TRINUCLEOTIDE REPEATS AND NEUROPSYCHIATRIC DISORDERS

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ABSTRACT

Expansions of trinucleotide repeats at the level of genomic DNA are increasingly recognized as a cause of a number of neuropsychiatric disorders. Triplet repeat disorders are commonly classified into two groups, those with moderate CAG expansions that result in a polyglutamine stretch in the gene products and those with very long expansions, usually non-CAG, that are not translated. The triplet repeat intergeneration and intra-generational instability, and genetic anticipation characterize disorders. Most of the diseases caused by expanded CAG repeat share common features, which include neurodegeneration, a dominant pattern of inheritance and widespread expression of the gene products with neuronal loss restricted to distinct subset of neurons. Neurodegenerative changes associated of CAG expansion disorders is explained in terms of intra and extra cellular aggregation of mutant gene products, the insoluble nature of the protein(s) being attributed to the presence of polyglutamine stretches, hence their neurotoxic effects. Methods based on polymerase chain reaction have become handy in the diagnosis of these genetic disorders. Progress in transgenic animal models for these disorders will be critical for understanding the progress of these disorders and for testing new therapeutic strategies.

KEY WORDS: CAG repeats, polyglutamine, neuropsychiatric disorders

INTRODUCTION

Recent progress in molecular biology has enabled to explain the molecular basis of the several genetic disorders at genomic level. While there has been a spate of discovery of newer genes for many of the maladies, a group of genetic disorders, most of them being neuropsychiatric in nature, are characterized by the expansion of a trinucleotide or triplet repeats in the genomic DNA. In the normal population, the trinucleotide repeat units are polymorphic but are transmitted stably from one generation to the next. An increase or expansion in the number of repetitions of a particular trinucleotide repeat unit is now recognized as the

cause of a growing number of inherited disorders (1). This class of mutations was first identified in 1991 as the cause of spinal and bulbar muscular atrophy and fragile X syndrome (2). Since then trinucleotide repeat expansions have been found to be associated with several other neurologic disorders. Triplet repeat disorders are commonly classified into two groups, those with moderate CAG expansions that result in a polyglutamine stretch in the gene products and those with very long expansions, usually non-CAG, that are not translated. The age of onset and severity of the disorder depends on the location and the number of triplet repeats present within the genomic DNA. The disease associated expanded repeats tend to increase in length from generation to generation and do not adhere strictly to rules of Mendelian inheritance. The expanded repeats can be identified by PCR amplification of the genomic DNA samples followed by sequencing the PCR products or by short tandem repeat polymorphism analysis.

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General features

Trinucleotide repeat diseases share several similar features (3)

1. Expansions arise from CG-rich triplet repeats that are polymorphic in the normal population.
2. Expanded triplet repeats are unstable; changing in size when transmitted to successive generations. Although either further expansions or contractions may occur with transmission of expanded repeats, expansions are more common.
3. The diseases frequently show anticipation; there is a tendency for disease severity to increase in successive generations.
4. Inheritance is often autosomal dominant or X-linked
5. Expanded repeat diseases are generally neurological disorders affecting one or more regions of the neuraxis
6. Disease severity varies widely and correlates directly with increasing repeat length. The longer expansions result in earlier onset and more severe clinical manifestations.

Classification

Trinucleotide repeat diseases fall into two groups. The first groups designated as type I by La Spada et al (1) consist of diseases confined to the nervous system and are caused by small, constrained CAG repeat expansions that encode polyglutamine tracts in the disease gene product. They are also called polyglutamine disorders. Despite the widespread tissue distribution of the protein for each of these genes, the affected region is primarily the brain, and the regions of neuronal loss are highly specific for each disease. These proteins containing expanded polyglutamine repeats appear to precipitate by self-aggregation and as a result, produce a core-disease-related phenotype; neuronal cell death or degeneration. At present, nine inherited neurodegenerative disorders are identified to be caused by polyglutamine-coding CAG triplet expansions in the responsible genes. They include Huntington's disease (HD), spinal and bulbar muscular atrophy (SBMA), dentatorubral-pallidoluysian atrophy (DRPLA), autosomal dominant pure spastic paraplegia (ADPSP), and spinocerebellar ataxia

(SCA) type 1, 2, 3, 6 and 7. Except for S .MA, all of these disorders demonstrate dominant inheritance, and late onset of their manifestations.

The second group, designated as Type 2, is characterized by much larger expansions of trinucleotide repeats that fall outside the protein coding region. To date, these have been non-CAG repeats. The underlying mechanism of disease does not seem to be altered protein function but instead altered expression of the gene. These are not progressive neurodegenerative disorders; instead, CNS manifestation of these disorders may in part reflect abnormalities in brain and neuromuscular development. Another feature distinguishing this group from CAG repeat diseases is marked somatic mosaicism; repeat sizes vary widely in somatic tissues (4). Relatively little somatic mosaicism occurs in CAG repeat diseases (5). The type 2 diseases include fragile X syndrome, Friedreich's ataxia and myotonic dystrophy.

Huntington's disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder of variable onset and severity characterized by chorea, personality change and dementia. Clinical symptoms are more severe in early onset cases (before the age of 20 years) where as late onset (after age of 65) is less severe. Death usually occurs 15-20 years after the onset of motor symptoms. Inheritance of this disease is usually associated with anticipation, worsening of symptoms and earlier times of onset with succeeding generations. The genetic defect causing HD has been mapped to chromosome 4p16.3. In 1993 a novel gene IT-15 containing a highly polymorphic CAG repeat in the 5' region, which is expanded and unstable in HD, was described (6). This gene which encodes huntingtin, a 350 kDa protein widely distributed both within and outside the central nervous system, consists of 67 exons spread over 180 kb of DNA. It contains an open reading frame for a polypeptide of >3140 residues, one of the longest known. The CAG repeat is part of the first exon and is followed by CCG, CCA and CCT repeats that encode prolines. In normal population the number of CAG repeats ranges from 11-34 whereas in individuals

affected by HD the repeat length ranges from 36-121 (7). The repeat length has been shown to correlate with the age of onset and rate of progression of clinical symptoms. It is essential for embryonic neurogenesis, but its exact function there is unknown (8).

The neuropathologic changes in the brain are centered in the basal ganglia affecting particularly the caudate nucleus and putamen and characterized by a loss of neurons containing GABA, substance P and opioid peptides.

X-Linked Spinal and Bulbar Muscular Atrophy

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy disease, is a rare progressive, X-linked neuromuscular disorder characterized by proximal muscle weakness, atrophy, and fasciculations. The X-linked pattern of inheritance, adult-onset, and bulbar involvement distinguish it from autosomal recessive forms. The gene for SBMA codes for the androgen receptor (AR). La Spada et al (2) found that a trinucleotide repeat, CAG was expanded in the first exon of the AR gene in all SBMA patients. This CAG repeat, which normally encodes a polyglutamine tract of 11-34 amino acids, is expanded to 40-62 amino acids in SBMA patients (3). SBMA is characterized pathologically by degeneration of the anterior horn cells, bulbar neurons, and dorsal root ganglion cells.

Spinocerebellar ataxia

The autosomal dominant spinocerebellar ataxias (SCA) are a heterogeneous group of dominantly inherited neurological disorders characterized by progressive ataxia that results from degeneration of the cerebellum, spinal cord, and brain stem. These disorders have been classified into several subtypes based on the clinical features. A genetic heterogeneity has been identified with six different loci (SCA1,2,3,4,6 and 7). A pathological expansion of a CAG sequence has been identified in SCA1,2,3, 6 and 7 (9). SCA type 1 is caused by unstable expansion of a CAG repeat in the protein coding region of a gene located on chromosome 6p23. In normal individuals SCA 1 repeat encodes a polyglutamine tract 6 to 39 repeats long whereas

in affected individuals it is expanded to 41 to 81 repeats (10). The expanded SCA 1 repeat is an uninterrupted sequence of CAG repeats, whereas the normal SCA 1 allele is usually interrupted midway by one to three CAT trinucleotides. The interruptions are thought to contribute to allele stability (11). This gene is expressed ubiquitously in neurons and its transcript (ataxin-1) localizes mostly in the nucleus. Ataxin-1 with expanded glutamine repeat is highly ubiquitinated and forms aggregation within the nucleus. SCA 1 usually begins in the third to fifth decade, progresses over 10 to 20 years and is characterized by ataxia, dysarthria, dysphagia and pyramidal signs.

SCA type 2 is caused by expansion of a CAG repeat in the SCA 2 gene located in chromosome 12q24. The function of the gene product, ataxin is as yet unknown (12). Ataxia, dysarthria, sensorimotor neuropathy and rarely dementia characterize this disorder.

SCA type 3, also known as Machado-Joseph disease (MJD), is often clinically indistinguishable from SCA type 1. This disorder is caused by expansion of a CAG repeat in chromosome 14 (13). This polyglutamine repeat is 13 to 36 repeats in normal alleles but 68-79 repeats in the disease alleles. The MJD gene product, ataxin 3, is a small hydrophilic protein of 359 amino acids. The polyglutamine tract lies near the carboxyl terminus.

In SCA type 6, the number of CAG repeats is smaller than the other types, (Table 1) and the gene in chromosome 19p13 encodes an α_1 subunit of a voltage-dependent calcium channel (14). Slowly progressive ataxia, dysarthria, nystagmus and hyporeflexia are the most common clinical features of this disorder. An CAG expansion in the SCA 7 gene located in chromosome 3p14-21.1 has been reported in SCA type 7 (15). This gene encodes ataxin 7 whose function is unknown.

Dentatorubral-Pallidoluysian Atrophy

Dentatorubral-Pallidoluysian atrophy (DRPLA) is characterized by ataxia, choreoathetosis, dementia, myoclonus, and epilepsy. A CAG repeat is expanded

in all DRPLA patients from a normal range of 7 to 25 copies to 49 to 88 copies (16). The repeat encodes a polyglutamine tract in a middle of a 124 kDa protein, atrophin-1. The exact function of this protein is unknown.

Familial Spastic Paraparesis

Familial spastic paraparesis is inherited as either an autosomal dominant or X-linked condition that selectively affects upper motor neurons. Linkage studies point to genetic heterogeneity with loci on chromosomes 2p, 14 q, and 15 q (17). One form is reported to be associated with an increased number of CAG repeats in a gene on chromosome 2p21-24 (18), but this association has not been confirmed.

Friedreich ataxia

Friedreich ataxia (FDRA), the most common of the inherited ataxias, is an autosomal recessive degenerative disorder, characterized clinically by onset before the age of 25 years of progressive gait and limb ataxia, absence of deep tendon reflexes and loss of position and vibration sense in the lower limbs. Most patients have skeletal deformities and hypertrophic cardiomyopathy. They also have an increased incidence of blindness, deafness and diabetes mellitus, suggesting that the disorder is of systemic as well and not limited to the nervous system. FRDA is caused by GAA triplet expansion in the first intron of the FRDA gene on chromosome 9q13 in 97% of patients (23). The FRDA gene encodes a widely expressed 210-amino acid protein, frataxin, which is located in the mitochondria and is severely reduced in FRDA patients. The function of frataxin is still unknown but the current hypothesis is that it is a mitochondrial protein important for normal production of cellular energy. A defect in its action may result in abnormal accumulation of iron in the mitochondria followed by death of the vulnerable neurons (24).

Fragile X syndrome

This X-linked disorder named after a folate-sensitive fragile site on the X chromosome, is the

most common cause of inherited mental retardation. The mental retardation is associated with dysmorphic features such as enlarged ears, head, and testicles. The primary genetic defect in fragile X syndrome is an expanded CGG repeat. The repeat is found in the 5'untranslated region of the gene called FMR-I (25). The gene product is a member of a family of RNA-binding proteins and is widely expressed in fetal and adult tissues, most abundantly in the neurons. The fragile site associated with the CGG repeat in FMR-1 is designated as FRAXA. In normal population, the repeat exists as a stable polymorphism of between 6 and 52 repeats, with a mode of 30. In FRAXA families, the repeat is expanded over a broad range, from premutations of ~60-200 repeats to full mutations of several thousand repeat or more. Expansions in the premutation range usually do not cause disease but are highly prone to expand to the full mutation, resulting in the full-blown manifestations of disease. Expansion is associated with increased methylation of both the repeat and the adjacent CpG island, leading to transcriptional silencing of the gene and decreased expression of the FMR-1 protein (26). The CGG repeat expansion and subsequent methylation likely account for the chromosomal fragility at the FRAXA locus. As the CGG repeat number increases in the 5'region of the FMR-I gene, mRNA and protein levels decrease, and the fragile X syndrome worsens. Although transcriptional silencing seems to be the principle cause of decreased FMR-I expression, translational inhibition may also play a role with the largest repeats (27).

Another folate-sensitive fragile site (FRAXE) 600kb downstream to FRAXA has been shown to contain a CGG repeat that is expanded in mildly retarded individual (28). The degree of mental retardation is milder in these patients. The mechanism of disease in FRAXE may be similar to that in FRAXA. FRAXE disease severity also correlates with increasing repeat size and hypermethylation of a nearby CpG dinucleotide island. The FRAXF site, located on the X chromosome ~1200kb downstream to FRAXA, is a GCC repeat that is normally 6-29 repeats but expanded to 300-500 repeats in patients expressing the fragile site (29).

Myotonic dystrophy (DM)

DM is a dominantly inherited neuromuscular disease, highly variable and multisystemic, which is caused by the expansion of a CTG repeat located in the 3'untranslated region of the DM gene (30). DM is characterized by progressive weakness and myotonia, often in association with cataracts, cardiac arrhythmias, cognitive impairment and endocrinopathies.

The DM gene product was given the name myotonin-protein kinase because sequence analysis showed it to have homology to members of the serine-threonine protein kinase family (31). In the muscle this protein is predominantly localized in the neuromuscular junction. Normal alleles show a copy number of 5-37 repeats on normal chromosomes, amplified to 50-3000 copies on DM chromosomes. The DM gene's kinase activity raises the possibility that in DM, myotonia may be due to altered phosphorylation of a protein involved in the muscle action potential or excitation-contraction coupling.

Schizophrenia and other psychiatric disorders

Genetic anticipation, manifested by increased severity and earlier age-at-onset of the disease over successive generations, has been reported in schizophrenia. Recently, studies suggest that enlarged CAG/CTG repeats are over represented in schizophrenic patients compared to normal controls (28). Polyglutamine-expanded tracts, encoded by CAG repeats, has been observed in total protein extracts derived from lymphoblastoid cell lines of schizophrenic patients (29). A recently cloned K⁺ channel, which might be linked to schizophrenia, shows CAG polymorphism. (30).

Recently, data generated by the repeat expansion detection method (RED) shows a significant association between bipolar disorders and large CAG/CTG repeat sequences in the genome (31). Since RED method cannot detect the specific triplet expansion, the expansion may be unrelated to the disease. Further studies are required in this area.

Molecular mechanisms of CAG diseases

The polyglutamine sequence in CAG-diseases seems to be the major factor involved in neurodegeneration, as the severity of the disease correlates with the length of the sequence. Transgenic mice that carried the gene for hypoxanthine phosphoribosyltransferase in which a 146-unit CAG repeat had been introduced developed neurodegenerative disease with a unique profile clearly indicating the key role of polyglutamine (32). In several of the CAG repeat disorders were found to have intra or intercellular aggregates of the involved protein in the neurons from the affected area of the nervous system. The presence of nuclear inclusions has been commonly observed (33). Similar changes were seen in transgenic mice, cultured cell lines transfected with genes encoding expanded CAG repeats, and *Caenorhabditis elegans* neurons that express these genes (34). Aggregates are not seen in normal individuals or in flies or mice expressing genes that carry only a normal number of CAG repeat. It has been suggested that these aggregates could be in some way responsible for the toxic gain of function that produces the disease. Two mechanisms have been suggested to explain the formation of aggregates:

- i. Expanded glutamine repeats can act as hydrogen-bonded polar zippers, forming pleated sheets of strands that are held together by hydrogen bonds between their amides (35).
- ii. Aggregates of glutamine-repeat proteins could be stabilized by transglutaminase catalysed cross-linking (36).

The aggregates found in HD and the other disorders may also contain other elements in addition to the proteins containing repeats. Ubiquitination of the proteins in aggregates has been observed. Further, the ability of huntingtin and DRPLA protein to bind a variety of proteins in proportion to stretch of polyglutamine could alter the function(s) of the concerned protein (37). Ataxin 1 aggregates in SCA 1 have been shown to contain a nuclear matrix protein and leucine-rich acidic nuclear protein, 20S proteasome protein and molecular chaperone (37).

Recently, a few proteins that contain glutamine repeats (huntingtin, atrophin-I, ataxin-3, and the androgen receptor) have been shown to be cleaved by the caspases (38), a group of cysteine protease cell death executioners. Cleavage of atrophin-I at aspartate 109 by caspase has been shown to modulate cytotoxicity and aggregate formation in DRPLA (38). Mutant atrophin-I that is resistant to caspase cleavage is associated with significantly decreased cytotoxicity and suppression

of aggregate formation (39). This might be of particular significance to suggest the probable target for therapeutic manipulation aimed at attenuating the neuronal death produced by polyglutamine containing proteins.

Repeats of other amino acid residues have also been reported to cause disease. Expansion of an alanine repeat in the polyadenine-binding protein 2 has been linked to oculopharyngeal muscular dystrophy (40). The nuclei of skeletal

Table 1. Neuropsychiatric disorders associated with triplet repeats

Disease	Repeat Sequence	Gene product	Normal repeat range	Expanded repeat range	References
Spinobulbar muscular Atrophy	CAG	Androgen receptor	11-34	40-62	1-3
Huntigtons	CAG	Huntingtin	11-34	36-121	6-8
Spinocerebellar ataxia SCA1	CAG	Ataxin-1	6-39	41-81	3,9-11
SCA2	CAG	Ataxin-2	14-32	33-77	9,12
SCA3(Macado-Joseph Disease)	CAG	Ataxin-3	13-36	68-69	3,13
SCA6	CAG	A1A voltage Dependent Ca ²⁺ channel	1-18	21-30	14
SCA7	CAG	Ataxin-7	7-17	38-120	15
Dentatorubral Pallido Lusylian atrophy	CAG	Atrophin-1	6-31	49-88	3,16
Schizophrenia	CAG	Ca ²⁺ -activated K ⁺ channel	<19	>19	28-30
Fragile X syndrome					
FRAXA	CGG	FMR-1	6-52	60->2000	3,21-22
FRAXE	GCC	FMR-2	6-25	>200	3,24
Myotonic dystrophy	CTG	Myotonin protein Kinase	5-37	50-3000	3,26-27
Friedreich's ataxia	GAA	Frataxin-	-	-	19-20

muscle fibres of patients with this disease contain filamentous inclusions. Poly-L-alanine chains have been shown to adhere to each other through non-polar interactions between their methyl side chains.

A likely mechanism by which expansion of the number of repeats may occur is through slippage of replicating strands (41). In this model, repetitive DNA sequences have a tendency to slip out of alignment during replication and incorporate more or fewer units of repeat. The longer the triplet repeats more prone to slippage in the future leading to the incorporation of successively larger numbers of additional repeats.

CONCLUSION

The dynamically expanding trinucleotide repeats is an important cause of an abnormality that causes genetic disorders, many of them being neurodegenerative in character. The number of these diseases that are being identified continues to grow and the coming years may witness the emergence of a common mechanism which could underlie these diseases. Recent progress in the development of cellular and animal models has provided invaluable insights and resources for studying the disease mechanisms, and will be useful for screening and evaluating newer therapeutic strategies.

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