0014-4754/94/121099-07$1.50 + 0.20

Reviews

Heritable trinucleotide repeats and neurological disorders

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Received 13 April 1994; accepted 22 July 1994

Abstract. In the past 3 years, seven human neurological disorders have been found to be associated with an abnormal number of unstable trinucleotide repeats within exons or non-expressed regions of a gene. These forms of mutations are called dynamic mutations. The expansion in copy number of trinucleotide repeats may represent a large number of hereditary disorders. The correlation between the length of the repeated size and the disease severity and variable onset has provided some genetic explanation for a phenomenon called anticipation. However, there are numerous questions which cannot be explained by anticipation. Many other factors such as genomic imprinting and variable DNA methylation may also contribute to the puzzling features of these phenotypes.
Key words. Anticipation; triplet expansion; unstable DNA, hereditary; dynamic mutation.

Introduction

One of the most exciting challenges in neuroscience is to understand the molecular basis for certain major neurological disorders such as Huntington's disease, Alzheimer's disease, schizophrenia and manic depressive illness. These disorders have unusual genetic features in that there is an extreme variability between different members of the same family, time of onset and increase in severity over generations. These kinds of genetic events are hard to explain by the rules of classical Mendelian genetics. Applications of molecular genetic techniques are successful in many single gene disorders such as cystic fibrosis and muscular dystrophy, but until recently, they were of little help in understanding complex disorders. However, recent molecular cloning of certain neurological disease genes has changed this perspective and opened up new opportunities by which the mechanisms underlying the pathogenesis can now be undertaken. To date, candidate genes for seven neurological disorders have been shown to contain an unusual mutation, that of triplet repeats[5, 57]. Four of these disorders are of the autosomal and three of the X-linked type. In this short review, an attempt is made to summarize this important discovery. For the purpose of discussion, five widely known disorders, whose candidate genes have been isolated, are selected and the remaining disorders are summarized in a table.

Spinal and bulbar muscular atrophy (SBMA)

This is a rare X-linked recessive disorder that is also known as Kennedy's disease[27]. The disorder is characterized by progressive muscle weakness accompanied by mental retardation. It is an adult late-onset form of motorneuron disease where the affected males also show reduced fertility. Most female carriers do not show any symptoms. Linkage analysis has localized the disease gene to Xq11–12 which has been assigned to the human androgen receptor gene[9, 34]. An investigation on the androgen receptor gene revealed a small expanded CAG triplet in the coding domain of the gene (fig.). The repeat length in the patient was double that in several control samples (table), and segregated with the disease. Moreover, the degree of expansion correlates with the severity of the disease[35, 53], suggesting that the phenotype is determined by this unusual mutation. The disease-associated alleles are also found to be unstable from generation to generation.

The functions of polyglutamine residues encoded by the expanded triplet repeats are currently unknown. Based on the anology that a number of transcription factors contain a long stretch of polyglutamine[26, 37, 42] in their activation domain, it has been speculated that the amplified CAG repeats in the androgen receptor gene could be involved in transcriptional regulation. Since androgen is known to affect motorneuron growth and development[33, 64], it is possible that an alteration in the structure of the multifunctional androgen receptor may impose an impaired interaction with androgen in motorneurons, thereby contributing to the degeneration of the cell. However, it should be noted that in this disorder, large expansion does not occur to the extent seen in other disorders (see below) and this mutation does not have any apparent effect on the sexual development in the patient.

Fragile X-syndrome (FMR-1)

The fragile X-syndrome is one of the most common X-linked recessive genetic disorders that results in familial

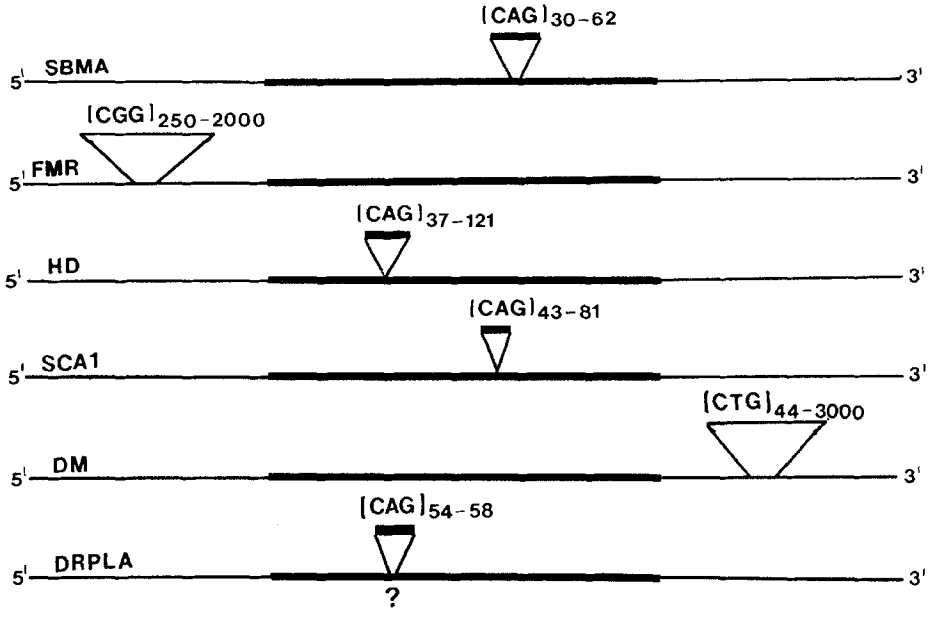

Figure. A schematic representation of genes containing trinucleotide repeats which are implicated in neurological diseases. Thick and thin bars denote coding and non-coding sequences, respectively. Approximate location of triplet repeats is shown as inverted triangles. The numbers give their abnormal repeat ranges. The exact location of the repeats in DRPLA disorder is not known. Maps are not drawn to scale.

mental handicap[48]. It affects one in 2000 births[56]. In addition to mental retardation, testicular enlargement, connective tissue abnormalities and anatomical defects (large head and ears, long face) have also been described[20]. Males are more severely affected than females. The segregation of the disease trait is unusual with transmission by normal males. These males do not harbor a fragile site, considered to be normal and are referred to as normal transmitting males. The females who carry the fragile X are minimally affected. The disorder shows an increasing severity in successive generations and hence genetic counselling is difficult. Linkage analysis[60] has localized the defective region to the long arm of the X-chromosome (Xq27.3). The disorder has recently been shown to be associated with a point mutation, a deletion or a heritable DNA sequence that

Table. Neurological disorders of trinucleotide repeat

Disorder	Chromosome linkage	Gene	Trinucleotide repeats	Amino acid repeats	Normal number of copies	Range of repetition in disease	Region of repetition	Incidence %	Ref.
Spinal and bulbar muscular atrophy	Xq21.3	Androgen receptor	CAG	Glutamine	13–33	30–62	coding	0.0017	34
Fragile X-syndrome	Xq27.3	FMR-1	CGG	–	6–54	250–2000	non-coding	0.050	12,30,46
FRAXE	Xq28	FRAXE	GCC	Alanine	6–25	25–200	?	-	28
Huntington disease	4p16.3	IT15	CAG	Glutamine	9–37	37–121	coding	0.010	23
Spino-cerebellar ataxia Type 1	6p24	unknown function	CAG	Glutamine	25–36	43–81	likely to be coding	0.001	49
Myotonic dystrophy	19q13.3	Myotonin kinase	CTG	–	5–37	44–3000	non-coding	0.013	8,16,40
Dentato-rubral-Pallidoluysian atrophy (DRPLA)	12p	unknown	CAG	Glutamine	8–25	54–58	likely to be coding	?	29,45

consists of an abnormal number of triplet repeats in a 5′-untranslated region of a FMR-1 gene[12, 15, 30, 46, 62, 65, 66]. Although the function of this gene is unknown, analysis reveals that there are 6–54 tandemly repeated CGG triplets in the normal population whereas affected individuals contain 250–2000 repeats. The premutation alleles present in the normal population do not cause disease, but, when amplified, lead to the disorder. Both female and male fragile X carriers contain 70–200 copies. The disease-associated allele varies within the family and is unstable when amplified beyond a certain size. How this unstable sequence becomes responsible for the clinical disorder remains unclear.

The FMR-1 gene is found to be highly expressed in the brain, testis and the eye. Interestingly, male patients show greatly reduced levels of FMR-1 transcript[50]. This reduced level of FMR-1 RNA could be due to the methylation of the CpG island located upstream from the transcription site[7, 28, 63]; furthermore, methylation could play a critical role in the transcriptional activity of the FMR-1 gene. The function of polyarginine-containing FMR-1 gene is unknown. Based on the observation that similar polyarginine rich proteins such as histones and protamines bind to DNA, it has been proposed[62] that the FMR-1 product may be a DNA binding protein. However, how this alteration in the gene causes the phenotypic consequences remains to be elucidated.

Huntington's disease (HD)

One of the most serious and common progressive neurodegenerative disorders of the central nervous system is Huntington's disease. It is an autosomal dominant syndrome and affects one in 10,000 individuals in Europe[23]. The disease is characterized by movement disorders and dementia. Interestingly, in the case of juvenile HD, it is the paternal allele that causes the disease in the child. The linkage analysis has localized the defective region to the chromosome 4p (ref. 19) and a gene from this region was recently isolated[23]. It was shown to contain a small, expanded, unstable CAG triplet in the 5′-coding region of the gene (fig.). Normal individuals contain 9–37 repeats whereas patients have 37–121 copies[23]. There is also an association between the expansion of the triplet and the age of onset of the disorder[23, 39]. The trinucleotide repeat is highly stable in somatic tissues, but the expanded allele is markedly unstable in sperm[5, 38]. In addition, a CCG rich sequence located 3′ to the expanded CAG repeats has been identified[4] which is suggested to be also responsible for HD. The gene is widely expressed[23, 24, 36, 59] in the brain and in non-neural tissues (pancreas, colon, liver and sperm). Its transcript is also present in homozygotes. Therefore, its expression pattern does not appear to clarify the specificity of pathology which involves a selective loss of neurons and is most severe in caudate and putamen. Since cell death in HD is confined to specific neurons in a particular region of the brain, it is not clear at present whether HD pathology is due to HD gene function. In a small number of cases, it has also been observed that some HD patients do not carry an expanded CAG in the disease range[5] which could be due to misdiagnosis. Alternatively, mutations in other unidentified genes could be responsible for HD pathology in these families.

Spinocerebellar ataxia type 1 (SCA1)

Spinocerebellar ataxia is a heterogeneous group of disorders that affects the cerebellum, spinal cord and brain stem[14, 49]. It is a late-onset disease (second and fourth decade) and the affected individuals have a life span of 10–20 years. SCA1 exhibits more severity in successive generations (anticipation). Linkage analysis mapped the defective region to the short arm of chromosome 6. An expanding CAG repeat was recently identified[49] in a gene of unknown function and hence this disorder represents another example of an expanding triplet sequence. The length of the repeat size is greater in the juvenile onset form of SCA1 compared to the adult form. Healthy persons contain 25–36 repeats and affected patients have between 43–81 copies (table). The repeat instability also leads to intergenerational variation[13] and an increase in repeat numbers is associated with paternal transmissions. Furthermore, the unexpanded alleles show an interrupted repeat whereas the expanded alleles reveal contiguous repeats which may suggest that the loss of an interruption causes a stable allele to become unstable. It is not known at present whether the other two ataxias (SCA2 and Machado-Joseph disease), which are linked to chromosome 12 and 14 respectively (also exhibit variable onset of disease), are also due to trinucleotide expansion in some other genes.

Myotonic dystrophy (DM)

Myotonic dystrophy is a hereditary neuromuscular disorder with a highly variable expressivity and age of onset. It is the most common muscular dystrophy of adult life. This multisystem disorder is characterized by progressive muscle weakness and atrophy. The other symptoms include high risk of miscarriage, premature balding, drowsiness, cataract, mental retardation and cardiovascular manifestations. It affects one in 7500 people at adulthood[21] and the severity increases over the generations. The disease trait is transmitted through an autosomal dominant mode with the disease gene mapping to chromosome 19. The disorder is extremely variable even between affected members of the same family. There are no reported sporadic cases of DM. A

new CTG trinucleotide repeat expansion has been described recently in an untranslated region of a protein kinase gene (DM-1) which is called myotonin protein kinase[3,8,11,16,21,22,40]. In the normal population, the length of the repeat is approximately 5–37, whereas in patients, it is 44–3000. In nearly all cases of DM, patients exhibit a dramatic expansion of CTG trinucleotide. There is also a good correlation between the repeat length, severity and earlier onset of the disorder. The sequence can also expand in successive generations of the same family[22]. It is also noteworthy that larger expansion of the repeat takes place in muscle than in lymphocytes[1] and the allele size in the muscle is stable for a period of 10–15 years[5]. On the other hand, an extensive mitotic instability of the repeat has been observed in all other tissues. Additionally, it has been found that in the case of severe congenital DM, it is the paternal allele which is stable while the maternal allele is unstable and almost exclusively transmitted[16]. Interestingly, a reverse mutation or rare contraction of an expanded allele leading to a normal phenotype has been described[10,47]. This contraction has been found to occur only through paternal transmission. Why these repeats are unstable, and expand and shrink spontaneously is not known. Additionally, a second gene, termed DMR-N9, has been isolated and is expressed in brain and testes[25]. Based on the expression pattern of DM-1 and DMR-N9 genes, it has been speculated that both genes may contribute to the clinical manifestation of DM patients. However, the function of DMR-N9 gene remains to be understood.

The DM (DM-1) gene has a region of strong homology to cAMP dependent serine-threonine protein kinase[8,40] and is expressed in brain, heart and muscle with a low level expression in most other tissues. Since the repeat is in the 3′-untranslated region of the DM kinase, it is hard to imagine how this structural abnormality causes the dominant condition of DM. On the other hand, it is possible that the expanded structure of the DNA may interfere in the expression of other genes in the vicinity through alterations in chromatin structure (e.g. DMR-N9 gene). In any event, how a stable harmless triplet nucleotide reiteration produces a pathological condition in muscle and other symptoms and how this mutation is responsible for the severity of the disease when transmitted from one generation to another remains to be understood.

Concluding remarks

It is known that the human genome contains many genes in which 1–6 nucleotide stretches are tandemly repeated numerous times[6,32,43,52], but they are not known to cause pathology unless the repeat length exceeds a critical threshold. Since only a handful of genes containing the expanded triplet have been studied to date, a definitive conclusion regarding their function in pathogenesis needs further investigation. On the other hand, the finding that seven neurological disorders contain an abnormal number of triplet repeats suggests that other neurological disorders such as autism and schizophrenia, which also exhibit the same type of unusual inheritance pattern, could also be due to a similar kind of mutation. Although it is believed that the molecular basis of variability and severity (anticipation) in a complex inherited disorder has been genetically explained, the above studies present many new questions. For instance, 1) it is important to know the basis of conversion of normal premutation into pathological form (after doubling in size) when it is transmitted from one generation to another, and 2) what factors determine early versus late-onset of illness in different individuals containing CAG repeats; 3) it is necessary to determine the molecular basis of normal sexual development in SBMA disorder containing triplet repeat expansions, and 4) we need to understand the null effect of other mutations (point mutations and deletions) in the androgen receptor gene[41] to produce SBMA. In Huntington's disease, although the inheritance pattern is Mendelian, their rapid expansion from one generation to another does not appear to be so. Moreover, in fragile X and congenital DM, the amplification occurs when the gene comes from the mother, whereas in Huntington's, it is the paternal allele that undergoes expansion. This type of parental sex preference cannot be explained by anticipation phenomenon alone[44]. Other factors such as genomic imprinting and variable methylation[51,55] should be taken into consideration. In addition, we need to explain why the fragile X-syndrome occurs in patients with a normal number of CGG repeats in FMR-1 gene[2]. Also noteworthy is the case where two members of a family contain the same number of repeats, but only one has mental retardation[31]. Is it possible that the FMR-2 gene which also contains CGG repeats is responsible for the disorder in these patients? The functional role of the FMR-1 gene, on the other hand, is further supported by the fact that an extensive deletion of the FMR-1 gene also causes fragile X-syndrome and mental retardation in some patients[61]. However, this does not exclude the possibility of involvement of FMR-2 in these patients.

The mutations described above are not fixed, but undergo large and small changes from generation to generation and occasionally are corrected spontaneously to normal size. They also do not occur at a fixed site, but at different positions in the gene. Although the strand slippage and errors in heteroduplex repair[32,54,58] of DNA polymerase during and post replication and unusual sister chromatide exchange[13] have been proposed as a possible mechanism, specific triplet amplification (not even flanking sequences) and its reversion requires further explanation. It is also not understood how an expanded DNA contributes to such a dramatic change

that causes the degenerative disease. Assuming that extra glutamine stretches cause a functionally abnormal protein and activate transcription abnormally[17], it is still unclear how one can explain the variable clinical onset and progression of the disorder[18]. Whatever the mechanisms and functions may be, the above findings and the availability of cloned genes may provide useful DNA probes for more advanced studies in the future and molecular diagnosis of these and other similar disorders.

Note added in proof

While this article is in press, another disorder, Haw River Syndrome, is also found to be associated with the same trinucleotide repeat as DRPLA(1).

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Acknowledgments. I am grateful to Dr. M. K. Hartzer of Oakland University for critically reading the manuscript. This work was supported in part by an Oakland University Research Fellowship Grant.

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