GUEST EDITORIAL

NEW INSIGHT INTO GENETIC DISEASE. THE ROLE OF TRINUCLEOTIDE REPEAT EXPANSIONS

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The development of genetics in the last few decades is replete with surprise phenomena and new findings. Some of these offer explanations for old observations which had previously been controversial and puzzling. One such phenomenon is the trinucleotide repeat expansion, a new type of mutation first discovered in 1991. This type of mutation is found in a number of diseases including myotonic dystrophy, familial mental retardation (Fragile X syndrome), Huntington's disease, spinocerebellar ataxia type I (Machado-Joseph disease) and spinobulbar muscular atrophy (Kennedy Disease). These genetic diseases usually appear late in life and show the unusual phenomenon of anticipation in which the disease appears earlier and increases in severity in subsequent generations. Furthermore anticipation is sex-dependent occurring if the transmitting parent is the father in Huntington's disease and spinocerebellar atrophy and if the transmitting parent is the mother in myotonic dystrophy and the fragile X syndrome.

Trinucleotide repeats occurring in the middle of a gene are normal and quite frequent findings. The most common trinucleotide repeats are $(CAG)_n$, $(CGG)_n$ or $(CTG)_n$. A mutation arises when the segment of repeats becomes unstable and expands during meiosis; the disease occurs when the repeat length exceeds a critical size. The trinucleotide repeat $(CAG)_n$ is present in the huntingtin gene (the gene responsible for Huntington's disease) on chromosome 4p; in normal individuals 2 to 35 repeats are present but individuals with Huntington's disease have 40 to 120 repeats.[1] Similarly Machado-Joseph disease is associated with expansion of $(CAG)_n$ repeats in the ataxin gene on chromosome 6p from the normal range of 6-39 to the disease range 40-81 repeats.[2] In myotonic dystrophy the trinucleotides $(CTG)_n$ in the myotonin kinase gene may be expanded up to 1500 repeats.[3,4]

Anticipation

Myotonic dystrophy is the classic example of anticipation. The disease usually appears in early adult life causing weakness of the facial and distal limb muscles, myotonia, cataracts, and other symptoms. Since the turn of the century it was known that myotonic dystrophy can manifest itself with increasing severity in successive generations and sometimes appears as the congenital type with severe respiratory and feeding problems and impaired psychomotor development. The striking feature is that congenital myotonic dystrophy is almost exclusively transmitted by an affected mother, whereas the paternally transmitted cases are the adult onset type. The expanded trinucleotides $(CTG)_n$ are transmitted virtually unaltered by affected fathers but are unstable during meiosis in affected women and may undergo expansion by several hundred times. A mildly affected mother having 50 to 100 repeats may have a child with congenital myotonic dystrophy and over 1000 repeats.[5]

A similar but less marked form of anticipation is noted in Huntington's disease in which the disease usually appears between 25 and 70 years but occasionally starts in childhood as juvenile chorea. In this case anticipation occurs when the father is affected but is gradual, being noticeable over several generations. In paternal transmission of Huntington's disease an average expansion of 3 CAG repeats occurs in each generation.[6]

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Pre-mutations

In Familial Mental Retardation type 1 (FMR1 or the Fragile X syndrome) there is a special situation because the condition is X-linked. The trinucleotide $(CGG)_n$ in the FMR1 gene on the X chromosome has a length of 6 to 54 repeats in normal individuals while affected boys, who are mentally retarded and have mild facial dysmorphic features, and normal carrier females have 250 - 800 repeats [7,8]. When the number of $(CGG)_n$ repeats is between 70 and 200 it is termed a pre-mutation since it is not large enough to cause mental retardation in boys. The pre-mutation is transmitted by normal carrier males to all their daughters in whom it may expand during meiosis causing mental retardation in half of their sons.

Heterogeneity

At present ten diseases are known to be caused by trinucleotide repeat expansions [9] but the list is expected to grow longer. Genetic heterogeneity often exists in diseases which clinically appear to be very similar but are recognisable as distinct entities by genetic studies. In some cases of fragile X syndrome it was immediately apparent that the classical trinucleotide $(CGG)_n$ expansion at the FRAX A (FMR1) gene locus was absent. However, a similar $(CGG)_n$ expansion was identified in another locus distinct from the FRAX-A and known as FRAX-E [10]. More than 60 X-linked mental retardation syndromes are known[11] and it is possible that other trinucleotide expansions may come to light as these genes are further characterised.

A similar situation has been discovered in some families with clinical features resembling Huntington's Disease in which the expected CAG expansion was lacking. This led to the identification of the new syndrome of dentatorubral-pallidoluysian atrophy (DRPLA), so called from the brain nuclei involved. Like Huntington's disease it is caused by a (CAG)n expansion, not in the huntingtin locus on chromosome 4p but in the DRPLA locus on chromosome 12p. There is, as expected, a remarkably high correlation between the size of the repeat and the age of onset and other clinical manifestations of DRPLA.[12]

Genetic implications

The implications of trinucleotide expansions as a cause of genetic diseases, are very far-reaching. Firstly this is a type of mutation which differs radically from the classical concept of mutations. Classically a mutation is a single chance event in which a genetic message is altered by a change in the sequence of nucleotides. The mutant gene is subsequently transmitted without further change through generations. However, trinucleotide expansions are dynamic mutations because they continue to change as they are transmitted through generations.[9] Besides, they do not alter the sequence of the nucleotides which constitute the genetic message. Indeed the trinucleotide repeats are often situated in the introns or non-coding segments of the gene.

The significance of trinucleotide repeats in the normal genome is still largely unknown. There are puzzling problems, but ones of tremendous importance, as to how these expansions give rise to such severe diseases and what causes the repeat segments to become unstable. Possible answers to the first problem have begun to emerge. Trinucleotide expansions are known to be associated with excessive methylation, and hypermethylation in its turn may inactivate or alter the expression of the gene.[9,13] Since methylation is a gradual process it might also partly explain the late onset of this class of genetic diseases.

The mechanism of expansion of the gene during meiosis is more difficult to explain. The instability of the gene is not limited to meiosis but may also occur during mitosis. There is ample evidence of variations in the size of the repeats in different tissues of the same individual.[14,15] However, the factors affecting the stability of trinucleotide repeats are still largely unknown.

Clinical implications

There are profound clinical and practical implications of direct concern to affected families, physicians and general practitioners. Direct identification of the trinucleotide expansions enables, not only confirmation of the mutation in affected individuals, but also presymtomatic testing of their relatives who are at risk of developing a late onset inherited disease such as Huntington's disease or spinocerebellar ataxia. Presymptomatic DNA testing must be accompanied by a well-organised program of genetic counselling, psychological preparation and support ensuring that the individual is aware of the possible serious implications of a positive result and is able to cope with them. For this reason predictive testing is not usually performed on children. Prenatal testing of foetuses is also possible but raises deeply controversial ethical issues.

The genetic facts are of direct concern to clinicians as well as geneticists. Thus a physician who recognizes mild symptoms of myotonic dystrophy in a young woman is responsible for referring her for genetic testing and counselling regarding her high risk of having a seriously affected child. Similarly, the genetic diagnosis of familial mental retardation in a boy carries with it the obligation of counselling the rest of his family particularly regarding the risk to his normal sisters of having a severely affected child and the possibility of DNA testing for the presence of a mutation or pre-mutation.

In the present age of molecular genetics all physicians must be informed and educated about the implications of genetic diseases.[16] Equally, there must be appropriate facilities for genetic testing and counselling and it is the responsibility of health authorities to ensure that such facilities are available and adequate.

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