

1. Sample used to estimate penetrance figures presented in chapter 5.

CALCULATION OF PENETRANCE		QUALITY EVALUATION
Method	Assumption(s)	
cumulative risks calculated for female FDR of carrier cases, of non-carrier cases, and of both types of controls based on fam hx of Br ca using life table analysis; PN estimates by kin-cohort method risks calculated separately for probands with dx<50 and probands carrying <i>BRCA1/2</i> mutations	half of the FDR of carriers were also carriers; relatives of probands could carry other <i>BRCA1/2</i> mutations at their frequency of occurrence in the AJ population no within-family association	ca dx in relatives depends on recall in probands (no pathological confirmation); selection of prevalent cases could affect PN estimate
1. case-control approach for Ov only: calculated OR by comparing mutation frequency in 208 AJ Ov ca cases with that in 5318 controls (Struewing <i>et al.</i> [1997] data + used SEER gp risk) 2. fam hx approach for Br and Ov: cumulative risks for female FDRs of carriers compared to female FDRs of controls using actuarial survival method + Cox proportional hazards model; PN estimates by kin-cohort method	method #1, baseline gp lifetime risk used for Ov ca; method #2: half of the FDR of carriers were also carriers relatives of carriers could carry other mutations at their frequency of occurrence in the AJ population	ca dx in relatives depends on recall in probands (no pathological confirmation); small number of cases, thus large CIs (not reported) and less accurate Ov ca risk estimates from method #2 than from method #1
case-control: PN determined by age-specific incidence rates of Br ca for AJ female carriers of <i>BRCA1/2</i> mutations using: 1. age specific prevalence of the 3 mutations in AJ gp using controls; 2. age-specific RR of Br ca in carriers versus controls; 3. gp incidence rate (using SEER data)	overall incidence rates of Br ca in AJ non-carriers similar to those of the SEER-US as a whole	controls not randomly selected: possible selection bias in favour of women with a fam hx of ca; only tumour tissue analysed (could be somatic, non-inheritable mutations); not based on fam hx data; large CI for PN estimates; aggregated data across centres; sensitivity analyses performed using 10 and 20% higher risk for AJ gp

Table D (continued).

REFERENCE / ETHNICITY OR REGION(S)	SAMPLE CHARACTERISTICS				
	Mode of ascertainment	Setting	Inclusion criteria	Sample size ¹	Gene(s) analysed and method(s)
Satagopan <i>et al.</i>, [2002] / AJ	1. consecutive Ov ca cases dx and treated 1986-2000 at a New York City cancer centre 2. invasive Ov ca cases dx 1995-96 from gynecology and oncology departments of 11 medical centres in North America and Israel [Moslehi <i>et al.</i> , 2000] controls: unaffected volunteer AJ women recruited in Washington DC area [Struwing <i>et al.</i> , 1997]	cases: hospital based controls: community based	cases: invasive epithelial Ov ca and self-identified AJ origin (not selected for age at dx nor fam hx), no prior dx of Br ca controls: no Br or Ov ca	382 cases (total, in 2 series), 3434 controls	3 <i>BRCA1/2</i> AJ founder mutations cases: technique depends on series (details in other articles); tumour tissue analysed in #1 controls: direct mutation analysis of blood DNA by ASO or AS-PCR ₂
King <i>et al.</i>, [2003] / AJ	female + male incident invasive Br ca cases, dx September 1996-December 2000 at 12 major cancer centers, New York metropolitan area identified by review of hospital tumour registries or chart review, and referred within 12 months of chart review by oncologists/ surgeons	hospital based	females or males with Br ca, not selected for age at dx nor fam hx, AJ origin	212 carrier relatives of female index cases	3 <i>BRCA1/2</i> AJ founder mutations by automated SEQ (for some deceased relatives, genotype was inferred if no archived tissue specimens available)
Dorum <i>et al.</i>, [1999b] / Norway	1. consecutive Ov ca series from a defined geographic area (in southern Norway) dx May 1993-January 1996 2. fam either self- or MD-referred	population based and clinic based	case series: not selected for age at dx nor fam hx (n=615); fam: Ov ca + 1 FDR (or SDR through a male) with Ov ca, or Br ca dx<60, and/or 1 fam member with both Br ca dx<60 and Ov ca (n=222)	relatives of 30 carrier index cases	<i>BRCA1</i> 1675delA and 1135insA by multiplex PCR-based fragment analysis

1. Sample used to estimate penetrance figures presented in chapter 5.

CALCULATION OF PENETRANCE		QUALITY EVALUATION
Method	Assumption(s)	
case-control: PN determined by age-specific incidence rates of Ov ca for AJ female carriers of <i>BRCA1/2</i> mutations using: 1. age specific prevalence of the 3 mutations in AJ gp using controls; 2. age-specific RR of Ov ca in carriers versus controls; 3. gp incidence rate (using SEER data) adjusting for Ov ca incidence among those with prior Br ca	2 hospital AJ series representative of the gp series of AJ cases; controls representative of the AJ gp; overall incidence rates of Ov ca in AJ non-carriers similar to those of the SEER-US as a whole	controls were not randomly selected: possible selection bias in favour of women with a fam hx of ca; tumour tissue analysed in one series; not based on fam hx data; large CI for PN estimates; sensitivity analyses performed using 10% higher risk for AJ gp
cumulative risks by age for Br and Ov ca in <i>BRCA</i> female carrier relatives of carrier Br cases by Kaplan-Meier method only female relatives with confirmed genotypes were included (212 carriers > 30 years); all probands excluded	hospital AJ series representative of the gp series of AJ cases	all cancer dx confirmed by records; evaluated possibility of biased ascertainment of probands with more severe ca hx; genotyped relatives to rule out phenocopies; excluding those with unknown carrier status may mean fewer unaffected carriers were included, and overestimation of PN
Kaplan-Meier analysis done 3 ways using ca hx among relatives: 1. including only carriers (demonstrated, obligate or carrier by state) 2. including all unaffected sisters of unknown mutation status as carriers 3. intermediate between 1 and 2, based on probabilities that untested unaffected sisters are carriers; excluded 1 index case per family	mutation carriers identified by direct mutation analysis or presumed carriers due to affected state or identified as unaffected obligate carriers	authors consider PN estimates at >50 years to be possibly influenced by presence of phenocopies + small numbers; age at death used when age at dx not available; authors examined role of censoring & competing risks of Br and Ov ca: results very sensitive to these

Table D (continued).

REFERENCE / ETHNICITY OR REGION(S)	SAMPLE CHARACTERISTICS				
	Mode of ascertainment	Setting	Inclusion criteria	Sample size ¹	Gene(s) analysed and method(s)
Heimdal <i>et al.</i>, [2003] / Norway	1. consecutive Ov and Br ca patients not selected for fam hx from southern (dx from May 1993 to January 1996) and middle (dx 1999) regions of Norway 2. fam followed in several ca clinics in Norway according to published or European consensus (the latter since 1999) criteria	population based and clinic based	female FDRs (>20 y as of April 1, 2000) of 99 index cases who carried 1 of the 4 <i>BRCA1</i> Norwegian founder mutations	962 FDR of 99 carrier index cases	4 <i>BRCA1</i> founder mutations (1675delA, 1135insA, 816delGT, 3347delAG) using a PCR-based method
Thorlacius <i>et al.</i> [1998] / Iceland	1. female Br ca (dx dates not specified) identified in national ca registry and tissue samples randomly collected over 2 time periods 2. all male Br ca (dx 1955–96), identified in national ca registry	population based	female tissue samples randomly collected and not selected for fam hx of ca (n=541); tissue samples from all male Br ca (n=34)	252 FDRs of 56 carrier cases; 2,156 FDRs of 485 non-carrier cases	<i>BRCA2</i> 999del5 mutation by PCR amplification and polyacrylamide gel electrophoresis
Loman <i>et al.</i>, [2003] / Sweden	Br ca cases dx ≤40, not selected for fam hx, dx in 1995 in the south Swedish health care region	population based	index cases with a <i>BRCA1/2</i> mutation, relatives identified using census registries and personal and fam hx of ca obtained from ca registries	82 FDRs of 22 carrier index cases	<i>BRCA1/2</i> EX/SP by SSCP (1 st half of samples) and DHPLC
Durocher <i>et al.</i>, [2004] / French Canadian	families recruited from different regions of Quebec for research purposes	research centre based	1. fam with 3 Br and/or Ov ca cases dx at an early age in FDRs 2. fam with 4 Br and/or Ov ca cases in FDRs or SDRs 3. fam with a <i>BRCA1/2</i> mutation fam included if an initial mutation carrier was identified + >1 other relative was subsequently tested	25 <i>BRCA1</i> mutation positive fam; 27 <i>BRCA2</i> mutation positive fam	<i>BRCA1/2</i> EX/SP by specific mutation screen or by direct SEQ

1. Sample used to estimate penetrance figures presented in chapter 5.

Br: breast; Ov: ovarian; ca: cancer; pop: population; dx: diagnosis; fam hx: family history; FDR: first degree relative; SDR: second degree relative; BCLC: Breast Cancer Linkage Consortium; PN: penetrance; y: years; NA: North America; RR: relative risk; F/U: follow-up; bil: bilateral; gp: general population; ind: individual

CALCULATION OF PENETRANCE		QUALITY EVALUATION
Method	Assumption(s)	
Kaplan-Meier analysis done 3 ways using ca hx among female FDR of carriers: 1. including only carriers (demonstrated, obligate or carrier by state) 2. including all relatives of unknown mutation status as carriers 3. intermediate between 1 and 2, based on probabilities that untested FDRs are carriers; mothers excluded if pedigree analysis or mutation testing indicated that the father might have been the carrier; index cases excluded from analysis	deceased affected with Br and/or Ov ca designated 'carriers by state'; in the pedigree, unaffected women located between demonstrated carriers and carriers by state considered obligate carriers among untested unaffected persons, same proportion of carriers present as among the tested unaffected	reported ca dx verified using records or ca registry when possible; attempted to test as many fam members as possible to determine mutation-carrying lineage; 'carrier by state' assumption overestimates mutation frequency; did not consider prophylactic oophorectomy
comparison of ca hx among female FDRs of carriers and non-carriers; cumulative incidence of Br ca in each age group of FDRs estimated using Kaplan-Meier method	mutant allele frequency set at 0.25%	the 541 females with Br ca did not differ in terms of incidence, age at onset, or fam hx from the ca series in the registry (all ca in Iceland since 1955; all Br ca since 1910)
cumulative Br ca incidence calculated for FDR of index cases with identified <i>BRCA1/2</i> mutation index cases excluded; testing performed in FDRs not uniform, thus carrier status data not included in analysis	relied on quality of ca registries for fam hx	risks calculated without knowing FDRs true carrier status; all individuals and all dx included in the estimation verified using independent registries (unverified dx excluded)
maximum likelihood (modified segregation analysis) to estimate risks in carriers compared to gp		lack of information for evaluation

Appendix E

STATISTICAL MODELS FOR RISK ASSESSMENT: STUDY DESIGNS

Table E1.

Development and preliminary testing of mutation risk models among Ashkenazi Jewish persons (since 1999): study designs

REFERENCE	STUDY SAMPLE	N / % CARRIERS	MOLECULAR METHODS	STATISTICAL METHODS
Hodgson <i>et al.</i>, [1999] <i>UK</i>	Population-based; women with Br and/or Ov ca; 169 had Br ca; 13 Ov ca; 2 both	184 / 15.2	HDA for 4 founder mutations	Variables: 1° & 2° family hx, age at dx, ca site; log reg with factors significant in bivariate analysis
Hartge <i>et al.</i>, [1999] <i>USA</i>	Population-based; women (70.4%) and men (sample of Struewing <i>et al.</i> , [1997]); 20% had ≥ 1 relative with Br ca; 8% of women had Br ca; 3% of men had prostate ca	5318 / 2.3	PCR-based assays for 3 founder mutations	Variables: personal hx, 1° family hx, decade of age at dx, ca site; classification and regression tree regression
Hopper and Jenkins [1999]	Re-analysis of Hartge [1999] data	see Hartge <i>et al.</i> , [1999]		Multiple log reg (nested models tested using likelihood ratio), considering # of 1° affected relatives, age at dx/ testing
Foulkes <i>et al.</i>, [1999]	Re-analysis of Hartge [1999] data; (results confirmed with 412 prevalent Br ca cases in Toronto and Montreal, Canada)	see Hartge <i>et al.</i> , [1999]		Stratified analysis (by decade) for affected and unaffected women; Mantel-Haenszel odd ratios
Apicella <i>et al.</i>, [2003] <i>UK (Hodgson 1999 sample) & Australia</i>	Population-based; UK: 184 women with Br and/or Ov ca Australia: 240 women either with Br or Ov ca, or a family hx of Br or Ov ca (1° or 2°) 250 had Br ca; 22 Ov ca; 4 both; 148 no ca	424 / 15.1	UK: HDA for 3 founder mutations; Australia: seq of exons 2 & 20 (<i>BRCA1</i>), seq of part of exon 11 (<i>BRCA2</i>)	Variables: 1° & 2° family hx, age at dx, ca site; log reg (likelihood ratio test) with same age groups as Hartge <i>et al.</i> ; internal goodness of fit tested for model using study data

Br=breast; Ov=ovarian; ca=cancer; hx=history; dx=diagnosis; log reg=logistic regression; seq=sequencing

Table E2.

Development and preliminary testing of mutation risk models among non-Ashkenazi Jewish persons (since 1999): study designs

REFERENCE	STUDY SAMPLE	N / % CARRIERS	MOLECULAR METHODS	STATISTICAL METHODS
Ligtenberg <i>et al.</i>, [1999] <i>the Netherlands</i>	Clinic-based; self- or MD-referred; ≥ 3 1°, 2° or 3° relatives with Br or Ov ca; 79 Br ca; 25 both	104 / 29.8	PTT + single base seq for <i>185delAG</i> + PCR analysis of exons 13 & 22 for frequent deletions	Variables: family hx (1°–3°), age at dx, ca site; log reg
Vahteristo <i>et al.</i>, [2001] <i>Finland</i>	Clinic-based; ≥ 3 1° or 2° relatives with Br or Ov ca, identified through patient interviews	148 / 19.6	n=95: screen of coding seq + EX/IN; n=53: ASO/RFLP for 19 mutations + PTT for n=36 at higher risk	Variables: family hx (1°, 2°), age at dx, ca site; log reg with factors significant in bivariate analysis
de la Hoya <i>et al.</i>, [2002] <i>Spain & Portugal</i>	Clinic-based; cases from 94 Spanish (2 sites) + 8 Portugese families; ≥ 3 1° or 2° relatives with Br or Ov ca, ≥ 1 dx <50; 62 Br ca; 1 Ov ca; 39 both; 5 with male Br ca	102 / 30.4	coding seq, EX/IN; for n=48 (S) + 8 (P): DGGE + f-CSGE; for n=46 (S): SSCP + PTT	Variables: family hx (1°–3°), age at dx, ca site; log reg with factors significant in bivariate analysis
Arver <i>et al.</i>, [2001] <i>Stockholm, Sweden</i>	Clinic-based, consecutive persons; families referred ¹ for testing, with cases of Br or Ov ca + case or carrier tested; 92 Br ca; 12 Ov ca; 56 both	160 / 16.9	n=first 100: SSCP + selective PTT & DHPLC; n=58: seq; n=2 AJ: 3 founder mutations (seq or RFLP)	No multivariate analysis; Variables: family hx (1°, 2°), age at dx, ca site; Mann Whitney U test for median # ca cases/ family & age at dx
Frank <i>et al.</i>, [2002] <i>Myriad Genetics</i>	Laboratory-based, consecutive persons; 91% women; 30% AJ; referral based on personal & family hx; 4679 Br ca; 584 Ov ca; 240 both; 76 men with Br ca	10,000 / 15.1 & 23.7 (for AJ)	n=7,461: seq of coding & EX/IN (737 of these AJ); n=2,539 AJ: 3 founder mutations	No multivariate analysis; Variables: personal & family hx (1°, 2°), age at dx, ca site; tests of association & binomial probabilities

Br=breast; Ov=ovarian; ca=cancer; seq=sequencing; hx=history; dx=diagnosis; log reg=logistic regression; EX/IN=exon-intron boundaries; AJ=Ashkenazi Jewish

1 Referral criteria: Br + Ov ca in 1 person or 1 family; ≥ 3 Br ca, ≥ 1 dx <51; 2 Br ca, ≥ 1 dx <41; 1 Br ca, dx <36; ≥ 2 Ov ca; 1 Ov ca, dx <51

Table E2 (continued).

REFERENCE	STUDY SAMPLE	N / % CARRIERS	MOLECULAR METHODS	STATISTICAL METHODS
Ozcelik <i>et al.</i>, [2003] <i>Canada</i>	Registry-based*; women & men with incident Br ca, recruited by age group ¹ ; 84.6% northern European; 12.7% AJ	314 / 9.9	PTT coding seq; for AJ, 3 founder mutations by HDA + complete PTT analysis for those negative	Variables: personal & family hx (1°–3°), age at dx, ca site; reported % carriers for each eligibility criterion; log reg with separate models for <i>BRCA1</i> & 2
Aretini <i>et al.</i>, [2003] <i>Italy</i>	Clinic-based; high risk families carrying <i>BRCA1/2</i> mutations in 6 centres (common testing criteria not given) probands: 89 Br ca, 34 Ov ca, 23 both, 22 bilateral Br ca, 11 male Br ca	179 / 58.1 (<i>BRCA1</i>), 41.9 (<i>BRCA2</i>)	At 3 centres: direct seq + PTT-SSCP; at 1 centre each: PTT-SSCP + FAMA, direct seq only, PTT-SSCP only	Variables: family hx (1°, 2°), age at dx, ca site; log reg using most informative variables; outcome= <i>BRCA2</i> (versus <i>BRCA1</i>) status
Gilpin <i>et al.</i>, [2000] <i>Canada</i> Referral tool: uses Br or Ov ca risk cut-offs	To develop tool: 26 hypothetical pedigrees sent to 16 clinical geneticists To test tool: 184 families with a female case with Br or Ov ca, referred for testing due to family hx; participants identified through registry (n=77), local MDs (n=92) or a research study (n=15)	184 / 19 (inferred)	PTT coding regions + <i>185delAG</i> test	Variables: family hx (1°–3°), age at dx, ca site; compared # persons who would be referred using FHAT (cut-off: score ≥ 10) to: Claus (cut-off: ≥ 22% life Br ca risk), BRCAPRO (3 cut-offs: ≥ 22% Br ca risk, ≥ 3.2% Ov ca risk, ≥ 20% mutation probability)
Evans <i>et al.</i>, [2004b] UK (2 regions) Referral tool	Clinic-based; cases from high risk Br and/or Ov ca families (various criteria) or enrolled in studies (early onset Br ca or male Br ca families) Validation sample 1: 192 cases from high risk Br and/or Ov ca families Validation sample 2: 258 cases from moderate risk families (≥ 2 Br ca with 1 dx <50, ≥ 3 Br ca any age, 1 Ov + 1 Br or Ov ca any age)	472 / 9.0†	PTT-SSCP (422 tested for <i>BRCA1</i> , 318 for both <i>BRCA1</i> and 2); some direct seq Validation sample 1: various methods, only 110 for <i>BRCA2</i> Validation sample 2: CSGE coding seq & EX/IN for both <i>BRCA1</i> and 2	No multivariate analysis; score for each ca in one lineage depending on type (Br, Ov, male Br, pancreas, prostate) and age at dx; scores derived from stratum-specific mutation frequencies; compared performance at 10% cut-off and area under ROC curve with Couch, Frank (1998, 2002) & BRCAPRO using validation samples

Br=breast; ca=cancer; AJ=Ashkenazi Jewish; seq=sequencing; hx=history; dx=diagnosis; log reg=logistic regression; Ov=ovarian; EX/IN=exon-intron boundaries

* methods described in previous publication by Knight *et al.*, [2002]

1. Eligible if AJ; Br ca dx <36; male case or male Br ca in family (2° or 3°); bilat Br or Br+Ov ca in case or family (2° or 3°); ≥ 1 Br or Ov ca in 1° (or 2 in 2°) relatives; ≥ 1 Br ca <36 or Ov ca <61 in 2° or 3° relatives; ≥ 3 1° relatives with Br, Ov, colon, prostate, pancreas ca or sarcoma, ≥ 1 dx <50.

† in validation sample #2 (n=258), where complete mutation testing was carried out

Table E3.

Validation or examination of existing risk models (since 1999): study designs

REFERENCE	STUDY SAMPLE	N / % CARRIERS	MODEL(S)	MOLECULAR TESTS (IF TESTED) AND METHODOLOGY
Chang-Claude <i>et al.</i>, [1999] <i>UK, France, Germany, Italy, Norway</i>	8 European centres, heterogeneous sample; families from research studies and/or clinics Various inclusion criteria: 1 early onset case up to multiply affected families	618 / 21.4	Adapted Claus (age-specific penetrances from Easton <i>et al.</i> , [1993] and Narod <i>et al.</i> , [1995]; mutation freq=0.33%)	Model versus <i>BRCA1</i> mutation testing in coding regions + EX/IN (SSCP, CSGE, DGGE, FAMA, PTT + direct seq) Software classified relatives based on status, age at dx/last observation, ca site
Bansal <i>et al.</i>, [2000]	None: estimated PPV and NPV using Frank 1998 model factors, Frank pre-test prob & assumed sens=85%, spec >99.95% for <i>BRCA1/2</i> seq		Frank (1998), Bayesian application	No new molecular testing Frank 1998 model considers <i>BRCA1/2</i>
Becher and Chang-Claude [2002]	Same sample as Chang-Claude <i>et al.</i> , [1999]; developed a maximum likelihood estimate of clinical sens (assumed spec=100%)		Adapted Claus (used iterative analysis and pre-test model prob)	Model versus previous <i>BRCA1</i> mutation testing (various methods); only functionally relevant mutations considered
Berry <i>et al.</i>, [2002] <i>USA (6 sites)</i>	Clinic-based; families with ≥ 1 member tested in 6 high risk clinics (various testing criteria), not selected for family hx; 71% ≥ 3 Br or Ov ca cases	301 (42% AJ) / 56	BRCAPRO (assumed same penetrance in model for AJ & non-AJ)	Model versus full <i>BRCA1/2</i> seq except AJ tested for 3 founder mutations only; Model versus test results based on BRCAPRO prob
Euhus <i>et al.</i>, [2002a] <i>USA</i> Exception: risk of disease	Clinic-based; women who had comprehensive risk assessment 74% of sample problematic for Gail model because had family hx of early Br ca or bilateral ca, <u>or</u> Ov or Br ca in 2° relatives	213 / n/d	BRCAPRO, Claus, modified Gail (includes Blacks, invasive ca only + updated population data)	No <i>BRCA</i> mutation testing Considered model yielding highest risk as the reference Gail risk used if risk by BRCAPRO higher but there was no ca hx in family
Euhus <i>et al.</i>, [2002b] <i>USA (8 sites)</i>	Clinic-based; families of women with Br or Ov ca & clear test results at 8 clinics All families (n=64, 3 sites), consecutive families (n=62, 2 sites), convenience sample (n=22, 3 sites)	148 (10% AJ) / 42.6	BRCAPRO versus 8 genetic counsellors (1/site; blind to <i>BRCA</i> results)	Model versus counsellors versus complete seq of <i>BRCA1/2</i> ; Each pedigree given to counsellors twice (one set with model info); pedigrees distributed evenly in 5 risk levels

freq=frequency; EX/IN=exon-intron boundaries; seq=sequencing; dx=diagnosis; ca=cancer; PPV, NPV=positive and negative predictive values, respectively; prob=probabilities; sens=sensitivity; spec=specificity; Br=breast; Ov=ovarian; n/d=not done; info=information

Table E3 (continued).

REFERENCE	STUDY SAMPLE	N / % CARRIERS	MODEL(S)	MOLECULAR TESTS (IF TESTED) AND METHODOLOGY
Fries <i>et al.</i>, [2002a] <i>USA</i>	Hospital-based; self- or MD-referred patients at 2 army hospitals Of >250, 101 met Myriad testing criteria ¹ (of prior to 1998) & 90 chose test	90 / 15.5	Couch and testing criteria (using 1° and 2° family hx information)	Model and testing criteria versus <i>BRCA1/2</i> coding region testing by SSCP + direct seq (for n=10, full gene seq)
Shannon <i>et al.</i>, [2002] <i>USA</i>	Hospital-based; consecutive incident Br ca cases, unselected for family hx 56% had no affected relatives 34% had 1 Br or Ov ca in family	50 (12% AJ) / n/d	Couch, Frank 1998, BRCAPRO	No <i>BRCA</i> mutation testing Collected family ca information for 3 generations (age at dx/death/interview, ca site, cause of death)
Stuppia <i>et al.</i>, [2003] <i>Italy</i>	Clinic-based; 68 eligible among 128 persons who received genetic counselling, with ≥ 3 1° or 2° relatives with Br or Ov ca at any age or ≥ 2 1° or 2° relatives with Br ca dx <age 50, or with bilateral Br ca, Ov ca, or male Br ca at any age, <u>OR</u> “sporadic cases” with early onset ca ² or male breast ca	68 / 11.7	BRCAPRO	Model versus <i>BRCA1/2</i> mutation testing of entire coding region/splice sites by combination of PTT, direct seq, SSCP
de la Hoya <i>et al.</i>, [2003] <i>Spain</i>	Clinic-based; 2 sites; high risk families; ≥ 3 1° or 2° relatives with Br or Ov ca in the same lineage	109 / 33.9	Couch (tables for non-AJ), Frank 2002 (tables for non-AJ), de la Hoya, Vahteristo	Models versus <i>BRCA1/2</i> mutation testing (for n=80, PTT/SSCP; for n=29, DGGE)
Marroni <i>et al.</i>, [2004] <i>Italy</i>	Clinic-based; 5 sites; families with multiple cases of Br or Ov ca or early onset Br ca (excluded AJ and Ov ca only families) <u>and</u> tested for mutations in <i>BRCA 1</i> and <i>2</i>	428 / 24.5	Empirically-derived models (Couch, Shattuck-Eidens, Frank 2002 tables, de la Hoya, Vahteristo) and Mendelian models (Claus, BRCAPRO, ‘adapted BRCAPRO’)	Models versus <i>BRCA1/2</i> mutation testing (mix of techniques: direct seq, SSCP/PTT, FAMA or a combination)

hx=history; seq=sequencing; Br=breast; Ov=ovarian; ca=cancer; n/d=not done; dx=diagnosis

1. These criteria were: (1) female Br ca & ≥ 2 1°/2° relatives with Br ca; (2) female Br or Ov ca & ≥ 2 1°/2° relatives with Br or Ov ca, *or* 1 Br ca dx <age 49; (3) female Br or Ov ca dx <age 49 *or* bilateral/multiple primary ca; (4) any male Br ca; (5) unaffected relative of *BRCA1/2* mutation carrier. There is some confusion in the article as to whether the early onset age cut-off was indeed 49 or actually 45.

2. Defined as Br ca dx <age 40, Ov ca dx <age 47, bilateral Br ca dx <age 43, Br + Ov ca dx <age 52, bilateral Br ca + Ov ca dx <age 56.

Appendix F

SUMMARY OF GUIDELINES ON CLINICAL INDICATIONS FOR *BRCA1/2* MUTATION TESTING

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2004	The French National Ad Hoc Committee [2004] <i>Eisinger et al. Bull Cancer 2004; 91 (3): 219-37.</i>	France	<i>Identification and management of hereditary predisposition to cancer of the breast and the ovary (2004 update)</i> update of recommendations published by the French National Ad Hoc Committee in 1998, based on advances in the field (2500 new references had been published); a group of 13 experts reviewed research evidence over a 12-month period; the report's first draft was reviewed by 5 additional experts

SUMMARY

Criteria for referral to an oncogenetic consultation:

The committee proposes a score method as a guideline. This simple additive score is based on family history, which is calculated for maternal and paternal branches separately (the highest score is retained), and counts multiple cancers in a single individual as separate cancers. Its limitations are recognised and flexibility is suggested to take into account the reliability of available diagnoses, the degree of family history, and the number of unaffected relatives.

Total score ≥ 5 : excellent indication for referral to a family cancer clinic
 Total score = 3 or 4: possible indication for referral
 Total score ≤ 2 : limited medical utility

Scoring system (points):

5: documented *BRCA* mutation in family
 4: female breast cancer before age 30 OR male breast cancer
 3: female breast cancer between ages 30 and 40 OR ovarian cancer
 2: female breast cancer between ages 40 and 50
 1: female breast cancer between ages 50 and 70

Indications for mutation testing:

The pre-test probability of a cancer susceptibility mutation being present in the family is one of the elements that provides legitimacy for genetic testing, along with the individual's participation in decision-making and informed consent. Additional conditions discussed concern availability of follow-up, psychological support, quality control mechanisms and other organisational and regulatory issues. In the previous (1998) guidelines (included in Appendix F), the Committee recommended that "a prior probability of a cancer susceptibility mutation of 25% or more justifies proposing molecular testing while a prior probability of 5% or less justifies the decision not to have the test performed". In the present report, the Committee states that "[a prior probability of a deleterious mutation in a major cancer susceptibility gene (*BRCA1,2,X*) of 25% or more justifies proposing molecular testing while a prior probability of less than 10% justifies the decision not to have

the test performed. These values correspond to expected detection rates of *BRCA1/2* mutations of 15% and 6% respectively].”

Risk factors for carrying a BRCA1/2 mutation:

The Committee lists the factors having a significant predictive value for assessing the *a priori* carrier probability, but provides no indications as to the means of combining risk factor information into a quantitative overall risk estimate. Among the personal factors, breast adenocarcinoma in a male and breast adenocarcinoma in a woman before age 30 have strong predictive value, the latter being considered a sufficient criterion to establish a **high prior probability**. Multiple tumours in a single individual in the following configurations are also sufficient: breast cancer *and* cancer of either ovary, or of the Fallopian tubes, pancreas, or prostate; or breast (or ovarian) cancer in combination with two other cancers (except for those with little or no association with *BRCA1/2* cancer susceptibility genes, such as lung and cervical cancer). Family history factors include the tumour spectrum (breast, ovary, Fallopian tubes, pancreas), the pattern of familial distribution (branch, degree, numbers of affected and unaffected individuals), that age at onset (particularly important for breast cancer), and ethno-geographic origin. In addition, the authors discuss the contribution to risk assessment of various pathological features of breast and ovarian tumours.

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2004	National Institute for Clinical Excellence (NICE) and The National Collaborating Centre for Primary Care [2004]	UK	<i>Familial Breast Cancer: The classification and care of women at risk of familial breast cancer in primary, secondary and tertiary care</i> based on review of the evidence according to the NICE guideline development process; comprehensive systematic reviews were not undertaken for each topic; where evidence is lacking, recommendations are based on consensus methods; recommendations for referral criteria outlined below are grade D, i.e. based on expert opinion/clinical experience or extrapolated from scientific studies

SUMMARY

This report aims at providing clinical guidelines to help health care professionals in primary, secondary and tertiary care address the needs of women aged 18 and older at risk of breast cancer because of family history (and not women diagnosed with breast cancer). It covers a wide range of clinical questions as well as organisational issues. The central focus is on the classification of women into risk categories to orient women at near-population risk, moderate risk and high risk towards primary, secondary and tertiary care, respectively. **Moderate risk** corresponds to a probability of developing breast cancer between ages 40 and 50 of 3 to 8% or a lifetime risk between 17 and 30%. **High risk** corresponds to a greater than 8% risk between ages 40 and 50, to a greater than 30% lifetime risk, or to a 20% or more probability of carrying a *BRCA1/2* or *TP53* mutation. However, professionals in primary and secondary care are not expected to calculate risks; they are instead provided with referral algorithms based on family history of breast and ovarian cancer and guidelines regarding mammographic surveillance and support mechanisms. Formal risk assessment is to be carried out in tertiary care, as are the offers of genetic testing and risk-reducing interventions.

Indications for mutation testing:

This report does not include a detailed discussion of formal risk assessment and genetic testing is said to be indicated if formal risk assessment reveals a **20% or greater probability of a *BRCA1/2* or *TP53* mutation** being harboured in the family.

Criteria for referral from a primary to secondary care setting (women likely to be at moderate risk, who do not require referral to tertiary care) – one of the following patterns of female breast cancer:

- 1 first degree relative diagnosed with breast cancer before age 40
- 2 first degree or second degree relatives diagnosed after average age 50
- 3 first degree or second degree relatives diagnosed after average age 60
- Formal assessment has given risk estimates of 3-8% of developing breast cancer between the age of 40 and 50, or a lifetime risk of developing breast cancer of 17% or greater, but less than 30%

Other criteria for referral to the secondary care setting – one of the following present in family:

- 1 first degree male relative diagnosed with breast cancer at any age
- 1 first degree relative diagnosed with bilateral breast cancer, first primary before age 50
- 2 first degree relatives, OR 1 first degree and 1 second degree female relative, diagnosed with breast cancer at any age
- 1 first degree or second degree relative diagnosed with ovarian cancer at any age (at least 1 first degree relative affected) AND 1 first degree or second degree relative diagnosed with breast cancer at any age
- 3 or more first or second degree relatives diagnosed with breast cancer at any age

Criteria for seeking additional advice about appropriateness of referral:

bilateral breast cancer, male breast cancer, ovarian cancer, sarcoma in a relative before age 45, glioma or childhood adrenal cortical carcinoma, complicated patterns of multiple cancers at a young age, a very strong paternal history (e.g. 4 relatives diagnosed before age 60), and Jewish ancestry.

Criteria for referral to tertiary care – one of the following present in family:

- Known *BRCA1/2* mutation in the family

Female breast cancer only in the family

- 2 first or second degree relatives diagnosed before average age 50 (at least 1 affected first degree relative)
- 3 first or second degree relatives diagnosed before average age 60 (at least 1 affected first degree relative)
- 4 relatives diagnosed at any age (at least 1 affected first degree relative)

One family member diagnosed with ovarian cancer (at any age) and, on the same side of the family:

- 1 first or second degree relative with a diagnosis of breast cancer before age 50 (if a first degree relative, this could be the same person as was diagnosed with ovarian cancer)
- 2 first or second degree relatives diagnosed with breast cancer before average age 60
- another ovarian cancer diagnosed at any age

Family containing bilateral cancer, with:

- 1 first degree relative with cancer diagnosed in both breasts before average age 50
- 1 first or second degree relative diagnosed with bilateral breast cancer AND 1 other first or second degree relative diagnosed with breast cancer before average age 60

Family containing male breast cancer (at any age) and, on the same side of the family:

- 1 first or second degree relative diagnosed with breast cancer before age 50

- 2 first or second degree relatives diagnosed with breast cancer before average age 60

Formal risk assessment has given estimates of 8% or greater risk of developing breast cancer in the next 10 years, or 30% or greater lifetime risk of breast cancer, or 20% or greater chance of a *BRCA1/2* or *TP53* mutation in the family.

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2004	The Association of Breast Surgery Family History Guidelines Panel (ABS) [2004] <i>Sauven et al. Eur J Cancer 2004; 40</i> (5): 653-65.	UK / USA	<i>Guidelines for the management of women at increased familial risk of breast cancer</i> based on evidence or expert consensus (from surgeons, radiologists, geneticists, and clinical psychologists) and produced by an international panel of experts on behalf of the Association; each recommendation is rated, but the specific referral and genetic testing criteria are not; risk assessment references include Eccles <i>et al.</i> [2000], Claus <i>et al.</i> [1994] and Ford <i>et al.</i> [1994].

SUMMARY

The panel recommends that **risk stratification** be used to identify women at low, moderate and high risk of breast cancer according to family history (and to guide clinical management). This recommendation is rated as **grade C**, based on level of evidence III (i.e., well-designed non-experimental descriptive studies, such as comparative, correlation and case studies). It is stated that the Claus tables are well validated and “give a good estimate of [cancer] risk in a simple pedigree”; the Cyrillic computer program based on Claus (which gives lower risks than the Claus tables) or the Tyrer-Cuzick program are also suggested for cancer risk assessment, although these are recognized as not being fully validated. It is recommended that risk assessment and referral to genetics clinics use the guidelines published by the UKCFSG (with lifetime risks based on the Cyrillic 3 version of the Claus model), included in appendix F [Eccles *et al.*, 2000].

With respect to **referral**, it is recommended that “women at **high risk** of familial breast cancer should be referred to a genetics clinic according to an agreed protocol”. This recommendation is rated as **grade D**, based on level of evidence IV (i.e., expert committee reports or opinions and/or clinical experiences of respected authorities). Referral to a family history clinic can be considered for women at moderate risk. It is also noted that “ovarian cancer is a marker of higher genetic risk and so brings most women into the high risk category and will result in recommendations for genetic referral”.

It is stated that “in general, the criteria for [genetic] testing in the UK is that there should be at least a **20% probability of the presence of a mutation**”.

Candidates for BRCA mutation testing:

- 1 case of breast cancer diagnosed before age 40 if Ashkenazi
- 2 cases of breast cancer diagnosed before age 40
- 3 cases of breast cancer diagnosed before age 50
- 4 cases of breast cancer diagnosed before age 60
- ≥ 4 cases of breast cancer diagnosed at any age
- ovarian and breast cancer in a family (if only one ovarian and one breast cancer, the breast cancer being diagnosed before age 50)

- male breast cancer diagnosed at any age AND female breast cancer diagnosed before age 60

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2004	National Comprehensive Cancer Network (NCCN) [2004]	USA	<i>Genetic/familial high-risk assessment: Breast and ovarian. NCCN Clinical Practice Guidelines in Oncology (v1.2004)</i> genetic risk research summarized but no specific sources cited for criteria

SUMMARY

Recommended inclusion criteria in order to proceed to an initial assessment of patient needs, a detailed medical, surgical and family history, and a physical examination:

- early-onset breast cancer (diagnosed before or at age 40, or before or at age 50*)
- bilateral breast cancer and/or breast and ovarian cancer in the same individual or among close relatives on same side of family
- familial clustering of female breast cancer with male breast cancer, thyroid cancer, sarcoma, adrenocortical carcinoma, endometrial cancer, brain tumours, dermatologic manifestations, or leukemia/lymphoma
- family member with a known mutation in a breast cancer susceptibility gene
- at risk population (e.g. of Ashkenazi descent and diagnosed with breast cancer before age 50 or ovarian cancer at any age): in this case, inclusion criteria can be made less strict

Criteria suggestive of hereditary breast/ovarian cancer syndrome that warrant further professional evaluation:

- known *BRCA1/2* mutation in the family

Personal history of breast cancer and one or more of the following:

- diagnosed before or at age 40, with or without family history (*if clinical situation warrants, may consider an age at diagnosis of before or at age 50**)
- diagnosed before or at age 50 OR with bilateral disease, AND with ≥ 1 close relative with breast cancer before or at age 50* or ≥ 1 close relative with ovarian cancer
- diagnosed at any age, with ≥ 2 close relatives with ovarian cancer at any age or breast cancer, especially if ≥ 1 woman diagnosed before age 50 or with bilateral disease
- ≥ 1 close male relative with breast cancer
- personal history of ovarian cancer
- if of Ashkenazi Jewish descent and diagnosed before or at age 50, no additional family history required
- if of Ashkenazi Jewish descent and 1 close relative diagnosed with breast or ovarian cancer, no age at diagnosis restriction required

Personal history of ovarian cancer and one or more of the following:

- ≥ 1 close relative with ovarian cancer
- ≥ 1 close relative with breast cancer before or at age 50 OR with bilateral breast cancer
- ≥ 2 close relatives with breast cancer
- ≥ 1 close male relative with breast cancer
- if of Ashkenazi Jewish descent, no additional family history is required

Personal history of male breast cancer and one or more of the following:

- ≥ 1 close male relative with breast cancer

- ≥ 1 close female relative with breast or ovarian cancer
- if of Ashkenazi Jewish descent, no additional family history

Family history only: close family member meeting any of the above criteria

*Italicized points represent updates since the 1999 NCCN guidelines (also included in Appendix F)

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2003	American Society of Clinical Oncology (ASCO) [2003] <i>ASCO Working Group on Genetic Testing for Cancer Susceptibility.</i> <i>J Clin Oncol 2003; 21 (12): 2397-406</i>	USA	<i>American Society of Clinical Oncology policy statement update: Genetic testing for cancer susceptibility</i> update of 1996 statement based on advances in the field and changes in regulations and public policy

SUMMARY

In addition to asserting the same three conditions as outlined in 1996 for genetic counselling and testing (suggestion of genetic susceptibility due to personal or family history; adequate interpretation of tests; use of results in diagnosis or management), ASCO states that “none of the cancer susceptibility tests currently available is as yet appropriate for screening of asymptomatic individuals in the general population”.

In contrast to the 1996 suggestion of a 10% cut-off in the definition of likely mutation carriers, ASCO 2003 states the following: “Given the known limitations and wide variations inherent in models for estimating mutation probability in a given family or individual, and the lack of such models for many cancer predisposition syndromes, **it is neither feasible nor practical to set numerical thresholds for recommending genetic risk assessment services.** ASCO therefore recommends that evaluation by a health care professional experienced in cancer genetics should be relied on in making interpretations of pedigree information and determinations of the appropriateness of genetic testing.”

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2003	Scottish Intercollegiate Guidelines Network (SIGN) [2003]	Scotland	<i>Epithelial ovarian cancer: A national clinical guideline</i> based on a review of evidence but no specific sources cited for the criteria

SUMMARY

According to these guidelines, women who appear to be at high risk of developing ovarian cancer “should be offered referral to a Clinical Genetics Service for assessment and confirmation of their family history”.

Criteria for defining high risk using family history:

- ≥ 2 individuals with ovarian cancer, who are first degree relatives of each other
- one individual with ovarian cancer at any age, and one with breast cancer before age 50 (these can be first degree relatives, or second degree if related in the paternal line)
- one individual with ovarian cancer at any age, and two with breast cancer before age 60 (these can be first degree relatives, or second degree if related in the paternal line)
- an individual with both breast and ovarian cancer

- ≥ 3 family members with colon cancer, or two with colon cancer and one with stomach, ovarian, endometrial, urinary tract or small bowel cancer in two generations; one cancer must be diagnosed before age 50
- known carrier of a *BRCA1/2* mutation, or untested first degree relative of a carrier

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2003	Cancer Genetics Service in Wales (CGSW) [2003]	Wales	<i>Referral guidelines for individuals with a family history of cancer</i> based on a multidisciplinary consensus group meeting and feedback from general practitioner focus groups

SUMMARY

The Cancer Genetics Service in Wales provides risk assessment and screening recommendations for individuals who meet referral criteria and thus may be at significantly increased risk for cancer due to inherited predisposition. On the basis of family history patients are categorised as low, moderate or high risk. For high risk patients genetic testing can be pursued if requested.

Criteria for referral to the Cancer Genetics Service:

- 1 first degree relative with breast cancer diagnosed before or at age 40
- 2 first degree relatives with breast cancer diagnosed before or at age 60 (on same side of family)
- 3 first degree relatives with breast cancer diagnosed at any age (on same side of family)
- 1 first degree male relative with breast cancer
- 1 first degree relative with bilateral breast cancer or with both breast and ovarian cancer
- ≥ 1 first degree relative with breast cancer AND ≥ 1 first degree relative with ovarian cancer (if only one relative with each cancer, breast cancer diagnosed before age 50)
- ≥ 2 relatives with ovarian cancer on the same side of the family AND ≥ 1 being a first degree relative

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2002	Department of Defense, Health Affairs [2002] <i>Fries et al. Mil Med 2002b; 167 (2): 93-8</i>	USA	<i>Guidelines for Evaluation of Patients at Risk for Inherited Breast and Ovarian Cancer: Recommendations of the Department of Defense Familial Breast/Ovarian Cancer Research Project</i> based on "four years of evaluation and care" (considered as expert opinion)

SUMMARY

These authors first report that Myriad Genetic Laboratories "supports the offering of testing if the patient has (1) one or more blood relatives with premenopausal breast or ovarian cancer; (2) breast cancer in her/himself, particularly if premenopausal; (3) ovarian cancer in herself, especially if blood relatives have had ovarian or breast cancer; (4) a known *BRCA1/2* mutation in the family; or (5) Ashkenazi Jewish ancestry with premenopausal breast/ovarian cancer in blood relatives.

Thus testing could be requested if a person is unaffected but has one relative with breast cancer diagnosed before age 49; using the Couch et al. (1997) or Shattuck-Eidens et al. (1995) tables, the probability of a *BRCA1* mutation using this criterion is 2.5 and 3%, respectively."

With cost-effectiveness in mind (assuming a comprehensive testing cost of \$2400), the Department of Defense Health Affairs recommends instead offering genetic testing if the **prior probability of having a mutation is 5%** using the Couch data **or 7%** using the Shattuck-Eidens data. According to the Department's recommendations, unaffected persons are typically not offered genetic testing unless a *BRCA1/2* mutation is known to be present in the family; in such cases testing should be obtained on an affected member first.

Criteria for genetic counselling and testing for BRCA1/2:

- patient with breast and/or ovarian cancer, plus ≥ 2 first or second-degree relatives with breast cancer OR ≥ 1 first or second degree relatives with ovarian cancer OR 1 first or second degree relative with onset of breast cancer before age 49
- patient with breast and/or ovarian cancer who had early onset of disease (before age 49) OR with multiple primary cancers or bilateral disease
- males with breast cancer at any age
- patient with breast and/or ovarian cancer who has Ashkenazi Jewish ancestry
- unaffected relatives of a person with a documented mutation in *BRCA1* or 2

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2001	Ontario Predictive Cancer Genetics Steering Committee (OPCGSC) [2001]	Ontario, Canada	<i>Criteria for genetic testing for BRCA1 and BRCA2 in the province of Ontario</i> source(s) not specified

SUMMARY

According to this committee, "genetic testing should be offered only when a significant increase in risk [for carrying a *BRCA1* or *BRCA2* mutation] is present."

Criteria for testing an individual affected with breast or ovarian cancer:

- breast cancer diagnosed before age 35 (or male breast cancer diagnosed at any age; test *BRCA2* only)
- invasive serous ovarian cancer diagnosed at any age
- for an individual of Jewish ancestry, breast cancer diagnosed before age 50 OR ovarian cancer diagnosed at any age
- for an individual of Jewish ancestry, breast cancer diagnosed at any age AND any relative diagnosed with breast or ovarian cancer
- breast cancer diagnosed before age 60 AND a first or second degree relative diagnosed with ovarian cancer or male breast cancer
- breast and ovarian cancer diagnosed in the same individual in the family, OR any family member with bilateral breast cancer and the first tumour diagnosed before age 50
- 2 first or second degree relatives diagnosed with breast cancer before age 50
- 2 first or second degree relatives diagnosed with ovarian cancer at any age
- ≥ 3 relatives on the same side of the family with breast or ovarian cancer at any age, in a pattern suggestive of an inherited form of breast/ovarian cancer

Criteria for testing an unaffected individual:

- a *BRCA1* or 2 mutation identified in a relative
- for an individual of Jewish ancestry, a first or second degree relative has been diagnosed with (1) breast cancer before age 50; OR (2) ovarian cancer at any age; OR (3) male breast cancer; OR (4) breast cancer at any age AND the affected relative has a family history of breast or ovarian cancer
- testing may be offered to a first degree relative of an affected individual with a family history strongly suggestive of hereditary breast/ovarian cancer (that is, **a probability of carrying a mutation of >10%**).

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2001	National Screening Committee (NSC) [2001]	UK	<i>National Screening Committee report on workshop: Screening for late onset genetic disorders: Breast and ovarian cancer</i> appears to be based on expert opinion and general literature review on genetic risk factors, penetrance, mutation frequency, risk assessment and surveillance methods, including guidelines by the Biomed 2 Demonstration Programme [Møller <i>et al.</i> , 1999]

SUMMARY

Regarding genetic screening of the population, it was generally agreed in this workshop that “a population based screening programme to identify women at increased risk of breast cancer because of family history is not justified.” Further, “none of the currently available tests for mutations in the cancer susceptibility genes are appropriate for the testing of asymptomatic persons in the general population, although the population-specific mutations described among Ashkenazi Jews and Icelanders may achieve that status in the future”.

Regarding a case-finding approach with women presenting to a general practitioner because of family history, “genetic testing currently will be restricted to cases with a high chance of carrying a mutation, due to constraints of availability and technology”. The report recommends that predetermined, nationally agreed upon criteria be used to refer individuals for consideration of genetic testing, after classification of such persons according to risk levels. The report does not provide details about risk levels beyond stating that “individuals with a proven *BRCA1* or *BRCA2* mutation” are at highest risk, and that those “with a family history suggestive of an increased genetic component” are at moderate risk.

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2001	The Cancer Research Campaign (CRC) [2001] endorsed by the Cancer Genetics Group of the British Society of Human Genetics	UK	<i>Familial breast cancer and ovarian cancer: A management guide for primary care</i> risk cut-offs are based on expert opinion; reference list includes guidelines by Eccles <i>et al.</i> [2000] (included in Appendix F as UKCFSG, [2000])

SUMMARY

This management guide for general practitioners lists referral criteria for risk assessment and presents information on what referred patients can expect at genetics/breast clinics. At such clinics patients are generally categorised as being at low, moderate or high risk according to cut-offs specified in the guide that are based on expert opinion:

Low risk: < 1 in 10 risk of carrying a dominant breast cancer predisposing gene; < three-fold increased risk of developing breast cancer by age 50 (relative to women in the general population); 10-14% lifetime risk (approximately) of developing breast cancer

Moderate risk: 1 in 4 to 1 in 10 risk of carrying such a gene; three to seven-fold increased risk for breast cancer by age 50; 15-24% lifetime risk of breast cancer

High risk: > 1 in 4 risk of carrying gene; > seven-fold increased risk by age 50; 25-80% lifetime risk

The guide states that “genetic testing is relevant for only a small minority of patients”, and will be offered at a genetics clinics if the risk of being a *BRCA* gene carrier “is **sufficiently high**”, based on evaluation by the geneticist.

Criteria for referral for further risk assessment:

- mother or sister with breast cancer diagnosed before age 40
- father or brother with breast cancer diagnosed before age 60
- 1 first or second degree relative with bilateral breast cancer, the first cancer diagnosed before age 50
- 2 first or second degree relatives with breast cancer diagnosed before average age 60 (on same side of family)
- 3 first or second degree relatives with breast cancer diagnosed at any age (on same side of family)
- 1 first or second degree relative with ovarian cancer at any age AND breast cancer diagnosed before age 50
- 1 first or second degree relative with ovarian cancer at any age AND ≥ 1 first or second degree relative with breast cancer diagnosed before age 50, from the same side of the family
- 1 first or second degree relative with ovarian cancer AND ≥ 2 first or second degree relatives with breast cancer diagnosed before average age 60, all from the same side of the family
- 2 first or second degree relatives with ovarian cancer diagnosed at any age, from the same side of the family AND ≥ 1 being a mother or sister

It is noted that persons with Ashkenazi Jewish heritage “are at higher risk and family histories which do not meet the guidelines may still warrant referral”.

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2001	CancerBACUP Medical Advisory Committee [2001]	UK	<i>Familial risk and genetics in common cancers: Bowel, breast and ovary</i> source(s) not specified

SUMMARY

Those at increased risk of developing cancer (due to a very strong family history) can be referred to family cancer clinics. The referral criteria often vary according to the clinic.

*Criteria for referral to a family cancer clinic due to a **moderate to high risk** of breast cancer:*

- 1 first degree female relative with breast cancer diagnosed before age 40, OR with bilateral breast cancer before age 60
- 1 second degree paternal female relative with breast cancer diagnosed before age 40
- 1 first or second degree female relative with breast and ovarian cancer diagnosed at any age
- 1 first degree male relative with breast cancer at any age
- 2 first or second degree relatives with breast cancer diagnosed before age 60, or ovarian cancer diagnosed at any age
- 3 first or second degree female relatives with breast or ovarian cancer at any age

*Criteria for referral to a family cancer clinic due to a **moderate to high risk** of ovarian cancer (two commonly used sets of criteria):*

Set 1

- 2 first degree relatives (or second degree if through the male line) on the same side of the family with ovarian cancer at any age

- ≥ 2 first or second degree relatives with ovarian cancer AND ≥ 2 first or second degree relatives with breast cancer on the same side of the family

Set 2

- 2 first degree relatives on the same side of the family with ovarian cancer at any age
- 1 first degree relative with ovarian cancer AND EITHER 1 first degree relative on the same side of the family with breast cancer diagnosed before age 50 OR 2 first or second degree relatives on the same side of the family with breast cancer diagnosed before age 60
- 3 relatives with colon cancer (one diagnosed before age 50) and 1 relative with ovarian cancer
- an inherited breast or ovarian gene mutation found in the family

There is an increased risk of a mutation if an individual has 4 or more relatives affected with breast or ovarian cancer on the same side of the family in 3 generations, or is an affected Ashkenazi woman.

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2001	Cancer Genetics Sub-Group of the Scottish Cancer Group (SCG) [2001]	Scotland	<i>Cancer genetics services in Scotland: Guidance to support the implementation of genetics services for breast, ovarian and colorectal cancer predisposition</i> source(s) not specified

SUMMARY

This Scottish guidance states that once at-risk persons are identified in primary or secondary care, they are to be referred to Regional Genetics Units, where risk stratification into low, moderate or high risk groups will be carried out. Management options based on these levels of risk is outlined in the guidance. After genetic counselling it is recommended that “gene testing should be available to all **high risk** families and predictive testing offered to all at risk individuals within these families”. Low risk categorisation applies to those not meeting the criteria for medium or high risk, below.

*Criteria for categorisation as **moderate risk** for breast cancer:*

- 1 first degree relative with bilateral breast cancer
- 1 first degree relative with breast cancer diagnosed before age 40
- 1 first degree male relative with breast cancer diagnosed at any age
- 2 first degree relatives or 1 first and 1 second degree relative with breast cancer diagnosed before age 60, or ovarian cancer at any age (on same side of family)
- 3 first or second degree relatives with breast or ovarian cancer on the same side of the family (≥ 1 of these being a first degree relative unless the family history is paternal)

*Criteria for categorisation as **high risk** for breast cancer:*

- a known mutation carrier (e.g. in *BRCA1*, *BRCA2*, *p53*, *pTEN*)
- an untested first degree relative of a mutation carrier
- 1 first degree relative with breast and ovarian cancer, OR 1 second degree relative with breast and ovarian cancer related via a first degree male relative
- women with an affected first degree relative (or an affected second degree relative via an intervening male relative) AND ≥ 4 relatives in three generations with breast cancer or ovarian cancer or male breast cancer (one case of bilateral breast cancer being counted as two affected relatives)

Criteria for categorisation as **moderate risk** for ovarian cancer:

- ≥ 2 first degree relatives OR first and second degree relatives with ovarian cancer at any age
- 2 first degree relatives or 1 first and 1 second degree relative with ovarian cancer at any age, OR ovarian cancer at any age and breast cancer diagnosed before age 50 (i.e. one of each type of cancer)
- 3 first degree relatives (or second degree relatives via a male) with a total of one ovarian cancer and two breast cancers (diagnosed before age 60) on the same side of the family
- 2 first or second degree relatives with colorectal cancer and an endometrial cancer and one ovarian cancer OR one relative with ovarian cancer and HNPCC family history

Criteria for categorisation as **high risk** for ovarian cancer:

- women in a family where a *BRCA1*, *BRCA2* or other predisposing gene mutation has been identified
- an untested first degree relative of a mutation carrier
- ≥ 1 first degree relative with breast and ovarian cancer

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2001	Oxford Regional Genetics Service (ORGS) [2001] <i>Lucassen et al. Fam Pract 2001; 18 (2): 135-40</i>	UK	<i>Guidelines for referral to a regional genetics service based on discussion with care providers (expert opinion) and evidence from national consensus when available</i>
SUMMARY			

Criteria for referral to a family cancer clinic (suggesting who might be at increased risk):

Breast cancer

- a mother or sister with breast cancer before age 40
- 1 first or second degree relative with bilateral breast cancer, or breast cancer at any age in a male
- 2 first or second degree relatives both with breast cancer before age 50
- 3 first or second degree relatives with breast cancer before age 60
- ≥ 4 relatives with breast cancer at any age
- ≥ 2 first or second degree relatives with breast cancer and ≥ 1 first or second degree relative with ovarian cancer

Women are categorized into **low, moderate or high genetic risk groups (0-10%; 10-25%; >25% risk of being a mutation carrier, respectively)**, based on the details of their family history. No extra prevention (screening) is offered to the low risk group.

Ovarian cancer

- 2 first or second degree relatives with ovarian cancer before age 60
- ≥ 3 first or second degree relatives with ovarian cancer at any age
- family history of both breast and ovarian cancer in first and/or second degree relatives

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2001	Kwaliteitsinstituut voor de Gezondheidszorg CBO [2001] <i>van Asperen et al. Ned Tijdschr Geneeskde 2001; 145 (3): 120-5</i>	the Netherlands	<i>Guidelines for breast cancer surveillance for women not eligible for population screening, by individual risk assessment</i> relative risks are cited from Ford <i>et al.</i> , [1998] and Claus <i>et al.</i> , [1994]; they also cite consensus guideline work by de Bock <i>et al.</i> , [1999], Blamey <i>et al.</i> , [1998] and Hoskins <i>et al.</i> , [1995] (the last of these is included in this appendix)

SUMMARY

For unaffected women, a cumulative risk for breast cancer of 20% or more (on the basis of family history) is recommended as an indication for surveillance; this cut-off is “not evidence-based yet”. If the **cumulative risk is 30% or more**, “consultation with a clinical geneticist involved in a family cancer clinic should be offered.”

Criteria for referral to a family cancer clinic:

- ≥ 2 first or second degree relatives with breast cancer before age 50
- ≥ 3 first or second degree relatives with breast cancer at age 50 or older
- breast cancer and ovarian cancer in one family
- breast cancer and prostate cancer (before age 60) in one family

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2000	SBU [2000] (Swedish Council on Technology Assessment in Health Care) <i>SBU Alert 2000; 1 (Oct 5): 1-6</i>	Sweden	<i>Presymptomatic diagnosis of hereditary breast cancer</i> systematic review; list of references includes Burke <i>et al.</i> , [1997] Dorum <i>et al.</i> , [1996] and Claus <i>et al.</i> , [1991]

SUMMARY

“Usual criteria” for the identification of high-risk individuals (eligible for testing) in Sweden:

- ≥ 3 first degree relatives in at least 2 generations affected with breast or ovarian cancer, where ≥ 1 case diagnosed before age 50 (for breast) or before age 60 (for ovarian)
- 2 affected first degree relatives where ≥ 1 diagnosed before age 40 (for breast) or before age 50 (for ovarian)
- males with breast cancer
- mutation in a breast cancer susceptibility gene in a relative
- the indications are further enhanced when bilateral breast disease or multiple primary breast tumours are present

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2000	American College of Obstetricians and Gynecologists (ACOG) [2000] <i>ACOG. Int J Gynaecol Obstet</i> 2001; 75 (3): 339-40	USA	<i>ACOG Committee Opinion: Breast-ovarian cancer screening</i> based on research (Ford, Claus) and consensus statements on clinical management from a task force convened by the Cancer Genetics Studies Consortium [Burke <i>et al.</i> , 1997]

SUMMARY

Genetic testing for *BRCA1* mutations is viewed by this organization as “an uncertain and incomplete science”. According to ACOG, *BRCA* mutation testing may be useful in selected families; that is, in a family containing multiple relatives affected with breast or ovarian cancer, or in a family in which a *BRCA* mutation has been identified. Testing of the general population is not recommended.

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2000	UK Cancer Family Study Group (UKCFSG) [2000] <i>Eccles et al. J Med Genet</i> 2000; 37 (3): 203-9	UK	<i>Guidelines for a genetic risk based approach to advising women with a family history of breast cancer</i> references to genetic research articles; criteria appear to be based on Claus model

SUMMARY

*The following criteria should be used to designate a woman as **moderate risk** (2-3 times the population lifetime risk: **risk of 1 in 6 to 1 in 4**); such women should be evaluated and screened at the ‘breast unit level’ (mutation testing unlikely but possible):*

- 1 female relative* with breast cancer diagnosed before age 40
- 1 female relative with breast cancer diagnosed before age 30 OR a male diagnosed with breast cancer at any age
- 2 female relatives with an average age at diagnosis for breast cancer of 40-49
- 3 female relatives with an average age at diagnosis for breast cancer of 50-60
- ≥ 1 relative with breast cancer diagnosed before or at age 50 AND ≥ 1 relative with ovarian cancer at any age OR 1 relative with both

*The following criteria should be used to designate a woman as **high risk** (>3 times the population lifetime risk: **risk of 1 in 4 to 1 in 3**); such women should be assessed further by specialized genetics services (mutation testing possible or probable):*

- 2 female relatives with an average age at diagnosis for breast cancer of 30-39
- 3 female relatives with an average age at diagnosis for breast cancer of 40-50
- ≥ 1 relative with breast cancer diagnosed before or at age 50 AND ≥ 1 relative with ovarian cancer at any age OR 1 relative with both cancers at any age
- ≥ 1 relative with breast cancer diagnosed before age 40 AND a relative with a childhood malignancy

*Notes: ‘relative’ includes first degree relatives and *their* first degree relatives; a relative with bilateral disease is viewed as 2 affected persons; Ashkenazi Jewish ancestry might mean genetic testing would be more helpful even with a less severe family history

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2000	Oncology Nursing Society (ONS) [2000] <i>Oncol Nurs Forum</i> 2000; 27 (9): 1349	USA	<i>Cancer predisposition genetic testing and risk assessment counseling</i> source(s) not specified

SUMMARY

The focus of these guidelines is on organizational and ethical issues, rather than on clinical indications for testing.

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
2000	National Health and Medical Research and National Breast Cancer Centre (NHMRC-NBCC) [2000]	Australia	<i>Advice about familial aspects of breast cancer and ovarian cancer: A guide for health professionals</i> based on best evidence if available or consensus expert opinion

SUMMARY

As in the NHMRC [1999] and RACGP [1997] documents which precede this guide (both included in Appendix F), family history factors are used to categorise women into risk groups. Here, categories apply to unaffected women, and breast and ovarian cancer risks are presented separately (although there is considerable overlap with respect to high risk features).

Regarding breast cancer risk, the categorisations are identical to those outlined by NHMRC [1999]. For those with **moderate risk (12-25% lifetime risk)** and **high risk (25-50% lifetime risk)**, or possibly higher if carrying a mutation) who wish to have further risk assessment, consultation with a family cancer clinic or specialist cancer service is recommended.

Regarding ovarian cancer risk the following categorisations are specified (identical to those in NHMRC [1999]), for unaffected women:

*Women considered to be at **moderately increased risk (lifetime risk 1 in 30 to 1 in 10)**:*

- 1 first degree relative diagnosed with ovarian cancer before age 50 (without *high risk features*; see list of these below)
- 2 first or second degree relatives on the same side of the family diagnosed with ovarian cancer (without *high risk features*)

*Women considered to be at potentially **high risk (lifetime risk 1 in 10 to 1 in 3)**:*

- ≥ 3 first or second degree relatives on the same side of the family with breast or ovarian cancer
- ≥ 2 first or second degree relatives on the same side of the family with breast or ovarian cancer plus any of the following *high risk features*: breast cancer diagnosed before age 40; ovarian cancer diagnosed before age 50; breast and ovarian cancer in same person; Jewish ancestry; breast cancer in a male
- ≥ 3 first or second degree relative on the same side of the family with cancers including colorectal cancer diagnosed before age 50 in particular, but also endometrial cancer, ovarian cancer, gastric cancer, other colorectal cancer or cancers involving the renal tract (features consistent with hereditary nonpolyposis colorectal cancer, HNPCC)
- a high risk ovarian cancer gene mutation identified in a relative

Women in either ovarian risk group described above may wish to pursue further risk assessment by consulting a family cancer clinic or specialist cancer service.

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1999	Society of Surgical Oncology (SSO) [1999] <i>Klimberg et al. Ann Surg Oncol</i> 1999; 6 (5): 507-9	USA	<i>Society of Surgical Oncology: Statement on genetic testing for cancer susceptibility</i> source(s) not specified

SUMMARY

SSO recommends that genetic testing should be offered when:

- the individual seeking testing has a strong family history of cancer or the characteristics of a hereditary cancer
- the test can be adequately interpreted
- results will influence the medical management or quality of life of the individual or other family members; in certain hereditary syndromes such as hereditary breast-ovarian syndrome, the medical benefit of identification of a mutation is presumed but not proven.

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1999	American College of Medical Genetics Foundation (ACMG) [1999b]	USA	<i>Genetic susceptibility to breast and ovarian cancer: assessment, counseling and testing guidelines</i> based on general literature review on penetrance, mutation frequency, and risk assessment, and/or consensus expert opinion

SUMMARY

ACMG does not recommend genetic testing in the absence of a personal or family history of breast or ovarian cancer. Widespread screening of specific populations is not endorsed.

Criteria for offering testing to an affected person:

- ≥ 3 first and/or second degree relatives on the same side of the family diagnosed with breast or ovarian cancer (regardless of age at diagnosis)
- 1 or 2 relatives diagnosed with breast or ovarian cancer, in the presence of any of the following:
 - an early diagnosis of breast cancer (before age 45) in the patient
 - ≥ 1 relative with ovarian cancer at any age AND ≥ 1 relative on the same side of the family with breast cancer at any age
 - multiple primary or bilateral breast cancers in the same individual (the patient or a relative)
 - breast cancer in a male (the patient or a relative)
 - Ashkenazi Jewish ancestry
 - a susceptibility mutation detected in the family

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1999	National Comprehensive Cancer Network (NCCN) [1999] <i>Daly et al. Oncology 1999; 13 (11A): 161-83</i>	USA	<i>NCCN practice guidelines: Genetics/familial high-risk cancer screening</i> genetic risk research summarized but no specific sources cited for criteria

SUMMARY

According to NCCN, the recommended inclusion criteria in order to proceed to an initial assessment of patient needs, a detailed medical, surgical and family history, and a physical examination are:

- a diagnosis of breast cancer before or at age 40
- multiple cases of breast and/or ovarian cancer in the same individual or among close relatives
- a family clustering of female breast cancer with male breast cancer, thyroid cancer, sarcoma, adrenocortical carcinoma, brain tumour, and/or leukemia/lymphoma
- family member with a known mutation in a breast cancer susceptibility gene

As a result of the initial assessment, women meeting the following criteria for hereditary breast/ovarian cancer syndrome should be given risk and psychosocial assessment and counselling, and may be offered BRCA mutation testing (unless risk is insufficient or testing is not indicated for psychosocial reasons):

- ≥ 1 close relative with breast cancer before or at age 40 OR with bilateral breast cancer
- ≥ 2 close relatives with ovarian cancer
- ≥ 2 close relatives with breast cancer, especially if ≥ 1 diagnosed before or at age 50
- ≥ 1 close relative with breast cancer and ≥ 1 with ovarian cancer
- ≥ 1 close male relative with breast cancer
- member of the family with a known mutation in *BRCA1/2*
- if of Ashkenazi Jewish descent, 1 close relative with breast or ovarian cancer

The modifications to the above criteria for a woman diagnosed with ovarian cancer are the following:

- ≥ 1 close female relative with breast cancer before or at age 50 OR with bilateral breast cancer
- ≥ 1 close relative with ovarian cancer
- if of Ashkenazi Jewish descent, no additional family history is required

The modifications to the above criteria for a woman diagnosed with breast cancer are the following:

- diagnosed before or at age 40, with or without family history
- diagnosed at or before age 50 OR with bilateral disease, AND with ≥ 1 close relative with breast cancer or ≥ 1 close relative with ovarian cancer
- diagnosed at any age, with ≥ 2 close relatives with ovarian cancer at any age or breast cancer, especially if ≥ 1 woman diagnosed before age 50 or with bilateral disease
- if of Ashkenazi Jewish descent and diagnosed before or at age 50, no additional family history required

Criteria for a man diagnosed with breast cancer are the following:

- ≥ 1 close male relative with breast cancer
- ≥ 1 close female relative with breast or ovarian cancer

Finally, eligibility for *BRCA1/2* genetic testing also depends upon the individual's prior probability of being a mutation carrier. Statistical models (described by NCCN as "yet to be validated") "are being used by some insurance companies to determine eligibility for coverage of testing costs (Shattuck-Eidens *et al.* 1997, Parmigiani *et al.* 1998)".

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1999	Biomed 2 Demonstration Programme [1999] <i>Møller et al. Dis Markers 1999; 15 (1-3): 207-11</i>	European (UK, Italy, Norway, Sweden, France, the Netherlands)	<i>Guidelines for follow-up of women at high risk for inherited breast cancer: Consensus statement from the Biomed 2 Demonstration Programme on Inherited Breast Cancer</i> based on compilation of results from “ongoing prospective studies” (considered as expert opinion)

SUMMARY

This collaborative group recommends that ongoing health programs in most programs may continue according to the guidelines by the UK Cancer Family Study Group and The French National Ad Hoc Committee (1998), with modifications as indicated.

*Definition of women at **high risk** for inherited breast cancer:*

- ≥ 2 first degree relatives (or second degree through males) with breast cancer before age 50 AND/OR
- a combination of early onset (<50) breast cancer and ovarian cancer in the family AND/OR
- multiple cases of breast cancers in the same lineage compatible with dominant inheritance in the family AND/OR
- a demonstrated *BRCA1/2* mutation (in females or males for *BRCA2*)

For inherited ovarian cancer:

- known mutation or strong indication of a *BRCA2* mutation carrier state, due to male breast cancer plus other relevant cancers in the family
- known mutation or strong indication of a *BRCA1* mutation carrier state, due to ≥ 2 cases of ovarian cancer AND/OR a case of both breast and ovarian cancer in the family

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1999	National Health and Medical Research Council (NHMRC) [1999]	Australia	<i>Familial aspects of cancer: A guide to clinical practice</i> references to genetic research articles; largely based on USA data (Claus and Gail models)

SUMMARY

Family history factors can be used to designate women at **moderate risk** of breast cancer (**12-25% lifetime risk**), or at potentially **high risk** of breast cancer (**25-50% lifetime risk**); such women may need more precise risk assessment or genetic testing. The recommended criteria are the same as those suggested by the Royal Australian College of General Practitioners, 1997 (see below), except that the list of *high risk features* in the 1999 guidelines includes Jewish ancestry and diagnosis of ovarian cancer before age 50. For ovarian cancer risks, these are the same as in NHMRC-NBCC [2000] (see above).

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1998	Stanford Program in Genomics, Ethics, and Society [1998] <i>Koenig et al. J Womens Health 1998; 7 (5): 531-45</i>	USA	<i>Genetic testing for BRCA1 and BRCA2: Recommendations of the Stanford Program in Genomics, Ethics, and Society</i> based on a review of the literature but no specific sources cited for testing criteria

SUMMARY

The Working Groups states that genetic testing for *BRCA1/2* mutations “may be useful for some, but not all, people in high-risk families. Testing is not appropriate for widespread clinical use or population screening but may be beneficial in some circumstances; for example, in high-risk families such as those experiencing multiple cases of cancer.”

“**High risk** may be reasonably defined in a number of ways, as long as all definitions require more than one case among near relatives. Testing may be appropriate for women who have been diagnosed with breast or ovarian cancer at an unusually early age. Men from high-risk families may also want to consider testing” (some members of the group dissented from this recommendation). “For people who are not at high risk for *BRCA1/2* mutations, the molecular tests provide little, if any, benefit” (although some members of the Working Group felt that preliminary approval for testing of those *not* at high risk was justified). “Outside the groups mentioned, testing should not be encouraged.”

“The focus should be on the safety and quality of a testing program, not on whether the program is called ‘research’.”

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1998	British Association of Surgical Oncology (BASO) [1998] <i>Blamey et al. Eur J Surg Oncol 1998; 24 (6): 464-76</i>	UK	<i>The British Association of Surgical Oncology Guidelines for surgeons in the management of symptomatic breast disease in the UK (1998 revision)</i> a consensus document which is evidence-based “where appropriate”; no genetic studies cited

SUMMARY

Definition of moderate risk group (relative risk at least 3 times that of general population; manage with surveillance):

- 1 first degree relative with breast cancer diagnosed before age 40
- 2 first or second degree relatives with breast cancer diagnosed before age 60, OR ovarian cancer at any age
- 3 first or second degree relatives with breast or ovarian cancer diagnosed at any age
- a first degree relative with bilateral cancer under age 60
- a first degree male relative with breast cancer at any age

Criteria for referral for specialist cancer genetic consultation (high risk group):

- 1 family member with both breast and ovarian cancer
- breast cancer (only) in the family, with 3 affected relatives with an average age at diagnosis under 40
- breast and ovarian cancer in the family, with 3 affected relatives with an average age at diagnosis of breast cancer under 60
- breast and ovarian cancer in the family, with ≥ 4 relatives on the same side of the family affected at any age

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1998	R&D Office of the Anglia & Oxford NHS Executive and the Unit for Public Health Genetics, Cambridge (PHGU) [1998]	UK	<i>Report of consensus meeting on the management of women with a family history of breast cancer</i> based on a meeting of experts that focussed on management of clinical risk in women who present with symptoms/signs of breast cancer or have a family history; risk groups appear to be based on Couch <i>et al.</i> , [1997]

SUMMARY

A number of interim conclusions were formed that are intended to be points for discussion (consensus was not reached at the meeting itself): (1) there should be no population-based screening of women for a family history of breast cancer; (2) it is appropriate to categorize risk into low, moderate and high groups based on family history; (3) genetic testing should only be offered in the high risk group under the guidance of a cancer geneticist in a regional genetics centre; low risk women should generally be managed within the primary care setting and not be referred for specialist consultation.

Further work is needed to achieve consensus on the criteria for separating women into the low, moderate and high-risk groups, and on the management of women in the moderate risk group (e.g., criteria for referred for possible genetic testing, or for annual mammography).

*Proposed criteria to identify families with a **20% or greater chance of having a BRCA1 mutation (high risk group; identical features to those recommended by BASO, 1998):***

- 1 family member with both breast and ovarian cancer
- breast cancer (only) in the family, with 3 affected relatives with an average age at diagnosis under 40
- breast and ovarian cancer in the family, with 3 affected relatives with an average age at diagnosis of breast cancer under 60
- breast and ovarian cancer in the family, with ≥ 4 relatives on the same side of the family affected at any age

*Proposed criteria to identify families with an increased risk of breast cancer, **at least 3 times the population risk (moderate risk group):***

- 1 first degree female relative with breast cancer diagnosed before age 40, OR bilateral breast cancer, OR 1 first degree male relative with breast cancer diagnosed at any age
- 2 first or second degree relatives with breast cancer diagnosed before age 60, OR ovarian cancer at any age, on the same side of the family
- 3 first or second degree relatives with breast or ovarian cancer diagnosed at any age on the same side of the family

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1998	The French National Ad Hoc Committee [1998] <i>Eisinger et al. Ann Oncol 1998; 9 (9): 939-50.</i>	France	<i>Recommendations for medical management of hereditary breast and ovarian cancer: The French National Ad Hoc Committee</i> based on systematic review of 3500 articles by experts at 11 workshops; for testing the references include Ford <i>et al.</i> , [1998], [1994], and Couch <i>et al.</i> , [1997], [1996]

SUMMARY

The committee believes that “population based, or even large scale, implementation of *BRCA1* and 2 mutation screening is not justified.”

Genetic cancer risk can be indicated by the number and pattern of familial distribution of cases, age at onset, the bilaterality of cancers, the occurrence of both breast and ovarian cancer within a family or in the same individual, and male breast cancer.

The Committee recommends that “a **prior probability of a cancer susceptibility mutation of 25% or more** justifies proposing molecular testing while a prior probability of 5% or less justifies the decision not to have the test performed. When laboratory resources are limited, priority should be given to families with the highest prior probabilities and/or to those for whom medical decisions have to be taken” (particularly if a request for prophylactic surgery has been made).

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1998	Scottish Intercollegiate Guidelines Network (SIGN) [1998]	Scotland	<i>Breast cancer in women: A national clinical guideline</i> based on a review of evidence but no specific sources cited for the criteria

SUMMARY

According to these guidelines, “only those women judged to be at substantially increased risk should be considered for detailed genetic assessment and follow-up in specialist clinics.”

*Criteria for identifying women at **substantial increased risk** for breast cancer (≥ 3 times the population risk):*

- 1 first degree relative with breast cancer and ovarian cancer, or bilateral breast cancer OR
- 1 first degree relative with breast cancer diagnosed before age 40, or 1 first degree male relative with breast cancer diagnosed at any age OR
- 2 first or second degree relatives with breast cancer diagnosed under the age of 60, or ovarian cancer at any age on the same side of the family OR
- 3 first or second degree relatives with breast or ovarian cancer on the same side of the family

*Criteria for identifying women at **very high risk** for whom direct gene testing may be appropriate:*

- families with ≥ 4 relatives affected with either breast or ovarian cancer in 3 generations and at least 1 affected individual available for testing

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1998	Health Technology Advisory Committee (HTAC) [1998]	Minnesota, USA	<i>Genetic testing for susceptibility to breast cancer</i> based on a survey of commercial laboratories

SUMMARY

This committee states that nearly all genetic tests take place within formal research protocols; as of February 1998 three commercial laboratories in USA were offering *BRCA1* and 2 mutation testing while continuing to participate in research studies.

Eligibility criteria for genetic testing by commercial laboratories:

- family history of breast or ovarian cancer, particularly if any diagnosed prior to menopause
- woman diagnosed with breast AND/OR ovarian cancer before age 45 or while pre-menopausal, or with bilateral disease
- woman diagnosed with breast AND/OR ovarian cancer with ≥ 2 close relatives (first or second degree in the same lineage) with breast or ovarian cancer
- woman diagnosed with breast AND/OR ovarian cancer with ≥ 1 close relative diagnosed with breast or ovarian cancer before age 45
- man diagnosed with breast cancer at any age
- woman or man in a family with a known mutation in a breast cancer susceptibility gene

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1997	National Action Plan on Breast Cancer and National Cancer Institute (NAPBC) [1997]	USA	<i>Genetic Testing for Breast Cancer Risk</i> source(s) not specified: information for patients

SUMMARY

A woman has an increased risk for breast and ovarian cancer in the presence of:

- ≥ 2 first or second degree family members affected with breast and/or ovarian cancer AND
- the breast cancer in the family members was diagnosed before age 50

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1997	Royal Australian College of General Practitioners (RACGP) [1997]	Australia	<i>Current best advice about familial aspects of breast cancer: A guide for general practitioners</i> based on published research (no specific references given)

SUMMARY

This organization states that “little Australian data exist on which to base familial risk assessments”.

Women in the following groups may wish to be referred to specialist services for a more accurate estimate of risk:

*Women considered to be at **moderately increased risk (lifetime risk 1 in 8 to 1 in 4):***

- 1 or 2 first degree relatives diagnosed with breast cancer before age 50 (without *high risk features*; see list of these below)
- 2 first or second degree relatives on the same side of the family diagnosed with breast or ovarian cancer (without *high risk features*)

Women considered to be at potentially **high risk (lifetime risk 1 in 4 to 1 in 2)**:

- ≥ 3 first or second degree relatives on the same side of the family diagnosed with breast or ovarian cancer
- ≥ 2 first or second degree relatives on the same side of the family with breast or ovarian cancer plus any of the following *high risk features*: bilateral disease; breast cancer diagnosed before or at age 40; breast and ovarian cancer in same person; breast cancer in a male
- 1 first or second degree relative diagnosed with breast cancer before or at age 45 AND another first or second degree relative on the same side of the family with sarcoma before or at age 45
- mutation in *BRCA1/2* identified in a relative

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1996	American College of Medical Genetics (ACMG) [1996]	USA	<i>Statement on population screening for BRCA-1 mutation in Ashkenazi Jewish women</i> source(s) not specified

SUMMARY

According to ACMG in 1996, “testing for the *BRCA1* mutation in high risk families, and population screening of Ashkenazi Jewish individuals ... is best performed by investigators working under ... research protocols ... additional ethnic-group specific mutations ... identified in *BRCA1* and 2 ... should be addressed in a similar fashion.”

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1996	National Breast Cancer Coalition (NBCC) [1996]	USA	<i>Presymptomatic genetic testing for heritable breast cancer risk</i> source(s) not specified

SUMMARY

This grassroots advocacy organization states that “because nothing is known as of yet about the sensitivity, specificity and reliability of the genetic tests, and little is known about the effectiveness of genetic or psychosocial counselling in this context, genetic screening should only be available to individuals who agree to join peer reviewed, approved research protocols.”

YEAR	ORGANIZATION	REGION	TITLE OF DOCUMENT / SOURCE OF GUIDELINES
1996	American Society of Clinical Oncology (ASCO) [1996] <i>ASCO Subcommittee on Genetic Testing for Cancer Susceptibility.</i> <i>J Clin Oncol</i> 1996; 14 (5): 1730-6	USA	<i>Statement of the American Society of Clinical Oncology: Genetic testing for cancer susceptibility</i> FitzGerald <i>et al.</i> , [1996] and Shattuck-Eidens <i>et al.</i> , [1995] cited

SUMMARY

“ASCO recommends that cancer predisposition testing be offered only when: (1) the person has a strong family history of cancer or very early age of onset of the disease; (2) the test can be adequately interpreted; and (3) the results will influence medical management of the patient [and] family member[s].” Genetic testing can be considered “for hereditary syndromes with a high probability of linkage to known cancer susceptibility genes, and for which the medical benefit of identifying a carrier is presumed but not established.”

The following criteria are currently used to define families with the highest likelihood of having a BRCA1 mutation (with these criteria, the prior probability of having a BRCA1 mutation is estimated at >10% in an affected family member):

- >2 relatives with breast cancer AND ≥ 1 relative with ovarian cancer diagnosed at any age
- >3 relatives diagnosed with breast cancer before age 50
- pairs of sisters with 2 of the following cancers diagnosed before age 50: 2 breast cancers, 2 ovarian cancers, or one of each

These ASCO guidelines recognize that testing will be carried out in both clinical and research settings; in the former case, enrolment in long-term research studies and the use of laboratories involved in test validation is encouraged.

Appendix G

METHODOLOGICAL DETAILS ON THE ESTIMATION OF CLINICAL SENSITIVITY DERIVED FROM STUDIES ON DISTRIBUTION OF *BRCA1/2* MUTATIONS

A. Heterogenous populations

1. Serova *et al.*, [1996]

Serova *et al.* [1996] searched for *BRCA1* mutations among 20 families at high risk for breast and ovarian cancer (19 French and one American family), and with at least an 80% probability of genetic linkage to *BRCA1*. The family inclusion criteria were: four or more cases of breast or ovarian cancer AND at least one case of each type. Ashkenazi Jewish persons were included in the study.

The results of the *BRCA1* mutation testing are presented in Table G-1 of this appendix, and include further results from these families published in complementary articles [Puget *et al.*, 1999b; Puget *et al.*, 1997]. Sequencing was used to screen for point mutations and small rearrangements in exons and intron-exon boundaries (the latter known as splice sites; hereafter all of these regions together will be referred to as EX/SP). Among the seven families who remained test negative after EX/SP sequencing, four families had an abnormal result on the basis of LHR (*Loss of Heterozygosity at the RNA level*) analysis in three families and SSCP analysis of cDNA in one family. The three other families remained test negative. Further analyses are necessary to confirm abnormalities detected by LHR: we identified confirmatory research for two of these three families [Puget *et al.*, 1999b, Puget *et al.*, 1997]. We did not find further studies on the three families which remained negative after all genetic analysis (the families in box C of Table G-1).

Table G-1 also summarizes the calculations for the proportion of large genomic rearrangements or non-coding mutations in *BRCA1* from the results by Serova *et al.*, [1996] and Puget *et al.* [1999b; 1997]. Given that the linkage analysis data are probabilities and carry a risk of error, the families that remained negative after all the genetic testing (in C) can be true negatives, false negatives, or a combination of both. From the data presented in Table G-1, we estimate a clinical sensitivity of 65 to 76.5% for *BRCA1* EX/SP screening by sequencing.

2. Puget *et al.*, [1999a]

Puget *et al.*, [1999a] tested for large genomic rearrangements in *BRCA1* among 27 American families that arose from a sample of 71 families ascertained primarily for linkage analysis (no linkage results were reported). Selection criteria for these families were: 2 or more first degree relatives with ovarian cancer OR 3 or more first degree relatives with breast or ovarian cancer (with at least one breast cancer before age 40) OR 4 relatives with breast cancer (at least 3 of these first degree OR 2 cases of breast cancer before age 40).

The results of the genetic analysis are presented in Table G-2 and include data from a complementary study [Puget *et al.*, 1999b]. The original 71 families were screened in the EX/SP regions of *BRCA1* and 2 but the same techniques were not used for all of the families [Serova-Sinilnikova *et al.*, 1997; Serova *et al.*, 1996]. Twenty-one families remained test negative (in C) after the EX/SP screening

and the diverse subsequent analyses of *BRCA1*: Southern blot, search for complete deletion of the gene, analysis of the 5' and 3' non-coding regions, analysis of the promotor regions and search for duplication of exon 13.

Table G-2 summarizes the calculations. From these data, we estimate that the proportion of large genomic rearrangements or non-coding mutations in *BRCA1* varies from 9.8 to 15%. We estimate that the clinical sensitivity of EX/SP screening for point, small rearrangement and truncated mutations in *BRCA1* varies from 55.7 to 85% (using a mix of HDA, SSCP/PTT, or sequencing; these values are thus not specific to one testing method in particular).

3. Ford *et al.*, [1998]

In this study, Ford *et al.* [1998] used multi-centre linkage analysis data to determine the proportion of high risk European and American families associated with *BRCA1* and 2 mutations. Selection criteria for these families were: at least 4 cases of female breast cancer diagnosed before age 60 OR any male breast cancer, with or without ovarian cancer. Direct mutation analysis was carried out among many of these families; the details of this analysis among 30 families with a strong probability of linkage to *BRCA1* (lod score >1.0) are presented in Table G-3.

The EX/SP screening completed on the 30 families used heterogeneous methods since the families arose from different sources. Among the nine families that remained test negative after the screening, some were investigated further for non-EX/SP *BRCA1* mutations: LHR detected genetic abnormalities in two families and RT-PCR analysis of cDNA found a deletion of exon 17 in one family. The status of the six other families is unknown as a result of non-informative or missing LHR analysis. For this study, the proportion of large genomic rearrangements or non-coding mutations in *BRCA1* varies from 10 to 12.5%. The estimated clinical sensitivity for the combination of the EX/SP screening methods used to detect point, small rearrangement and truncated mutations in *BRCA1* (sequencing, DSDI/PTT, SSCP-HA, SSCP alone, CSGE or CDGE) varies from 70 to 87.5%.

4. Unger *et al.*, [2000]

As pointed out by Unger *et al.*, [2000], families ascertained primarily for linkage analysis may not provide the information necessary to evaluate the frequency of genomic rearrangements in a more clinically relevant population of women seeking *BRCA* mutation testing. In the study by Unger *et al.*, [2000], mutation screening was carried out in the non-EX/SP regions of *BRCA1* in 42 American families (of European and Ashkenazi Jewish origin) with a family history of breast or ovarian cancer and ascertained in clinics. The sample arose from a clinic series of 106 families for whom study inclusion criteria were: at least one breast cancer case AND one ovarian cancer case in the same lineage; if male breast cancer was present, the family had to also have one female case of breast cancer plus at least one ovarian cancer case in the same lineage.

We present the results of *BRCA1/2* genetic analysis in two unpublished studies reported in the article by Unger *et al.*, [2000] (Table G-4a and G-4b). Martin *et al.* (Table G- 4a) carried out mutation analysis on the original 106 families from which arose the sample of 42 families studied by Unger *et al.*, [2000]. Forty-four of the 106 families who underwent EX/SP screening of *BRCA1/2* tested negative. Large *BRCA1* genomic rearrangements were detected in five of these families: a large deletion found by Southern blot analysis in four families and a duplication of exon 13 found by specific detection in one family. No analysis for large *BRCA2* genomic rearrangements was performed. Sinilnikova *et al.* [unpublished] (Table G-4b) analyzed *BRCA1/2* mutations in 48 families with breast and ovarian

cancer (the different molecular techniques used and the family inclusion criteria were not specified). Among the 16 families that remained test negative after EX/SP screening, five tested positive for a large rearrangement in *BRCA1*.

The proportion of large *BRCA1* rearrangements varies from 5.1 to 8.4% using the Martin *et al.* [unpublished] data, and from 11.1 to 14.7% using the Sinilnikova *et al.* [unpublished] data. We estimate that the clinical sensitivity of the EX/SP screening for point mutations and small rearrangements in *BRCA1* varies from 55.6 to 91.5% (for Martin *et al.*, using CSGE) and from 64.4 to 85.3% (for Sinilnikova *et al.*, method unknown).

5. Gad *et al.*, [2002]

This study involved analysis for large *BRCA1* genomic rearrangements among 68 families with a history of breast and ovarian cancer who tested negative after EX/SP screening by DGGE or DHPLC [Gad *et al.*, 2002]. The 68 families arose from a sample of 120 seen in cancer genetic clinics in France. The selection criteria were: at least one case of ovarian cancer plus two cases of breast or ovarian cancer (diagnosed at any age) OR at least two cases of breast or ovarian cancer among first degree relatives OR an index case with both breast and ovarian cancer.

The results of the *BRCA1/2* genetic analysis for the 120 families are presented in Table G-5 and include further results from a complementary study [Casilli *et al.*, 2002]. Fifty-two families tested positive in the EX/SP screen of *BRCA1/2*. Gad *et al.*, [2002] tested the 68 negative families with a Bar code approach on combed DNA and identified 4 families with large genomic rearrangements. Testing a subgroup of the families (from the genetics clinic of the Institut Curie), Casilli *et al.* [2002] were able to identify large rearrangements in the same four families using the QMPSF technique, as well as finding a large rearrangement in one additional family (that had remained negative in the study by Gad *et al.* [2002]). Combining the results of these two studies, the proportion of large genomic rearrangements or non-coding mutations in *BRCA1* varies from 4.7 to 11.6%. We estimate that the clinical sensitivity of EX/SP screening for point mutations and small rearrangements in *BRCA1* by a combination of DHPLC and DGGE varies from 35.8 to 88.4%.

6. Montagna *et al.*, [2003]

Among the 230 patients with breast and/or ovarian cancer attending a medical oncology clinic in Padua (Italy) over an 8-year period, 37 families were classified as HBOC families on the basis of the following criteria: three or more cases of breast or ovarian cancer at any age (including at least one ovarian cancer), among first-degree relatives (except for disease-transmitting males) on the same parental branch, over a minimum of two generations. Previous mutational screening, using SSCP + PTT and DHPLC, had revealed the existence of point mutations in *BRCA1* in nine families and point mutations in *BRCA2* in five families. A large rearrangement in the *BRCA1* gene had also been identified by Southern blot. Using multiplex ligation-dependent probe amplification (MLPA), Montagna *et al.*, [2003] identified five additional genomic rearrangements in *BRCA1* in the remaining 22 families. Of note, the genotyping of SNPs (single-nucleotide polymorphisms) located in the *BRCA1* gene was completed for 22 EX/SP negative families and was predictive of a large rearrangement (detected by MLPA) in 2 families. As shown in Table G-6, the proportion of large rearrangements thus varies from 18.8 to 40.0%, whereas the clinical sensitivity of EX/SP screening for point mutations and small rearrangements in *BRCA1* by a combinaison of SSCP+PTT and DHPLC can be estimated to vary from 28.1 to 60.0%.

7. Hartmann *et al.*, [2004]

Hartmann and colleagues performed a search for *BRCA1* large genomic rearrangements using multiplex ligation-dependent probe amplification (MLPA) in families classified as being at high-risk according to the categories defined by the German Consortium for Hereditary Breast and Ovarian Cancer. These families counted 2 or more cases of breast cancer with at least two cases diagnosed before age 50, or with at least one ovarian cancer case. Families with at least one male breast cancer also qualified for the study. Among 251 families investigated in Heidelberg, 140 families were considered as high-risk and underwent DHPLC analysis and sequencing. As shown in Table G-9, point mutations or small rearrangements were identified in *BRCA1* in 48 families and in *BRCA2* in 17 families. In the remaining 75 families, MLPA identified deletions in 4 families (2 deletions of exons 1a, 1b and 2 and 2 deletions of exon 17). Clinical sensitivity is thus estimated as 39.0% or 92.3% depending on the underlying assumption. Additional rearrangements were identified in families from Northern Germany, but available information on these families was insufficient to allow derivation of clinical sensitivity estimates.

B. Founder populations

Dutch

1. Petrij-Bosch *et al.*, [1997]

Petrij-Bosch *et al.* [1997] searched for large genomic rearrangements among 137 Dutch families at high risk for breast or ovarian cancer who tested negative on EX/SP screening of *BRCA1/2*. These families were part of a larger sample of 170 for whom the selection criteria were: at least three first degree relatives with breast or ovarian cancer, with at least one diagnosed before age 50. The families arose from three different studies [Peelen *et al.*, 1997, Peelen *et al.*, 1996, Hogervorst *et al.*, 1995]; these studies did not use the same analytic conditions (with respect to primers) for the EX/SP screening by PTT and PCR-FLA¹²⁶.

The results of the mutation testing for the 170 families are presented in Table G-7. Southern blot analysis for three specific large *BRCA1* deletions was carried out among the 137 families who were negative after EX/SP screening, and 13 families tested positive. Twelve of the 13 families carried 510 base pair or 3835 base pair deletions common to the Dutch population (the elevated frequency of these two mutations is thought to be the result of a founder effect); the other family had the exon 22 deletion caused by IVS22 +5G->A. The remaining 124 families were negative after all of the *BRCA1* genetic analysis (shown in C). Some of these families (n=58) had further analysis by Southern blotting in *BRCA2* but no large genomic rearrangements were detected [Peelen *et al.*, 2000b]. Based on these data, the proportion of large rearrangements or non-coding mutations in *BRCA1* varies from 8.2 to 38.2% for the three deletions. The clinical sensitivity of the EX/SP screening for point, small rearrangement and truncated mutations in *BRCA1* is estimated as 13.3 to 61.8%, using PTT and PCR fragment length analysis.

2. Hogervorst *et al.*, [2003]

This study reports on *BRCA1/2* mutation analysis in 805 families seen at two family cancer clinics in Amsterdam (the Netherlands) between 1995 and 2001 who agreed to be tested. On the basis of the available information, some degree of overlap with families from the above Petrij-Bosch study

126. FLA=fragment length analysis (a general term used to denote screening techniques such as SSCP, DGGE, etc.).

cannot be excluded. No precise eligibility criteria are provided but, in general, families had a prior probability of carrying a *BRCA1/2* mutation of 10% or greater. As shown in Table G-8, mutations in 144 families had been identified through DGGE, DHPLC, PTT or mutation-specific tests (A, A' and B'). Among these mutations, 28 were one of the two founder deletions involving exons 13 and 22 reported by Petrij-Bosch *et al.* [1997]. The remaining 661 families were tested for additional large rearrangements. Multiplex ligation-dependent probe amplification (MLPA) analysis revealed that 5 families carried aberrant gene copy numbers: 2 deletions, 2 duplications and one triplication. Overall, the proportion of large rearrangements in *BRCA1* is estimated as 4.3 to 27.3%, and these estimates would be 3.6% (28/777) and 23.1% (28/121) respectively if only the previously described founder mutations were considered. The estimated clinical sensitivity of EX/SP screening in *BRCA1* varies from 11.3 to 72.7%.

Ashkenazi Jewish (AJ)

1. Schubert *et al.*, [1997]

This study involved *BRCA1/2* mutation analysis among 17 American AJ families at high risk for breast and/or ovarian cancer. Families with at least four cases of breast or ovarian cancer were selected. The families were first tested for the 3 *BRCA1/2* mutations common in the AJ population (method not reported) and 8 families were positive (Table G-10). EX/SP screening was then performed among the remaining 9 families, using SSCP for *BRCA1* and SSCP plus PTT in exon 10-11 for *BRCA2*. Only one family was identified with a non-common *BRCA2* mutation. The proportion of *BRCA1/2* mutations that were not detected by screening for common AJ mutations therefore varies from 5.9 to 11.1%. The estimated clinical sensitivity of screening specifically for the 3 *BRCA1/2* mutations common in the Ashkenazi Jewish population varies from 47.1 to 88.9% in this study.

2. Frank *et al.*, [1998]

In this study, EX/SP screening of *BRCA1/2* by sequencing was carried out among 238 high risk families, of whom 47 had Ashkenazi Jewish ancestry. The study selection criteria were: women with breast cancer diagnosed before age 50 or with ovarian cancer AND at least one female relative (first or second degree) with breast cancer before age 50 or ovarian cancer at any age. The results of mutation testing are presented in Table G-11. Among the 47 AJ families, 18 carried one of the three founder *BRCA1/2* mutations and two families had a non-common *BRCA1/2* mutation. Twenty-seven families remained negative after EX/SP sequencing (in C). Based on these data, the proportion of non-common *BRCA1/2* mutations varies from 4.3 to 10%. The estimated clinical sensitivity of screening for the 3 *BRCA1/2* mutations common in the AJ population by sequencing varies from 38.3 to 90% in this study.

3. Phelan *et al.*, [2002]

Phelan *et al.* [2002] looked for common *BRCA1/2* mutations and EX/SP mutations among 245 AJ families at high risk for breast and/or ovarian cancer, with at least two cases of breast or ovarian cancer, diagnosed at any age among first or second degree relatives. Table G-12 presents the results. The specific analysis for the three common mutations was performed using multiplex PCR and 85 families tested positive. The 160 negative families had EX/SP screening of *BRCA1*, using PTT for exon 11 and DGGE for all other exons. One family was identified with a frameshift *BRCA1* mutation which is predicted to truncate proteins, and three families were identified with missense variants of unknown clinical significance. We carried out two sets of calculations according to the interpretation of these missense variants. If they are considered benign polymorphisms, the proportion of non-common

BRCA1 mutations varies from 0.4 to 1.2% and the clinical sensitivity of screening for the 3 common mutations varies from 34.7 to 98.8%. If we assume that the three missense mutations are deleterious, the proportion of non-common *BRCA1* mutations is higher and varies from 1.6 to 4.5%, and the clinical sensitivity of the common mutation screen is lower, with a maximum value around 95.5%.

Finnish

A number of founder *BRCA1/2* mutations have been detected in the Finnish population. We found two relevant studies that carried out genetic analysis among high risk families.

1. Huusko *et al.*, [1998]

This study involved *BRCA1/2* genetic analysis among 88 high risk families from the Oulu region of Finland. The study selection criteria were: at least two breast and/or ovarian cancer cases in first degree relatives or other HBOC characteristics (age at onset < 40, bilateral cancer or multiple tumours). Table G-13 shows that the EX/SP screening by a combination of PTT and CSGE identified 11 families with five *BRCA1/2* mutations. Seventy-seven families remained negative. Some of the detected mutations were recurrent either among the study families (3 mutations: *BRCA1* 3745delT, *BRCA1* 4216 2ntA->G and *BRCA2* 9346 2ntA->G); a fourth mutation has been found in many families in a different Finnish study (*BRCA2* 999del5) [Vehmanen *et al.*, 1997a; Vehmanen *et al.*, 1997b]. Together these four mutations appear to be founder mutations in the Finnish population on the basis of haplotype analysis [Vehmanen *et al.*, 1997a, Vehmanen *et al.*, 1997b]. In this study of Oulu families, then, the proportion of non-common mutations varies from 1.1 to 9.1% and the estimated clinical sensitivity of screening for the four common *BRCA1/2* mutations varies from 11.4 to 90.9%.

2. Vehmanen *et al.*, [1997a]

Vehmanen *et al.* [1997a] carried out *BRCA1* genetic analysis among 100 high risk families from different cohorts of patients diagnosed with breast cancer in the oncology department of Helsinki University Central Hospital (HUCH), or other hospitals in the southern region of Finland. Families with at least three breast and/or ovarian cancer cases in first or second degree relatives were selected (however, two of the analyzed families have one breast and one ovarian cancer case).

The test results are presented in Table G-14 and include data on *BRCA2* genetic analysis from a second study [Vehmanen *et al.*, 1997b]. The EX/SP screening was performed for *BRCA1/2* on the 100 families using a combination of HA, SSCP and PTT. Seventy families also had EX/SP screening in *BRCA1* by sequencing. *BRCA1* or 2 mutations were detected in 18 families as a result of the EX/SP screening. Among the 82 families that remained negative, linkage analysis was performed for 12 families: seven families were not linked, four were non-informative and one family showed linkage to *BRCA2*. In the last family, a *BRCA2* 7708C->T mutation was confirmed by sequencing following the linkage analysis. The entire sample of 100 families was then specifically tested for the *BRCA2* 7708C->T mutation and two additional families were found to be positive.

We carried out two sets of calculations for the proportion of common mutations found in this study (Table G-14): (1) considering the four recurrent mutations that were identified by Huusko *et al.* [1998] among high risk families from Oulu (a more northern region of Finland); and (2) considering all of the recurrent mutations identified by Vehmanen *et al.* [1997a, 1997b] among high risk families from Helsinki and southern Finland. In the first case the estimated clinical sensitivity when testing for four common mutations varies from 6.5 to 28.6%; regarding the eight recurrent mutations our estimate is

higher and varies from 14 to 61.9%. It appears that the clinical sensitivity of screening for the four founder mutations examined by Huusko *et al.* [1998] displays geographic variation within Finland. According to the Vehnamen data [1997a, 1997b], the proportion of non-common mutations varies from 8.6 to 38.1%.

In other work by Lahti-Domenici *et al.* [2001], search for large *BRCA1/2* genomic rearrangements by Southern blot analysis was carried out among 82 Finnish HBOC families who tested negative on EX/SP screening. These families arose from the study samples of Huusko *et al.* [1998] and Vehmanen *et al.* [1997a]. We do not know how the 82 families examined by Lahti-Domenici *et al.* [2001] were selected from the 149 who remained negative in the two previous studies. The Southern blot analysis did not detect any rearrangements [Lahti-Domenici *et al.*, 2001]. It seems that the proportion of large *BRCA1/2* genomic rearrangements is much lower among the Finnish, in contrast to American, French and Dutch populations.

Hungarian

Van der Looij *et al.* [2000] report that the *185delAG*, *5382insC* and *300T→G* mutations account for more than 80% of the *BRCA1* mutations in HBOC families of Hungarian origin [Van der Looij *et al.*, 2000]. We found only one study to explain this figure [Ramus *et al.*, 1997].

1. Ramus *et al.*, 1997

Ramus *et al.* [1997] analyzed *BRCA1* and 2 among 32 high risk families with at least two first degree relatives diagnosed with breast or ovarian cancer at any age (the authors further report that cases with a strong family history were selected preferentially). Table G-15 shows that the EX/SP screening by a mixture of SSCA/HDA and PTT identified 11 families with *BRCA1/2* mutations (no details were given about the exons targeted by PTT). Two *BRCA1* mutations were recurrent: *185delAG* and *5382insC* were found in two and four families, respectively. The two families with the *185delAG* mutation had Ashkenazi Jewish (AJ) ancestry and shared the haplotype which is common in AJ breast patients with the *185delAG* mutation. The four families with the *5382insC* mutation were not AJ in origin, but shared a haplotype common to northern and eastern European populations. Twenty-one families remained negative after the EX/SP screening. Based on this study, the proportion of non-common *BRCA1* mutations varies from 3.6 to 14.3%, and the clinical sensitivity of screening for the two recurrent *BRCA1* mutations is estimated as 21.4 to 85.7%.

Van der Looij *et al.* [2000] also report that the *BRCA2* *9326insA* and *6174delT* mutations account for more than 50% of the *BRCA2* mutations among Hungarian HBOC families, but we could not find further references for this figure.

Polish

1. Gorski *et al.*, 2004a

Three common founder *BRCA1* mutations have been described in Poland: *5382insC* (also common in the AJ population), *C16G*, and *4153delA*. In order to establish the relative contribution of founder and non-founder mutations, Gorski *et al.* [2004a] determined the spectrum of *BRCA1/2* mutations in 200 high risk families seeking genetic counselling in one of 18 cancer genetic centres across Poland. The study sample comprised 100 families with three or more cases of breast cancer but no ovarian cancer, and 100 families with cases of both breast and ovarian cancer for a total of at least three affected individuals. The entire coding sequence, and splice sites, of the *BRCA1* and *BRCA2* genes

were sequenced for one affected women per family. Table G-16 shows that common *BRCA1* founder mutations were identified in 111 families and less common mutations in another 11 families. In addition, non-common *BRCA2* mutations were identified in seven families, and 11 variants of unknown clinical significance were identified in *BRCA2*. (Nonsense and frameshift mutations were systematically considered as pathogenic mutations, while missense alterations were classified as variants of unknown clinical significance, except for one missense mutation previously described as pathogenic.) In sixty families, EX/SP sequencing revealed no alterations. We carried out two sets of calculations according to the interpretation of the missense variants. The proportion of non-common *BRCA1/2* mutations varies from 9 to 14% if unknown variants are considered as benign polymorphisms (14.5 to 20.7% if not), and the clinical sensitivity of screening for the three *BRCA1* founder mutations is estimated as 55.5 to 86%. If a few variants of unknown significance turned out to be pathogenic, the proportion of non-common mutations could in fact be slightly higher, but the maximum clinical sensitivity value would remain in the order of 80%.

Greek

The founder *BRCA1* 5382insC mutation is frequent in the Greek population. We identified one study on high risk families that allowed calculation of clinical sensitivity estimates.

1. Ladopoulou *et al.*, 2002

This study involved *BRCA1/2* genetic analysis in 85 high risk families. The study selection criteria were: at least two breast cases diagnosed before age 50 in first or second degree relatives or other HBOC characteristics (such as age at onset < 35, bilateral breast cancer). Table G-17 shows that the combination of techniques used for EX/SP screening was not the same for all families. Furthermore, the *BRCA2* analysis was restricted to exons 10 and 11 only. This combination of techniques identified 14 families with a *BRCA1/2* mutation among which 7 families carried the *BRCA1* 5382insC mutation. Seventy-one families remained negative. The estimated clinical sensitivity of screening for the common *BRCA1* 5382insC mutation varies from 8.2 to 50%.

French Canadian

Seven common *BRCA* mutations had initially been identified in the French-Canadian population [Tonin *et al.*, 1998]. More recently, two groups have presented or published data that allow for the calculation of clinical sensitivity estimates.

1. Simard *et al.*, 2004

Simard and colleagues [2004] (J. Simard)¹²⁷ analysed *BRCA1/2* genes in high-risk families of French-Canadian origin with at least 3 breast and/or ovarian cases at any age in first degree relatives, or with at least 4 first or second degree relatives with breast cancer and/or ovarian cancer. Among 399 eligible families that came from different regions of Quebec, 251 families participated in the study (counted as 256 in total because 5 families had a history of cancer on both sides). In 191 families, testing was performed on a member affected with cancer or an obligate carrier, whereas in 65 families, only unaffected relatives were available for testing. All families were tested for 24 specific *BRCA1/2*

127. Oral presentation "Familial breast/ovarian cancer in the French Canadian founder population", *Oncogenetics: Achievements and challenges. Les 17ièmes Entretiens du Centre Jacques Cartier*, Montréal, Québec, Oct 7-8, 2004. Personal communication with J. Simard, January 28, 2005.

mutations reported to date in the French-Canadian population, followed by direct sequencing of the EX/SP regions if negative.

Amongst the 256 families analysed for a *BRCA1/2* mutation in the EX/SP regions, 62 families tested positive and 15 distinct *BRCA1/2* mutations¹²⁸ were identified. However, we show results for the subset of 191 families with at least one affected individual tested (Table G-18). In this subset of families, there were 2 founder *BRCA1/2* mutations, *BRCA1* C4446C→T and *BRCA2* 8765delAG, as demonstrated by haplotype analysis and genealogical studies, which were present in 17 and 25 families, respectively. Two other *BRCA1* mutations were also recurrent but to a lesser degree (2244insA and 2953del3+C). The clinical sensitivity estimates for the two founder mutations (*BRCA1* C4446C→T and *BRCA2* 8765delAG) in the subset of 191 families vary from 22 to 75%. The clinical sensitivity for the 4 recurrent mutations varies from 24.1 to 82.1% (Table G-18).

An analysis of genomic rearrangements in the *BRCA1/2* genes was carried out by Southern blot and MLPA in some families. No genomic rearrangements were detected by MLPA analysis for the *BRCA1* gene in the 135 families negative after the EX/SP screen, nor for the *BRCA2* gene in 133 of these families (no MLPA analysis in *BRCA2* was done for two families). Southern blot analysis likewise revealed no large rearrangements in a sub-sample of 61 of the families negative after the EX/SP screen (in which 63 individuals were tested). These analyses suggest there are no recurrent *BRCA1/2* genomic rearrangements in the French Canadian population.

2. Oros *et al.*, [2004]

Oros *et al.* [2004] analysed 169 independent families with breast and/or ovarian cancer. The families were selected based on a history of ≥ 3 confirmed female Br diagnosed at or before 65 years, epithelial Ov or male Br cancer in 1st, 2nd and 3rd degree relatives occurring on the same branch of the family. Index cases reported grand-parental French-Canadian ancestry. Among the 169 families, 81 were seen before the cloning of the genes (i.e <1994) in various clinics across Quebec and 88 were seen after 1994 in one of 4 Montreal hospitals.¹²⁹ One index case per family was tested by SSCP for 20 *BRCA1/2* mutations¹³⁰ previously identified in the French-Canadian population and 74 families were positive for one of the mutations (Table G-19). Fifteen distinct mutations were identified, including 7 recurrent mutations. Haplotype analysis suggested a founder effect for 5 mutations (*BRCA1* 4446C→T and 2953del3insC, *BRCA2* 6085G→T, 8765delAG and 3398delAAAAG). The two most frequent founder mutations in this study are the mutations *BRCA1* 4446C→T and *BRCA2* 8765delAG (see Table G-19) and the clinical sensitivity of their specific detection is estimated as 25 to 57%. The clinical sensitivity values for the 5 founder mutations identified are 36.7 to 84%, and for the 7 recurrent mutations combined they are 39 to 89.2%. Among 95 families negative after the specific mutation search, 83 families had a complete analysis or partial analysis¹³¹ of the EX/SP regions in the *BRCA1/2* genes but no additional variants were identified.

128. *BRCA1*: C4446T, 2244insA, 2953del3+C, E352X, 1623del5, 2080insA, 3705insA, 5221delTG, 4160delAG
BRCA2: 2816insA, 3034delAAAC, 6503delTT, R3128X, 8904delA, 8765delAG.

129. The hereditary cancer clinics of McGill, the breast clinic of Hôtel-Dieu Hospital, the breast clinic of the Division de Gynécologie Oncologique, Notre-Dame Hospital and the hereditary cancer clinics affiliated with the Jewish General Hospital.

130. *BRCA1* 360C→T, 1081G→A, 1623delTTAAA, 2072insG, 2953delGTAAinsC, 3768insA, 3875delGTCT, 4160delAG, 4184delTCAA, 4446C→T, 5221delTG; *BRCA2* 2558insA, 2816insA, 3034delAAAC, 3398delAAAAG, 3773delTT, 6085G→T, 6503delTT, 7235G→A, 8765delAG

131. Sequencing in *BRCA1* or *BRCA2* or in both genes or PTT in *BRCA1* exon 11 and *BRCA2* exons 10-11 or PTT in *BRCA1* exon 11.

TABLE G-1.

Serova <i>et al.</i> , [1996] 20 French & American Br-Ov families: probability of linkage to <i>BRCA1</i> > 80% (complementary analysis: in Puget <i>et al.</i> , [1997, 1999b])			ASSUMPTION	Proportion of <i>BRCA1</i> large rearrangements or non-coding region mutations	Proportion of <i>BRCA1</i> point mutations/small rearrangements detected by EX/SP screen
EX/SP +ve (13 families)	EX/SP -ve (7 families)		C. 3 families † -ve after LHR, SSCP on cDNA	All C families false-ve	A/A+B+C= 13/20=65%
A. 13 families Point mutations/SR in <i>BRCA1</i> (SEQ)	B. 4 families +ve for large genomic rearrangements or non-coding region mutations in <i>BRCA1</i>	C. 3 families † -ve after LHR, SSCP on cDNA			
	1 family: del exon 5 (SSCP on CDNA), in Serova <i>et al.</i> , [1996]				
	1 family: exon 13 duplication (LHR and then PCR screen for splicing defects, in Puget <i>et al.</i> , 1999b 1 family: del exon 17 (1kb) Alu-mediated (LHR screen and then Southern blot, in Puget <i>et al.</i> , [1997]		†we did not find further molecular investigations for these families	All C families true-ve	A/A+B= 13/17=76.5%

Abbreviations: SR=small rearrangements

TABLE G-2.

Puget <i>et al.</i> , [1999a] 71 American Br-Ov families: selected for linkage studies but no results of linkage studies available (complementary analysis in Puget <i>et al.</i> , [1999b])				ASSUMP-TION	Proportion of <i>BRCA1</i> large rearrangements or non-coding region mutations	Proportion of <i>BRCA1</i> point mutations/small rearrangements detected by EX/SP screen
EX/SP +ve (44 families)		EX/SP -ve (27 families)			All C families true -ve	A/A+B= 34/40=85%
A. 34 families Point/ SR and truncated mutations in <i>BRCA1</i> (HDA or SSCP/PTT or SEQ)	A'. 10 families Point/SR and truncated mutations in <i>BRCA2</i> (HDA or SSCP/PTT or SEQ)	B. 6 families +ve for large genomic rearrangements or non-coding region mutations in <i>BRCA1</i>		C. 21 families -ve after Southern blot, entire gene deletion analysis, promoter and 5', 3' UTR analysis, exon 13 duplication screening (all done in <i>BRCA1</i>)		
		3 families: distinct internal deletions (quantitative Southern blot) in Puget <i>et al.</i> , [1999a]	3 families: exon 13 duplication (PCR using primers dup13F/R) in Puget <i>et al.</i> , [1999b]			
					B/A+B= 6/40=15%	A/A+B+C= 34/61=55.7%

TABLE G-3.

Ford <i>et al.</i> , [1998] 30 European and American Br-Ov families linked to <i>BRCA1</i> (lod score >1.0)				ASSUMPTION	Proportion of <i>BRCA1</i> large rearrangements or non-coding region mutations	Proportion of <i>BRCA1</i> point mutations/small rearrangements detected by EX/SP screen
EX/SP +ve (21 families)	EX/SP -ve (9 families)					
A. 21 families Point/SR and truncated mutations in <i>BRCA1</i> (SEQ, DSDI/PTT, SSCP-HA, SSCP alone, CSGE or CDGE)	B. 3 families +ve for large genomic rearrangements or non-coding region mutations in <i>BRCA1</i>	C. 6 families LHR analysis not informative or not done		All C families false-ve	B/A+B+C= 3/30=10%	A/A+B+C= 21/30=70%
		2 <u>families*</u> : inferred regulatory mutation (LHR screen) *we did not find further investigations	1 <u>family</u> : del of exon 18 (SSCP on cDNA analysis) *this family was reported to have an exon 17 deletion in Swensen <i>et al.</i> , [1997]			
				All C families true-ve	B/A+B= 3/24=12.5%	A/A+B= 21/24=87.5%

TABLE G-4a.

Martin <i>et al.</i> , [unpublished] 106 European Br-Ov families (reported by Unger <i>et al.</i> , [2000])				ASSUMPTION	Proportion of <i>BRCA1</i> large rearrangements or non-coding region mutations	Proportion of <i>BRCA1</i> point mutations/small rearrangements detected by EX/SP screen
EX/SP +ve (62 families)	EX/SP -ve (44 families) (only 42 of these families were analysed in Unger <i>et al.</i> , [2000], see text)					
A. 55 families Point mutations/SR in <i>BRCA1</i> (CSGE)	A'. 7 families Point mutations/SR in <i>BRCA2</i> (CSGE)	B. 5 families +ve for large genomic rearrangements or non-coding region mutations in <i>BRCA1</i>		All C families false-ve	B/A+B+C= 5/99=5.1%	A/A+B+C= 55/99=55.6%
		1 family: exon 13 duplication (specific PCR detection)	4 families: del exon 8-9, del exon 1-2, del exon 1 and 2, del exon 17-19 (Southern blot)			
		C. 39 families -ve after specific detection of the exon 13 duplication in <i>BRCA1</i> , and Southern blot analysis in <i>BRCA1</i>		All C families true-ve	B/A+B= 5/60=8.3%	A/A+B= 55/60=91.7%

TABLE G-4b.

Sinilnikova <i>et al.</i> , [unpublished] 48 Br-Ov families (reported by Unger <i>et al.</i> , [2000])				ASSUMPTION	Proportion of <i>BRCA1</i> large rearrangements or non-coding region mutations	Proportion of <i>BRCA1</i> point mutations/small rearrangements detected by EX/SP screen
EX/SP +ve (32 families)		EX/SP –ve (16 families)				
A. 29 families Point mutations/SR in <i>BRCA1</i> (technique not reported)	A'. 3 families Point mutations/SR in <i>BRCA2</i> (technique not reported)	B. 5 families +ve for large genomic rearrangements in <i>BRCA1</i> Types of rearrangements not described and technique not reported	C. 11 families –ve after EX/SP screen in <i>BRCA1/2</i> , and after screening for large rearrangements	All C families false-ve	B/A+B+C= 5/45=11.1%	A/A+B+C= 29/45=64.4%
				All C families true-ve	B/A+B= 5/34=14.7%	A/A+B= 29/34=85.3%

TABLE G-5.

Gad <i>et al.</i> , [2002] 120 French Br-Ov families (complementary analysis in Casilli <i>et al.</i> , [2002])				ASSUMPTION	Proportion of <i>BRCA1</i> large rearrangements or non-coding region mutations	Proportion of <i>BRCA1</i> point mutations/small rearrangements detected by EX/SP screen	
EX/SP +ve (52 families)			EX/SP -ve (68 families)				
A. 38 families Point mutations/SR in <i>BRCA1</i> (DGGE or DHPLC)	A'. 14 families Point/SR and truncated mutations in <i>BRCA2</i> (FAMA/PTT of exons 10-11 or DHPLC)	B. 5 families +ve for large genomic rearrangements or non-coding region mutations in <i>BRCA1</i>		C. 63 families -ve after Bar Code method and QMPSF† in <i>BRCA1</i> † QMPSF done by Casilli <i>et al.</i> , [2002]	All C families false-ve	B/A+B+C= 5/106=4.7%	A/A+B+C= 38/106=35.8%
		4 families: 2 families with del exon 8-13; 1 family with dup exon 3-8; 1 family with dup exon 18-20 (detected by the Bar Code method) in Gad <i>et al.</i> , [2002]	1 family*: del exon 1-22 (detected by QMPSF) [Casilli <i>et al.</i> , 2002] *this family was also analyzed by Gad <i>et al.</i> , [2002] but the deletion was not detected by the Bar Code method				
				All C families true-ve	B/A+B= 5/43=11.6%	A/A+B= 38/43=88.4%	

TABLE G-6.

Montagna <i>et al.</i> , [2003] 37 Italian Br-Ov families					ASSUMPTION	Proportion of <i>BRCA1</i> large rearrangements or non-coding region mutations	Proportion of <i>BRCA1</i> point mutations/small rearrangements detected by EX/SP screen
EX/SP +ve (14 families)		EX/SP –ve (23 families)					
A. 9 families Point mutations/SR in <i>BRCA1</i> (SSCP+PTT and DHPLC)	A'. 5 families Point mutations/SR in <i>BRCA2</i> (SSCP+PTT and DHPLC)	B. 6 families +ve for large genomic rearrangements or non-coding region mutations in <i>BRCA1</i>	C. 17 families -ve after MLPA in <i>BRCA1</i>	Uninformative for heterozygosity of SNPs (single-nucleotide polymorphisms) in the <i>BRCA1</i> gene	All C families false-ve	B/A+B+C=6/32=18.8%	A/A+B+C=9/32=28.1%
		<u>1</u> family: 3kb del exon 17 (Southern blot)	<u>5</u> families: del exon 1a-2 (twice), del exon 9-19, del exon 18-19, del exon 20 (MLPA)		All C families true-ve	B/A+B=6/15=40.0%	A/A+B=9/15=60.0%

TABLE G-7.

Petrij-Bosch <i>et al.</i> , [1997] 170 high-risk Dutch families				ASSUMPTION	Proportion of <i>BRCA1</i> large rearrangements or non-coding region mutations†	Proportion of <i>BRCA1</i> point mutations/small rearrangements detected by EX/SP screen
EX/SP +ve (33 families)	EX/SP –ve (137 families)					
A. 21 families Point/SR and truncated mutations in <i>BRCA1</i> (PTT/PCR-FLA) <i>FLA</i> denotes fragment length analysis, similar to SSCP, DGGE (no other details)	A'. 12 families Point/SR and truncated mutations in <i>BRCA2</i> (PTT/PCR-FLA)	B. 13 families +ve for large genomic rearrangements or non-coding region mutations in <i>BRCA1</i> B'. 1 family exon 22 del caused by IVS22 +5G->A (detected by routine PCR and confirmed by RT-PCR) B''. 12 families del 510bp (exon 22) or del 3835bp (exon 13) (specific detection by Southern blot)	C. 124 families* -ve after specific detection in <i>BRCA1</i> of del 510bp (exon 22), del 3835bp (exon 13) and del exon 22 caused by IVS22 +5G->A by Southern blot *58/124 families analyzed by Southern blot for <i>BRCA2</i> but all 58 were –ve (in Peelen <i>et al.</i> , [2000b])	All C families false-ve	B'+B''/A+B'+B''+C=13/158=8.2% B''/A+B'+C 12/157=7.6%†	A/A+B'+B''+C=21/158=13.3%
				All C families true-ve	B'+B''/A+B'+B''=13/34=38.2% B''/A+B'+B''=12/34=35.3%†	A/A+B'=21/34=61.8%

†the second set of figures refers to del 510bp and del 3835 bp only

TABLE G-8.

Hogervorst <i>et al.</i> , [2003] 805 Dutch Br (79%), Br-Ov (18%) and Ov (3%) cancer families				
EX/SP +ve (116 families)		EX/SP –ve		ASSUMPTION
A. 88 families Point mutations/SR in <i>BRCA1</i> (DGGE, DHPLC or PTT)	A'. 28 families Point mutations/SR in <i>BRCA2</i>	B. 33 families +ve for large genomic rearrangements or non-coding region mutations in <i>BRCA1</i> B' : 28 families del 13 or del 22 (mutation-specific tests)	B'' : 5 families del exons 8; del exon 20-22; dupl exon 13; dupl exon 21-23; tripl exon 17-19 (MLPA)	
		C. 656 families -ve after MLPA		Proportion of <i>BRCA1</i> large rearrangements or non-coding region mutations
				Proportion of <i>BRCA1</i> point mutations/small rearrangements detected by EX/SP screen
				A/A+B+C= 88/777=11.3%
				A/A+B= 88/121=72.7%

TABLE G-9.

Hartmann <i>et al.</i> , [2004] 140 High risk Br+/-Ov families from southern Germany				
EX/SP +ve (65 families)		EX/SP -ve (75 families)		ASSUMPTION
A. 48 families Point mutations /SR in <i>BRCA1</i> (DHPLC + SEQ)	A'. 17 families Point mutations /SR in <i>BRCA2</i> (DHPLC + SEQ)	B. 4 families +ve for large genomic rearrangements or non-coding region mutations in <i>BRCA1</i> <u>2 families:</u> exons 1a, 1b, and 2 deletion (MLPA)	C. 71 families -ve after MLPA	

TABLE G-10.

Schubert <i>et al.</i> , [1997] 17 AJ Br-Ov families				ASSUMPTION	Proportion of non-common <i>BRCA1/2</i> mutations in AJ population	Proportion of <i>BRCA1/2</i> common mutations detected
+ve after screening for 3 common AJ <i>BRCA1/2</i> mutations (8 families)	-ve after screening for 3 common AJ <i>BRCA1/2</i> mutations (9 families)					
A. 8 families 6 families: <i>BRCA1</i> 185delAG 1 family: <i>BRCA1</i> 5382insC 1 family: <i>BRCA2</i> 6174delT (technique not reported)	B. 1 family +ve for a non-common <i>BRCA1/2</i> mutation	C. 8 families -ve after EX/SP screen for <i>BRCA1</i> by SSCP and for <i>BRCA2</i> by SSCP/PTT of exons 10-11		All C families false-ve	B/A+B+C= 1/17=5.9%	A/A+B+C= 8/17=47.1%
	Point mutation in <i>BRCA2</i> : 6425 delTT (EX/SP screen by SSCP/PTT of exons 10-11)			All C families true-ve	B/A+B= 1/9=11.1%	A/A+B= 8/9=88.9%

Abbreviations: AJ=Ashkenazi Jewish

TABLE G-11.

Frank <i>et al.</i> , [1998] 47 AJ Br-Ov families				ASSUMPTION	Proportion of non-common <i>BRCA1/2</i> mutations in AJ population	Proportion of <i>BRCA1/2</i> common mutations detected
+ve using EX/SP (20 families) SEQ of <i>BRCA1</i> and <i>BRCA2</i>		-ve after EX/SP (27 families)				
A. 18 families	B. 2 families	C. 27 families		All C families false-ve	B/A+B+C= 2/47=4.3 %	A/A+B+C= 18/47=38.3%
+ve for one of the 3 common mutations (<i>BRCA1</i> 185delAG; <i>BRCA1</i> 5382insC; <i>BRCA2</i> 6174delT)	+ve for a non-common <i>BRCA1/2</i> mutation			All C families true-ve	B/A+B= 2/20= 10%	A/A+B= 18/20=90%

TABLE G-12.

Phelan <i>et al.</i> , [2002] 245 AJ Br-Ov families					
	-ve after screening for 3 common AJ <i>BRCA1/2</i> mutations (160 families)		ASSUMPTION	Proportion of non-common <i>BRCA1/2</i> mutations in AJ population†	Proportion of <i>BRCA1/2</i> common mutations detected†
+ve after screening for 3 common AJ <i>BRCA1/2</i> mutations (85 families)	+ve after screening for EX/SP in <i>BRCA1</i> (4 families)				
A. 85 families	C. 156 families		All C families false-ve	1. B/A+B+B'+C= 1/245=0.4% 2. B+B'/A+B+B'+C= 4/245=1.6%†	1. A/A+B+B'+C= 85/245=34.7%
44 families: <i>BRCA1</i> 185delAG 16 families: <i>BRCA1</i> 5382insC 25 families: <i>BRCA2</i> 6174delT (PCR multiplex)	B. 1 family Frame shift mutation in <i>BRCA1</i> : 4572del22 (PTT of exon 11 and DGGE for other exons)	B'. 3 families 3 single missense variants of unknown clinical significance			
	All C families true-ve		1. B/A+B= 1/86=1.2% 2. B+B'/A+B+B'= 4/89=4.5%†	1. A/A+B= 85/86=98.8% 2. A/A+B+B'= 85/89=95.5%†	

[†] the second set of figures consider the B' variants to be clinically significant

TABLE G-13.

Huusko <i>et al.</i> , [1998] 88 Finnish Br and/or Ov families, Oulu region				ASSUMPTION	Proportion of non-common <i>BRCA1/2</i> mutations	Proportion of <i>BRCA1/2</i> common mutations detected
+ve after EX/SP screen (11 families) <i>BRCA1</i> : CSGE/PTT exon 11; <i>BRCA2</i> : CSGE/PTT exon 10-11		-ve after EX/SP screen (77 families) C. 77 families				
A. 10 families +ve for 4 common mutations: 3 families: <i>BRCA1</i> 3745delT 3 families: <i>BRCA1</i> 4216 2ntA->G 3 families: <i>BRCA2</i> 9346 2ntA->G 1 family: <i>BRCA2</i> 999del5	B. 1 family +ve for a non-common mutation: <i>BRCA2</i> 6503delTT			All C families false-ve	B/A+B+C= 1/88=1.1%	A/A+B+C= 10/88=11.4%
				All C families true-ve	B/A+B= 1/11=9.1%	A/A+B= 10/11=90.9%

TABLE G-14.

Vehmanen <i>et al.</i> , [1997a] 100 Finnish Br and/or Ov families, Helsinki and southern regions (complementary analysis in Vehmanen <i>et al.</i> , [1997b])					ASSUMPTION	Proportion of non-common <i>BRCA1/2</i> mutations	Proportion of <i>BRCA1/2</i> common mutations detected
+ve after EX/SP screen (18 families)		-ve after EX/SP screen (82 families)					
EX/SP screen: All 100 families: <i>BRCA1</i> by HDA and SSCP/PTT of exon 11; <i>BRCA2</i> by HA and SSCP/ PTT of exons 10-11 Also, in only 70 families: SEQ of <i>BRCA1</i>		Linkage analysis for <i>BRCA1</i> and 2 done in 12 families -ve after EX/SP screen			All C families false-ve	B/A+A'+A''+B+C=8/93=8.6% 1. 4 mutations reported by Huusko <i>et al.</i> , 1998 (A) 2. all 8 mutations (A+A'+A'')	1. A/ A+A'+A''+B+C=6/93=6.5 % 2. A+A'+A''/ A+A'+A''+B+C=13/93=14%
A. 6 families +ve for common mutations reported by Huusko 1998:	A'. 4 families +ve for other common mutations:	B. 8 families +ve for non-common mutations:	A''. 3 families +ve for one other common mutation: <i>BRCA2</i> 7708C->T (1 family: confirmed by SEQ after linkage; 3 families: ASO done on all families)	C. 72 families -ve after EX/SP screen	D. 7 families excluded for <i>BRCA1</i> and 2 by linkage analysis (true-ve)		
1fam.: <i>BRCA1</i> 3745delT 1fam.: <i>BRCA1</i> 4216 2ntA>G 2 fam.: <i>BRCA2</i> 9346 2ntA>G 2 fam.: <i>BRCA2</i> 999del5	1family: <i>BRCA1</i> 3446C->T' 1family: <i>BRCA1</i> 5370-CT' 2 families: <i>BRCA2</i> 8555T->G	8 distinct <i>BRCA1/2</i> mutations unique to each family				B/A+A'+A''+B=8/21=38.1% All C families true-ve	1. A/A+A'+A''+B=6/21=28.6% 2. A+A'+A''/ A+A'+A''+B=13/21=61.9%

TABLE G-15.

Ramus <i>et al.</i> , [1997] 32 Hungarian Br and/or Ov families				ASSUMPTION	Proportion of non-common <i>BRCA1</i> mutations	Proportion of <i>BRCA1</i> common mutations detected (2 <i>BRCA1</i> mutations)
+ve after EX/SP screen (11 families) <i>BRCA1/2</i> : PTT or SSCA/HDA		-ve after EX/SP screen (21 families)				
A. 6 families +ve for 2 common <i>BRCA1</i> mutations:	B. 5 families +ve for non-common <i>BRCA1/2</i> mutations	C. 21 families		All C families false-ve	B'/A+B'+C=1/28=3.6%	A/A+B'+C=6/28=21.4%
4 families: <i>BRCA1</i> 5382insC 2 families: <i>BRCA1</i> 185delAG	B'. 1 family: <i>BRCA1</i> glu755term B''. 4 families: <i>BRCA2</i> : 6174delIT, Ser2219ter, 8034insAG, 9326insA (unique to each family)			All C families true-ve	B'/A+B'=1/7=14.3 %	A/A+B'=6/7=85.7%

TABLE G-16.

Gorski et al., [2004a] 200 high-risk Polish families (100 Br. Only + 100 Br and Ov)					ASSUMPTION	Proportion of non-common <i>BRCA1/2</i> mutations [†]	Proportion of common <i>BRCA1</i> mutations [†]
+ve after <i>BRCA1/2</i> EX/SP sequencing (140 families)			- ve after EX/SP screen (60 families)				
A. 111 families +ve for three common founder mutations: <i>5382insC</i> (68 fam) <i>C16G</i> (31 fam) <i>4153delA</i> (12 fam)	B. 29 families +ve for non-common mutations or variants of unknown clinical significance		C. 60 families		All C families false-ve	1. B ⁺ B ⁺ /A+B+C = 18/200=9.0% 2. B ⁺ B ⁺ + B ⁺ /A+B+C = 29/200=14.5%	1. A/A+B+C = 111/200=55.5%
	B ⁺ . 11 families +ve for non- <i>BRCA1</i> mutations	B ⁺ . 7 families +ve for non-common <i>BRCA2</i> mutations					
	B ⁺ . 11 families with variants of unknown clinical significance in <i>BRCA2</i>						
	All C families true-ve						

[†] the second set of figures consider the B⁺ variants to be clinically significant

TABLE G-17.

Ladopoulos <i>et al.</i> , [2002] 85 Greek Br and/or Ov families			ASSUMPTION	Proportion of non-common <i>BRCA1/2</i> mutations	Proportion of <i>BRCA1</i> 5382insC common mutations detected
+ve after EX/SP screen (14 families) 1) in 60 fam: <i>BRCA1</i> PTT exon 11, SEQ other <i>BRCA1</i> exons, <i>BRCA2</i> PTT exon 10-11; 2) in 25 fam: SSCP all exons <i>BRCA1</i> , SSCP exon 10-11 <i>BRCA2</i>	-ve after EX/SP screen (71 families) C. 71 families				
A. 7 families +ve for the common mutation <i>BRCA1</i> 5385insC	B. 7 families +ve for other <i>BRCA1/2</i> mutations		All C families false-ve	B/A+B+C= 7/85=8.2%	A/A+B+C= 7/85=8.2%
		All C families true-ve	All C families true-ve	B/A+B= 7/14=50%	A/A+B= 7/14=50%

TABLE G-18.

Simard <i>et al.</i> , [2004] 191 French-Canadian Br and/or Ov families (testing done on an affected case or obligate carrier)				ASSUMPTION	Proportion of non-common <i>BRCA1/2</i> mutations	Proportion of common <i>BRCA1/2</i> mutations detected
+ve after EX/SP screen (56 families) Specific detection of 24 <i>BRCA1/2</i> mutations by targeted sequencing followed by full sequencing of EX/SP regions, if negative		C. 135 families -ve after EX/SP				
A. 42 families : founder mutations <i>BRCA1</i> 4446C→T (17 x) <i>BRCA2</i> 8765delAG (25x)	A' 4 families: other recurrent mutations <i>BRCA1</i> : 2244insA (2x) 2953del3+C (2x)	B. 10 families: unique, non-common mutations <i>BRCA1</i> : 6 mutations <i>BRCA2</i> : 4 mutations	C. 135 families -ve after EX/SP ➤ Southern blot (done in 61 -ve families) ➤ MLPA: <i>BRCA1</i> : done in all 135 -ve families <i>BRCA2</i> : done in 133 of the 135 -ve families		All C families false-ve	1. A/A+A'+B+C=42/191=22% 2. A+A'/A+A'+B+C=46/191=24.1%†
				All C families true-ve	B/A+A'+B=10/56=17.9%	1. A/A+A'+B=42/56=75% 2. A+A'/A+A'+B=46/56=82.1%†

† The second set of figures refer to the 4 recurrent mutations.

TABLE G-19.

Oros <i>et al.</i> , [2004] 169 French-Canadian Br ± Ov families				ASSUMPTION	Proportion of non-common <i>BRCA1/2</i> mutations	Proportion of common <i>BRCA1/2</i> mutations detected
+ve after EX/SP screen (74 families) Specific detection of 20 <i>BRCA1/2</i> mutations in EX/SP regions by SSCP		C. 95 families -ve after specific detection of 20 <i>BRCA1/2</i> mutations in EX/SP regions by SSCP 83 families –ve after: ➤ complete analysis of EX/SP in <i>BRCA1</i> or <i>BRCA2</i> or in both genes by sequencing or ; ➤ partial analysis by PTT in <i>BRCA1</i> exon 11 and <i>BRCA2</i> exons 10-11 or by PTT in <i>BRCA1</i> exon 11				
A. 62 families: founder mutations: A ^{***} : <i>BRCA1</i> 4446C→T (25x) <i>BRCA2</i> 8765delA/G (17x)	A [*] . 4 families: less recurrent mutations <i>BRCA1</i> : 3875delGTCT (2x) <i>BRCA2</i> : 6503delTTT (2 x)	B. 8 families: unique, non-common mutations 8 <i>BRCA1/2</i> mutations ⁺		All C families false-ve	B/A+A ⁺ +B+C 8/169=4.7%	1. A/A+A ⁺ +B+C=62/169=36.7% † 2. A+A [*] /A+A ⁺ +B+C=66/169=39% ‡ 3. A ^{**} /A+A ⁺ +B+C=42/169=24.8%*
Others: <i>BRCA1</i> 2953del3insC (6x) <i>BRCA2</i> : 6085G→T (10x) 3398del1AAAAAG (4x)				All C families true-ve	B/A+A ⁺ +B=8/74=10.8%	1. A/A+A ⁺ +B=62/74=83.8%* 2. A+A [*] /A+A ⁺ +B=66/74=89.2% ‡ 3. A ^{**} /A+A ⁺ +B=42/74=56.8%*

*The 2 founder mutations which are the most frequent in the study by Oros *et al.*, [2004] and in the study by Simard *et al.*, [2004] (Table G-18).+ *BRCA1*: 360C→T, 1081G→A, 5221delTG; *BRCA2*: 2558insA, 2816insA, 3034delAAAC, 3773delTT, 7235G→A.

† for 5 founder mutations; ‡ for 7 recurrent mutations

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