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DUCHENNE AND BECKER MUSCULAR DYSTROPHIES: FAMILY SCREENING AND MOLECULAR DIAGNOSIS
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SUMMARY

The transfer of knowledge from the field of genetics to new clinical applications needs to be evaluated to ensure successful implementation in the overall context of the health-care system. We have taken a keen interest in genetic technologies derived from molecular biology, which has made an increased number of diagnostic and prenatal tests available over the past few years. Of the different pathologies for which molecular genetics is changing medical practice, we have chosen a neuromuscular disease, our priority criteria being severity, incidence, test availability and reliability, and preventive measures. This report summarizes the scientific, medical and genetic knowledge about Duchenne and Becker muscular dystrophies.

Overview of the Disease

Duchenne muscular dystrophy (DMD) is the most serious and most common of the progressive muscular dystrophies. It is characterized by slow muscle-fiber degeneration, which causes a severe handicap and death at about age 20. It is a monogenic, sex-linked recessive disease occurring almost exclusively in boys. The mothers are generally asymptomatic carriers who pass on the disease.

Compared to this very severe form, Becker muscular dystrophy (BMD) follows a later and slower course. It becomes disabling during adult-hood but does not prevent a certain social and professional life. The mean age at death is about 42.

Presently, there is no curative treatment for these extremely serious and disabling diseases, which cause a severe physical and social handicap. Neuromuscular clinics do a multidisciplinary follow-up of these patients in cooperation with medical genetic services. Despite a substantial increase in the level of assistance, it is the families that shoulder most of the burden associated with the loss of autonomy caused by the disease and that suffer its psychological and social consequences.

One in 3,500 male newborns is affected with DMD. In the case of BMD, the figure is one in 20,000. In Québec, it is estimated that about 12 boys with DMD and two with BMD are born each year. There are reportedly about 200 families with a positive family history of DMD / BMD.

Contribution of Genetics

Up until the 1980s, knowledge about the Duchenne and Becker myopathies was limited to the clinical symptomatology, signs of muscle degeneration detected by electromyography and muscle biopsy, the marked elevation in the serum creatine kinase (CK) level, and the location of the causative gene on the X chromosome. The patho-physiologic mechanism and the responsible protein were unknown.

The gene was identified in 1987. It is now known that these two diseases are clinical variants due to mutations in the dystrophin gene, which is located on the short arm of the X chromosome. These

abnormalities in the gene prevent the normal production of dystrophin, a protein essential for nor-mal muscle-membrane function.

Since Duchenne and Becker muscular dystrophies are transmitted from one generation to the next, a preventive approach can be adopted to avert the recurrence of the disease within families. Identifying women who carry the mutated gene is particularly important in the case of X-linked diseases, since they can give birth to affected children and yet not present with any signs of the disease. All women related to an affected individual are, to varying degrees, at risk for being carriers and transmitting the disease to one or even several children.

Previously, the only intervention possible for families was genetic counselling for women at risk (mothers, sisters and maternal aunts of a myopathic individual) based on serum creatine kinase (CK) determinations. However, this strategy was of limited value because it did not permit a definite conclusion as to carrier status. At the time, many couples abstained from having children or used the only type of prenatal diagnosis possible in this situation¾fetal sex determination, which sometimes led to the aborting of male fetuses, even though the probability of their being affected was at most 50% and could not be determined with certainty.

Advances in molecular genetics led to the identification of the gene and its product, dystrophin. Most of the mutations in the dystrophin gene are major rearrangements, which can be detected directly by genotypic analysis. In 65% of cases, the mutations consist of large deletions (loss of one or more segments of DNA) and in about 5% of cases, duplications of DNA segments. The other mutations (30%) are point mutations, which are more difficult to detect. For now, use is made of an indirect method, linkage analysis, in which samples from several family members must be examined.

Thanks to these discoveries, the genetic abnormality can be detected directly in 70% of affected individuals, and with this accurate diagnosis, one can then identify with certainty the heterozygous (carrier) women and the affected fetuses in these families. For the 30% of families where direct mutation detection is not possible with the usual methods, linkage analysis can be used to determine the risk more accurately than if only pedigree data and the results of serum creatine kinase determinations are taken into account. Linkage analysis generally does not permit a definitive diagnosis. The results of such analysis are therefore expressed as the probability of being a carrier. The most notable contribution of molecular analysis is to permit an accurate diagnosis in the fetus. Previously, the only type of prenatal diagnosis available was fetal sex determination, which led to the aborting of male fetuses. Thanks to molecular analysis, male fetuses that are affected can be distinguished from those that are healthy.

Diagnosis-and-Screening Strategy

Generally speaking, it is the mother of an affect-ed child who seeks to find out what the risk of recurrence is for her other children. With an affected individual as a starting point, and based on mendelian laws of inheritance, the probability of each woman in the family (mother, sisters, aunts and cousins) being a carrier of the mutated gene is estimated by a pedigree analysis. Genotypic diagnosis makes it possible to refine the a priori risk, since direct deletion and duplication detection permits a definitive determination of carrier status. Even if linkage analysis has to be per-formed, the risk estimate can be refined considerably. For these families, this results in less uncertainty with regard to reproductive choices. Even if the risk is high, with prenatal diagnosis, pregnancy can be considered. If, on the other hand, the risk is low or nil, the woman is relieved of the anxiety associated with the risk of genetic transmission of the disease.

The screening strategy recommended is therefore family-based, proceeding in cascade from the affected individual. It is therefore intended only for high-risk individuals. This strategy entails both diagnosis and screening, diagnosis because it seeks to confirm or rule out the presence of the disease in a clinically affected person or in a fetus, and screening because it involves identifying the women within a given family who are potential carriers.

This family-based strategy includes three steps: 1) the genotypic diagnosis of the individual with DMD/BMD; 2) the identification of women who are potential carriers, and 3) possibly prenatal diagnosis. Several techniques are used to execute this strategy: multiplex PCR, quantitative PCR (gene dosage) and microsatellite- or RFLP-based linkage analysis. These methods have had a proven track record for several years now and are offered as clinical services elsewhere in Canada and in other countries. Even if these techniques are constantly evolving, they have a high degree of validity and can be applied in most cases.

Québec Context

In Québec, there are medical genetic services that provide genetic counselling and manage the care of families with hereditary diseases. These services work in close collaboration with neuro-muscular disease clinics, where individuals with muscular dystrophy are usually identified.

With support from the Muscular Dystrophy Association of Canada and the Réseau de médecine génétique du Québec, the medical genetics laboratory at Hôpital Sainte-Justine developed, from 1988 to 1991, expertise in Duchenne and Becker muscular dystrophy molecular diagnosis and screening. It was thus possible to offer, for the entire province, a service comprising testing and genetic counselling for the families concerned, and as part of this pilot clinical-research pro-gram, the map of the deletions in the Québec population was laid out. The service was discontinued when the funding ran out. For many families, the picture is still incomplete. Even most of those for whom a family study was done between 1988 and 1991 could benefit from more accurate results, given the new microsatellite-based linkage analyses and gene dosage technologies. Today, clinicians have to limit them-selves to ordering only the most essential tests and are generally obliged for test purposes, to send the specimens, to molecular genetics laboratories in Ontario or Alberta. The turnaround time is such that several women become pregnant before their carrier status is determined. As a result, a certain number of prenatal diagnoses are performed unnecessarily.

Presently, in Québec, public molecular genetics laboratory has been designated to offer the entire population the tests required for the genotypic diagnosis of Duchenne and Becker muscular dystrophies and for carrier screening. Unlike other laboratory services, for which there is an established infrastructure, the molecular genetics diagnostic tests for this disease are usually paid for with research funds or are performed outside Québec or in the private sector, a first centre (Biogénétique) having announced that it was offering genetic services

It is estimated that the demand for molecular tests for DMD/BMD will level off when all the affected families have been reached and when the testing that has already begun is completed. To determine the number of tests that would be needed, a distinction must be made between an initial phase, during which the backlog would be cleared, and a subsequent levelling-off phase. For the catch-up phase, the number of tests to be planned is not insignificant, but a laboratory devoted to this type of pathology and which has adequate resources could clear this backlog in one year. As for the levelling-off phase, the number of tests should remain relatively low, since the indications are based on familial risk.

Because of this low volume, and to ensure the maintenance of a quality service, all the tests should be performed in the same laboratory.

Conclusion

It is therefore important that families in which there is an individual with muscular dystrophy have access to family screening. The main objective of the proposed diagnosis-and-screening strategy is to prevent the recurrence of the disease in the affected families and to enable the women to have healthy children. These families should therefore have access to medical genetic services and a laboratory service providing the different tests required for the genotypic diagnosis of muscular dystrophy and for carrier screening.

Because of the complexity of hereditary diseases and of genetic testing, several conditions must be met. The offer of testing must be accompanied by genetic counselling: 1) so as to be able to coordinate the management of the whole of families; 2) so that the information is communicated to them properly; and 3) so that pedigree analysis remains the basis of the indication for testing. This practice requires compliance with certain ethical considerations, such as voluntary and informed consent, confidentiality and noninterference in private decisions.

To ensure that the tests are interpreted correctly, there must be close coordination between the medical genetic services and the laboratories. Furthermore, because of the rapid developments in molecular genetics, the necessary expertise cannot be maintained without close ties between the diagnostic laboratories and research laboratories. For these reasons, the quality of the service offered will depend on the organizational choice that will be made.

The organization of genetic services and laboratories is, for the future, an important and essential issue, as is the development of private laboratories. The planned organization of molecular genetics laboratories is extremely important if we are to avoid the needless proliferation of services and ensure the best possible integration of new applications into health care and optimal coordination between the different services.

Given the specificity of genetic analyses, the difficulties that their interpretation poses, and the importance of the geneticist's role, it seems that the laboratory-specialized-by-pathology model is advisable. However, because of the cost of setting up a molecular genetics diagnostic laboratory and the relatively small number of tests for each hereditary disease, this infrastructure could be used for several diseases. It would be important to assess the molecular diagnostic needs for the entire province and for all diseases.

A service was offered for a few years as part of a pilot project, and the expertise in molecular bio-logy and a research laboratory do exist in Quebec. Yet the patients' needs and especially those of their families are not being met at the present time. It is important to note that once the backlog is cleared, the demand for this service will level off and that an exponential increase in the demand or costs should therefore not be expected.

In conclusion, the *Conseil* considers that the conditions are in place for recommending that a service be instituted for the genotypic diagnosis of patients with Duchenne or Becker muscular dystrophy. The service would include screening for women carrying a mutation on the dystrophin gene and, if necessary, the prenatal diagnosis of the disease. A single laboratory

would suffice to meet the province's needs in this area. It would have to be accredited and meet the required quality standards .