The genetic background of anticipation

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Anticipation was controversial

Anticipation may be defined as the occurrence of a genetic disorder at progressively earlier ages in successive generations. The disease moreover occurs with increasing severity. The concept emerged early in this century mainly through descriptive clinical studies of myotonic dystrophy[1,2]. Later studies have added other disease entities to a list of states showing anticipation, the most notable being Huntington's disease[3]. In one form of inherited mental retardation, the fragile X syndrome, the term 'the Sherman paradox' describes a very similar phenomenon[4].

Towards the middle of this century, basic research in genetics had given us a much clearer understanding of Mendelian inheritance. It became increasingly difficult to reconcile the originally described phenomenon of anticipation with a concept of genes as stable elements only changed by the rare mutation. Leading geneticists therefore regarded the term with scepticism to say the least. Lionel Penrose in 1948 dismissed the clinical data, and concluded that postulated anticipation was the result of observational and ascertainment bias[5]. This view was largely accepted, perhaps in a rather uncritical manner, by leading geneticists. An important textbook of genetics denied the validity of the term as late as in 1986[6]. The era when geneticists solved any problem concerning inheritance with statistical treatment of data, good or bad, was coming to an end, however. Molecular genetics revealed that DNA is not as stable as was thought[7]. Even careful family studies could at this time show that anticipation was fact and not fiction[8].

Anticipation in the clinical setting

In myotonic dystrophy (MD) Fleischer[1,2] demonstrated that the disease showed remarkable evolution in subsequent generations. He studied families with common ancestors. In the first generations neither the family histories nor early deaths indicated the occurrence of MD. There often followed one or two generations with patients showing only senile or presenile cataracts. The subsequent generation then included patients with classical MD. In this, and also later generations, celibacy, childless marriages, mental impairment and increased infant mortality were observed. The sequence of events often ends in congenital MD with its severe clinical manifestation of mental retardation and muscular dystrophy. Later, clinical studies confirmed these observations and described a dominant inheritance pattern which could not be explained by classical Mendelian mechanisms[8].

Another phenomenon which did not fit easily into the concepts of genetics was the finding that congenital MD was transmitted almost exclusively via affected mothers[9].

In the fragile X syndrome, anticipation is manifested in a different manner. This is the most common cause of familial mental retardation. It segregates in families as an X-linked dominant disorder with reduced penetrance. When chromosomes are stained a fragile site on the X chromosome may be seen in a proportion of cells taken from a patient. Again, it was observed that the chances of having the disease increase in successive generations of a family. Approximately 30% of carrier females were affected, and 20% of males carrying the fragile X chromosome were phenotypically normal, but were able to transmit the disorder to their grandsons (the Sherman paradox)[4]. In addition to the mental retardation, which is variable in severity, affected males exhibit additional clinical features including macroorchidism and distinctive facies.

The clinical picture of Huntington's disease (HD) is one of distinctive choreic movements. The disease typically has a subtle, insidious onset in the fourth to fifth decade of life which gradually worsens with mental impairment. Death occurs after 10–20 years. It is inherited in an autosomal dominant fashion. Anticipation is less dramatically manifested, but progressively earlier age of onset may be observed in successive generations. When the disease manifests itself at a very early age the clinical picture may be more severe. Rigidity and a more rapid course may be seen. In this disorder anticipation is observed mainly when the entity is inherited through the father.

The molecular genetics of anticipation

Classical genetics, as it emerged through studies in the first half of this century, taught us that genes as units of inheritance were stable elements only changed by the rare mutation. These mutations were again stable and inheritable. Molecular genetics has revealed that this concept cannot be

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maintained. There are sequences on the genome that mutate frequently. They may, in some instances, change each time they are transmitted from parents to their children. In this discovery we can see an aspect of the effect of human genome mapping which is not often focused upon: the revelation of the totally unexpected. The geneticists found what they were not looking for, and the unstable genetic elements proved to be of immense interest with regard to our understanding of important inherited diseases.

Unstable DNA occurs in the repetitive DNA sequences which are common in the human genome. Each repeat may contain from two to hundreds of nucleotides repeating from a few to thousands of times: the term simple tandem repeat has been used. They often show a high degree of variability between individuals, in other words different individuals have different numbers of repeats on their chromosomes. There are even usually varying numbers of repeats on the two chromosomes of the same individual. Nothing is known of their normal function, but the interpersonal variability has been employed for the unique identification potential which they confer (genetic fingerprints)[7]. These extremely polymorphic systems have also been used extensively for gene mapping purposes.

The connection between this phenomenon of unstable DNA repeats and anticipation was made in 1991 when different groups identified, and subsequently characterized, the gene causing the fragile X syndrome[10–12]. Of the four different bases making up human DNA [adenine (A), cytosine (C), guanine (G) and thymine (T)] a repeating series of the trinucleotide containing CGG was found in this position at the fragile site. In the normal X chromosome between six and 50 repeating units were found. The chromosomes containing the fragile site have from 250 to several thousand units of CGG. In the intermediate range may be found the normal carriers. The repeating region of the carriers is highly unstable and tends to expand from generation to generation. Manifest disease thus appears. Expansion more often occurs when the repeat is transmitted from women to children of either sex. The repeat is located in the untranslated part of the gene. The function of the protein coded for by the gene is unknown, but expression of the fragile X syndrome is associated with direct inactivation of the gene.

In the next couple of years at least seven different disease states were shown to be caused by expanding trinucleotide repeats, the best known being MD and HD. The others include the rare entities spinobulbar muscular atrophy, spinocerebellar ataxia type I, dentatorubral and pallidoluysian atrophy and the so-called FRAXE syndrome.

The MD gene is located on chromosome 19. The gene was cloned and shown to contain a triplet repeat undergoing expansion in MD[13–15]. The trinucleotide repeat contains the base sequence cytosine–thymine—guanine (CTG). In a normal population sample of 282 individuals all chromosomes showed from five to maximally 27 repeats. The normal variability was large. In these individuals more than 75% were heterozygous, i.e. they had different numbers of repeats on their two chromosomes. In MD populations it has been demonstrated that patients with 50 to 80 repeats on the extended chromosome have very slight manifestations of disease, and symptoms and signs appear late in life. The more severely affected patients have from 100 to thousands of triplet copies. The genetic basis of anticipation can clearly be demonstrated. The patients with the largest repeats have earlier onset and more severe disease manifestations. Patients with congenital MD all have very large CTG repeats.

There is good evidence from MD population studies that instability is associated with certain haplotypes[16]. This means that certain chromosomes seem predestined to instability. One, or a few mutations may lay behind all observed cases in the populations studied so far. It is at present not possible to identify the normal-number triplet repeats that in the future will undergo expansion.

The MD triplet repeat is transcribed, but is situated in the untranslated part of the gene. The gene codes for a polypeptide that is a member of the protein kinase family. The expanded repeat seems to inhibit the expression of the gene[17,18]. Lack of the gene product may explain disease manifestations although little is known about exact disease mechanisms. A puzzling and unexplained aspect of MD genetics is the obervation that congenital MD is only inherited from the mother.

The HD gene has been mapped to the short arm of chromosome 4. The gene has been cloned and codes for an aminoacid sequence that does not correspond with any known protein[19]. The normal gene contains between six and 37 repeats of the nucleotide sequence containing cytosine–adenine–guanine (CAG). Individuals who inherit more than 37 repeats from one parent are at high risk of developing HD. There may be a slight overlap between normals and patients in the 30–39 range. The magnitude of the expansion is less dramatic in HD than in fragile X and MD. The largest repeats found are in the range 60–70. The repeat tends to expand through the generations, but the phenomenon of anticipation is less pronounced than in the two previously described disorders. Large repeats are, however, associated with juvenile onset and marked rigidity. Expansion leading to manifest disease is most often seen in HD when the gene is transmitted from the father[3].

The triplet repeat is located in the transcribed and translated part of the gene. CAG codes for the aminoacid glutamine, and the protein thus contains a polyglutamine stretch. The function of the protein is unknown.

There is evidence indicating that HD develops from one or a few predisposing ancestral mutations. The mutant gene

may be inherited for generations without undergoing expansion. There is, thus, a pool of dormant disease genes in the population.

Why do some triple repeats in the genome undergo expansion or what some authors call dynamic mutations? Nothing is known about the mechanisms that go wrong in the replication process. Simple meiotic recombination is not important. In the fragile X syndrome the risk of expansion correlates well with the size of the allele. It has been postulated that these repeats are predisposed to slippage. The replicating mechanism may thus copy the repeat a small number of times. Slippage during replication may also occur if the DNA strand being replicated breaks. It should, however, be obvious that triplet repeats in general are not inherently unstable. We all have many of them. An error in DNA repair mechanisms has been suggested as another possible predisposing factor.

In several of these conditions a striking parental sex bias has been observed. Again, nothing is as yet known about the mechanism behind the preference for triplet expansion in one of the sexes.

May these repeats leading to disease sometimes undergo contraction, become smaller, in the process of replication? There is indeed evidence both from the fragile X syndrome and MD that this may happen, but the phenomenon seems to be very rare.

Triplet expansion leading to disease has so far been seen in nervous system disorders only. Whether this mechanism may operate in genes mainly expressed in other organ systems remains to be seen. Geneticists are, however, eagerly looking for trinucleotide repeats in several different disorders, including cancer.

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