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Review Article

UNSTABLE EXPANSION OF TRIPLET REPEATS AS A NEW DISEASE MECHANISM FOR NEURODEGENERATIVE DISEASES

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Introduction

Neurodegenerative diseases have been defined as diseases characterized by gradual loss of neurons in particular regions of the central nervous system due to "unknown" causes. Substantial number of neurodegenerative diseases exhibit familial occurrence, suggesting that mutations in the causative genes are the primary cause of the diseases. Application of the molecular genetic strategy of "positional cloning" has enabled us to identify the causative genes without prior knowledge of the pathophysiology of the diseases. Among the causative genes which have been identified by the strategy of positional cloning, unstable expansions of triplet repeats have recently been discovered to be a common novel disease mechanism for neurodegenerative diseases. In this review I will focus on recent developments in the research on triplet repeat diseases with particular emphasis on non-Mendelian aspects of triplet repeat diseases.

1. Four Classes of Triplet Repeat Diseases Classified Based on the Location of the Triplet Repeats

A triplet repeat disease is defined as a disease caused by unstable expansion of a triplet repeat such as a CAG trinucleotide repeat. Interestingly, triplet repeats are present in normal individuals, with the number of repeat units ranging from several to nearly 40. Presence of expanded allele with the size of the trinucleotide repeats exceeding the normal range leads to expression of disease phenotypes. So far four classes of triplet repeat diseases have been identified based on the location of the triplet repeats in the causative genes. As shown in Fig. 1, the triplet repeat may be located in the 5′-untranslated region (5′-UTR), 3′-UTR, introns or coding regions.

Fragile X syndrome has been found to be caused by expansion of a CGG trinucleotide repeat in the 5′-UTR (Kremer *et al.*, 1991; Oberle *et al.*, 1991; Yu *et al.*, 1991; Verkerk *et al.*, 1991), while expansion of a CTG trinucleotide repeat

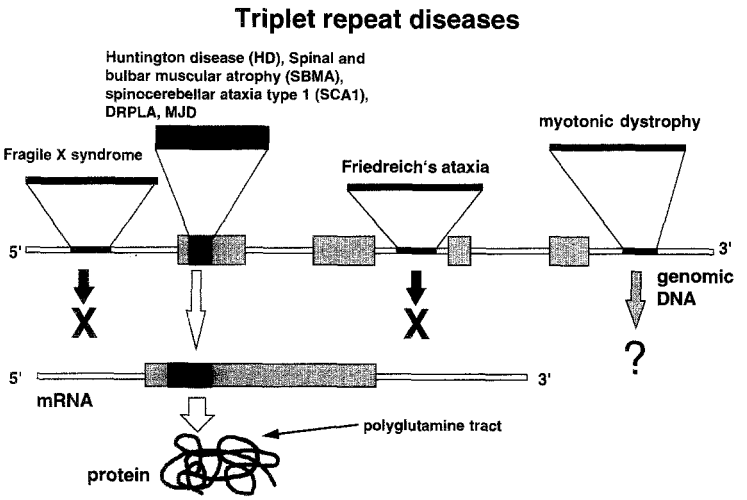

Fig. 1. Schematic representation of location of triplet repeats in the genes for various triplet repeat diseases.

in the 3'-UTR has been identified in myotonic dystrophy (Mahadevan *et al.*, 1992; Fu *et al.*, 1992; Brook *et al.*, 1992). Expansion of an intronic GAA trinucleotide repeat has recently been identified in Friedreich's ataxia (Campuzano *et al.*, 1996). The triplet repeats in these diseases are expanded to as many as several hundred to several thousand repeat units. Expansion of CAG repeats in protein coding regions was first identified as the causative gene for spinal and bulbar muscular atrophy (SBMA) (La Spada *et al.*, 1991). Since then expansion of CAG repeats in protein coding regions has been identified in Huntington's disease (HD) (The Huntington's Disease Collaborative Research Group, 1993), spinocerebellar ataxia type 1 (SCA1) (Orr *et al.*, 1993), dentatorubral-pallidoluysian atrophy (DRPLA) (Koide *et al.*, 1994; Nagafuchi *et al.*, 1994a) and Machado-Joseph disease (MJD) (Kawaguchi *et al.*, 1994) (Fig. 1). In contrast to those of fragile X syndrome, Friedreich's ataxia or myotonic dystrophy, the expanded CAG repeats range in size from approximately 40 to 100 repeat units.

Fragile X syndrome is an X-linked recessive disease and the leading cause for mental retardation in male (Kremer *et al.*, 1991; Oberle *et al.*, 1991; Yu *et al.*, 1991; Verkerk *et al.*, 1991). Friedreich's ataxia is an autosomal recessive neurodegenerative disorder characterized by ataxia, decreased or absent tendon reflexes and impairment of deep sensation (Campuzano *et al.*, 1996). Myotonic dystrophy is an autosomal dominant disease affecting multiple organs including muscle and other tissues (Mahadevan *et al.*, 1992; Fu *et al.*, 1992; Brook *et al.*, 1992). Clinical features of myotonic dystrophy are characterized by myotonia (prolonged contraction of muscle after voluntary contraction or percussion), weakness in distal muscles, cataract, frontal baldness and endocrine abnormalities. SBMA is an X-

linked recessive neurodegenerative disorder characterized by muscular atrophy and weakness in tongue and four extremities, muscle cramp and postural tremor associated with other endocrinological abnormalities such as gynecomastia and hypogonadism. Huntington's disease is an autosomal dominant neurodegenerative disorder characterized by involuntary movement and dementia. SCA1, DRPLA and MJD are autosomal dominant neurodegenerative diseases and belong to a group of diseases called spinocerebellar degeneration which are clinically characterized cerebellar ataxia and other accompanying neurological symptoms. Ages at onset of these diseases are usually in adulthood, but exhibits considerable wide range from childhood to late adulthood. The clinical presentations also exhibit considerably broad spectrum even in a single family.

It has been suggested that in fragile X syndrome and Friedreich's ataxia presence of hugely expanded triplet repeats either in the 5′-UTR or in introns results in decreased efficiency of translation of the causative genes, implying that the disease mechanism is a "loss of function" type of mechanism, which is in accordance with the mode of inheritance of these diseases. In myotonic dystrophy, however, it has been still controversial whether the mRNA levels of myotonin kinase gene, the causative gene for myotonic dystrophy, are increased or not in cases of myotonic dystrophy. In the case of triplet repeat diseases caused by expansions of CAG repeats, it has been suggested that the mutant proteins with expanded polyglutamine tracts have toxic effects on neuronal cells, implying that the disease mechanism is a "gain of function" type of mechanism.

The gene product of *FMR1* gene, the causative gene for fragile X syndrome, has been suggested to have a function as an RNA-binding protein (Kremer *et al.*, 1991; Oberle *et al.*, 1991; Yu *et al.*, 1991; Verkerk *et al.*, 1991), and that of the myotonin kinase gene to be a protein kinase based on the nucleotide sequence homology with other protein kinases (Mahadevan *et al.*, 1992; Fu *et al.*, 1992; Brook *et al.*, 1992; Campuzano *et al.*, 1996). The function of frataxin, the gene product of the gene for Friedreich's ataxia, remains to be elucidated. Among the gene products of the gene with expansions of CAG repeats, the function of the gene products remain unknown except for androgen receptor, the gene product of the gene for SBMA.

2. Expansion of CAG Repeats in Coding Region as a Common Mechanism of Neurodegeneration

As mentioned above, to date five diseases have been identified to be caused by unstable expansion of CAG repeats including SBMA, HD, SCA1, DRPLA and MJD (La Spada *et al.*, 1991; The Huntington's Disease Collaborative Research Group, 1993; Orr *et al.*, 1993; Koide *et al.*, 1994; Nagafuchi *et al.*, 1994a; Kawaguchi *et al.*, 1994). The mode of inheritance of these diseases is autosomal dominant one except for SBMA which is inherited as an X-linked recessive trait. There are many similarities among the diseases of this class. 1) The diseases are

caused by unstable expansion of CAG repeats in a coding sequence. 2) The CAG repeats are predicted to code for a polyglutamine tract. 3) The number of CAG repeat units is variable even among normal individuals, in whom it ranges from several to as many as 40. 4) The CAG repeat is expanded, with the number of repeat units exceeding 40 and ranging to as many as 100. The CAG repeats which are largely expanded with the number of repeat units exceeding 100 are exceptional. 5) There is a strong inverse correlation between the size of the expanded CAG repeat and the age at onset. 6) Genetic anticipation, i.e. decreasing age at onset in successive generations, has been identified as a common phenomenon unique to triplet repeat diseases. 7) Despite the fact that the causative genes are widely expressed, central nervous system is commonly and selectively involved with the distribution of neurodegeneration unique to each disorder. 8) The essential feature of neuropathology associated with the diseases is "neurodegeneration" or loss of neurons in particular regions of the central nervous system. For example spinal and brain stem motor neurons are selectively involved in SBMA.

There are a number of diseases exhibiting genetic anticipation of which causative genes have not been identified. These diseases include various forms of spinocerebellar ataxia, bipolar illness, and schizophrenia (Ranum *et al.*, 1994; Gouw *et al.*, 1994; Basset *et al.*, 1994; Mclnnis *et al.*, 1993). Therefore, there may be more diseases caused by CAG repeat expansion. Since the conventional approach to identification of causative genes by positional cloning requires large pedigrees for linkage studies, development of novel strategies for direct identification of expanded triplet repeats will be required.

3. Non-Mendelian Aspects in Triplet Repeat Disease

A. Variable age at onset

Figure 2 shows the correlation of the size of expanded CAG repeat of the DRPLA gene and the age of onset of DRPLA (Koide *et al.*, 1994; Ikeuchi *et al.*, 1995a, b, c). As has been demonstrated in diseases caused by expansion of CAG repeats, a strong inverse correlation between the size of expanded CAG repeats and the age at onset was demonstrated in DRPLA. The inverse correlation implies that the size of the expanded CAG repeat is strongly related to the pathophysiologic processes. It should be noted that the size of the expanded CAG repeat and the age at onset are more strongly correlated in the case of greatly expanded CAG repeats than in the case of mildly expanded CAG repeats. In the case of mildly expanded CAG repeats, the correlation is not as strong as in the case of greatly expanded CAG repeats. This result suggests that prediction of age at onset based on the CAG repeat size is difficult especially in the case of mild CAG repeat expansion and late onset. This should be carefully considered in genetic counseling.

B. Variable clinical phenotypes

DRPLA is characterized by a wide spectrum of clinical phenotypes depending on the age at onset (Naito and Oyanagi, 1982). To clarify the clinical spectrum of

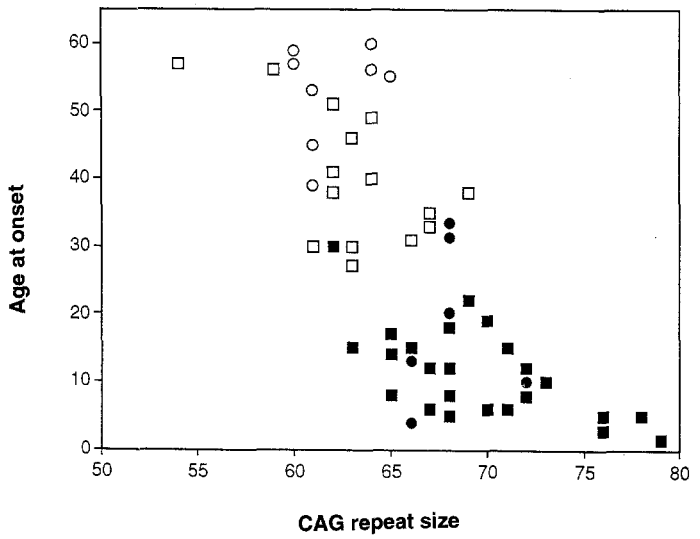

Fig. 2. Correlation between size of CAG repeat and age at onset of symptoms of DRPLA. There is a strong inverse correlation ($r=-0.737$). Filled squares represent cases of PME phenotype with paternal transmission, filled circles represent cases of PME phenotype with maternal transmission, open squares represent cases of non-PME phenotype with paternal transmission, and open circles represent cases of non-PME phenotype with maternal transmission.

DRPLA depending on age at onset, we analyzed 65 patients with DRPLA and determined the frequencies of the 6 characteristic clinical features, i.e. ataxia, dementia or mental retardation, myoclonus, epilepsy, choreoathetosis and psychiatric changes including character changes, delusion or hallucinations (Ikeuchi *et al.*, 1995a). We found that ataxia and dementia are cardinal features irrespective of age at onset. Furthermore, patients with onset before age 20 frequently exhibit myoclonus and epilepsy in addition to ataxia and dementia. The combination of these clinical features corresponds to the clinical entity of progressive myoclonus epilepsy phenotype (PME). On the other hand, patients with onset after age 20 frequently exhibit choreoathetosis and psychiatric changes in addition to ataxia and dementia. Since the age at onset is inversely correlated with the CAG repeat size, the above implies that various clinical features are strongly correlated with the CAG repeat size. Similar correlation of clinical phenotypes with the CAG repeat size has also been demonstrated in other diseases caused by expansion of CAG repeats.

C. Genetic anticipation

Table 1 summarizes the genetic anticipation observed in DRPLA (Ikeuchi *et al.*, 1995a, b, c; Takiyama *et al.*, 1995). Analysis of our data set as well as data in the literature revealed that paternal transmission results in decrease in age at onset of 26–29 years, while maternal transmission results in decrease in age at onset of 15 years on average (Ikeuchi *et al.*, 1995a, b). There are statistically significant

Table 1. Genetic anticipation in DRPLA (Koide *et al.*, 1994; Ikeuchi *et al.*, 1995a, b, c)

	Paternal	Maternal	
Our cohort	-25.6 ± 2.4 (n=27)	-14.0 ± 4.0 (n=9)	$p<0.05$
Literature	-28.8 ± 1.9 (n=27)	-14.8 ± 4.0 (n=11)	$p<0.05$

differences in the magnitude of decrease in age at onset between paternal and maternal transmissions. The parental bias in the genetic anticipation implies that the genetic anticipation is intimately associated with the differences in meiotic instability of the CAG repeats between paternal and maternal transmissions.

D. "Sporadic" cases

The diagnosis of "sporadic" cases of triplet repeat diseases, i.e. patients with no family history of similar cases, has been extremely difficult, since a high penetrance in these diseases makes diagnosis of such cases difficult unless the disease is proven to be transmitted as an autosomal dominant trait. We have so far encountered 6 sporadic cases of DRPLA, in only one of which we had the opportunity to analyze genomic DNA of both parents (Ikeuchi *et al.*, 1995a). The patient had generalized epilepsy at the age of 26, and began exhibiting ataxia and involuntary movements such as choreoathetosis at the age of 37. She was found to carry an expanded allele of 62 repeat units of the DRPLA gene. Although neither of parents showed any clinical abnormalities at the ages of 65 and 67, respectively, her father was found to carry a mildly expanded allele of 57 repeat units of the DRPLA gene. With this intergenerational increase of 5 repeat units during the paternal transmission, the patient was the first to cross the phenotypic threshold in this pedigree.

This shows that sporadic cases can occur even in the absence of family history. In the above family, the intergenerational increase in the number of the CAG repeat units in paternal transmission resulted in the appearance of the sporadic case. In the case of HD, expansion from an "intermediate range" allele to a fully expanded allele has been described. Although such cases of DRPLA with expansion from an intermediate allele to a fully expanded allele have not been described, a much more sporadic cases should be analyzed to clarify the molecular mechanisms for occurrence of sporadic cases.

E. Gene dosage effect

Recently we encountered a patient homozygous for the DRPLA mutation (Sato *et al.*, 1995). The parents are first cousins. The patient carries an expanded CAG repeat of 57 repeat units of DRPLA gene as a homozygous state. The parents, though asymptomatic at the ages of 62 and 63, respectively, had both a mildly expanded allele of 57 repeat units as a heterozygous state. Individuals carrying a CAG repeat with 57 repeat units as a heterozygous state are predicted to develop symptoms at the age of around 50 or later (Koide *et al.*, 1994; Ikeuchi *et al.*, 1995a, b, c). The age at onset of the patient, however, was 17, and the

clinical phenotype of the patient is a typical PME phenotype. Thus, the age at onset of the patient is much younger than that of patients heterozygous for the expanded allele with a CAG repeat of the same size and the clinical phenotype of this case is much severer than expected for those with heterozygous mutation with the similar sizes of CAG repeats. A quite similar gene dosage effect was reported in a case of MJD (Lang *et al.*, 1994). These findings suggest that there is a gene dosage effect in diseases caused by CAG repeat expansions. In the case of HD, however, it was demonstrated that the age at onset and the clinical severity do not differ between patients with a homozygous mutation and those with a heterozygous mutation (Wexler *et al.*, 1987). It is unclear whether the gene dosage effect is invariably present in diseases caused by CAG repeat expansion. Analysis of a large number of cases of CAG repeat expansion diseases with homozygous mutations will be required before we can conclude whether a gene dosage effect commonly occurs in these diseases caused by CAG repeat expansions.

F. Meiotic drive

In segregation analysis of DRPLA pedigrees, we found that the segregation rate of transmission of mutant DRPLA gene and that of wild type DRPLA gene were not equal in paternal transmission of DRPLA. As shown in Table 2, expanded alleles of DRPLA gene are significantly more frequently transmitted than normal alleles in paternal transmission (Ikeuchi *et al.*, 1966). Such segregation distortion was not observed in cases of maternal transmission. A quite similar phenomenon was also observed for MJD. Such segregation distortion specific to the gender of the affected parent is called "meiotic drive" (Lyttle, 1993; Raju and Perkins, 1991; Silver, 1993).

Meiotic drive has been found to occur in several human diseases including retinoblastoma (Munier *et al.*, 1992), split foot/hand disease (Jarvik *et al.*, 1994) and retinal cone dystrophy (Evans *et al.*, 1994). In the case of triplet repeat

Table 2. Segregation distortion in DRPLA and MJD (Ikeuchi *et al.*, 1996)

Disorder and parent of origin	No. Affected (%) [male:female]	No. Unaffected (%) [male:female]	Total [male:female]	χ^2	Probablility
DRPLA:					
Male	78 (63) [37:41]	47 (37) [27:20]	125 [64:61]	7.69	$p<0.01$
Female	36 (42) [22:14]	50 (58) [24:26]	86 [46:40]	2.28	$0.1<p<0.2$
Total	114	97	211	1.37	$0.2<p<0.3$
MJD:					
Male	24 (73) [9:15]	9 (27) [4:5]	33 [13:20]	6.82	$p<0.01$
Female	26 (55) [13:13]	21 (45) [10:11]	47 [23:24]	0.53	$0.3<p<0.5$
Total	50	30	80	5.00	$0.01<p< 0.05$

diseases, segregation distortion also occurs in myotonic dystrophy (Hurst *et al.*, 1995; Carey *et al.*, 1995), though the validity of the statistical analysis used to determine this has been disputed. Since genetic anticipation and meiotic instability of CAG repeats are frequently associated with paternal meiosis, similar molecular mechanisms may underlie the meiotic drive, which is also specific to male meiosis.

4. Instability of CAG Repeats

As described above, CAG repeats have been shown to exhibit meiotic as well as mitotic instability. The molecular mechanisms of the instability, however, are unknown. Recently we observed an interesting phenomenon regarding the meiotic instability of the CAG repeat of the *MJD1* gene, the causative gene for MJD. There are two polymorphic loci, one within the CAG repeat and the other immediately downstream of the CAG repeat of the *MJD1* gene (Kawaguchi *et al.*, 1994). We developed a novel method to distinguish these polymorphisms with use of allele-specific oligonucleotide probes (Igarashi *et al.*, 1995). The genomic fragments containing the CAG repeat of the *MJD1* gene were amplified by polymerase chain reaction (PCR) and the PCR products are separated through agarose or polyacrylamide gels and blotted onto nitrocellulose membranes, followed by allele-specific oligonucleotide hybridization. This method allows determination of haplotypes without the need for pedigree analysis. We investigated whether the polymorphism in *cis* or in *trans* to the expanded allele affects the intergenerational instability of the CAG repeat of the *MJD1* gene.

We found that the polymorphism in *trans* to the expanded CAG repeat has a substantial influence on the degree of intergenerational instability of the CAG repeat. This result indicates that inter-allelic interaction occurs between the normal and expanded alleles. Comparing the phenomenon with that observed for the human minisatellite MS32 (Jeffrey *et al.*, 1994), we hypothesize that a gene conversion event is involved in the intergenerational instability of the CAG repeat of the *MJD1* gene. The occurrence of a gene conversion event in the case of contraction of CTG repeats of the myotonin kinase gene has also been described (Hunter *et al.*, 1993; O'Hoy *et al.*, 1993). These findings suggest that gene conversion events occur as a fundamental mechanism of meiotic instability of the CAG repeat in diseases caused by triplet repeat expansion.

Somatic mosaicism has been described in various regions of autopsied tissues in CAG repeat expansion diseases. Presence of smaller CAG repeat in the cerebellum than those in other regions of the central nervous system was first detected in two cases of HD with unusually early ages at onset (Telenius *et al.*, 1994). Since the cerebellum is the least involved brain region in HD, it was suggested that the presence of smaller CAG repeat in the cerebellum than in other regions of the central nervous system may inhibit the pathological process in the cerebellum (Telenius *et al.*, 1994). Subsequent studies on DRPLA (Ueno *et al.*, 1995; Takano *et al.*, 1996), however, revealed that the CAG repeats of expanded alleles of the

DRPLA gene are also smaller in the cerebellum than in other regions of the brain. Moreover, Takano *et al.* analyzed the size distributions of expanded CAG repeats of the DRPLA gene by dissecting the cerebellum and cerebrum of autopsied DRPLA brains into the cortex and white matter, and found that the CAG repeats are consistently smaller in cerebellar and cerebral cortex than those in cerebellar and cerebral white matter, respectively. These results suggest that the CAG repeats of the DRPLA gene are smaller in neuronal cells than in glial cells. They further speculated that the presence of much smaller CAG repeats in the cerebellum than in other regions of the central nervous system reflects the presence of dense population of neuronal cells including granule cells in the cerebellar cortex (Takano *et al.*, 1996). The role of somatic mosaicism in the pathogenesis of DRPLA, however, remains to be elucidated.

5. Mechanisms of Neurodegeneration Caused by CAG Repeat Expansion

The molecular mechanisms of neurodegeneration have attracted the interest of many investigators, since there may be a fundamental mechanism underlying a number of neurodegenerative diseases. Although the entire structure of DRPLA cDNA has been elucidated (Nagafuchi *et al.*, 1994b; Onodera *et al.*, 1995), the physiological functions of the gene product (DRPLA protein, DRPLAP) as well as the mechanisms of neurodegeneration caused by CAG repeat expansion remain unknown. To date, a number of hypotheses regarding the molecular mechanisms of neurodegeneration caused by expansion of CAG repeats have been proposed, including position effect hypothesis, polar zipper theory, a hypothesis of the expanded polyglutamine tracts as a substrate for transglutaminase, and a hypothesis of neurotoxicity caused by the expanded polyglutamine tracts. Recently proteins which may interact with the polyglutamine tract were identified (HAP1 and GAPDH) (Li *et al.*, 1995; Burke *et al.*, 1996). Burke *et al.* proposed that stronger binding of mutant DRPLAP carrying expanded polyglutamine tract with GAPDH than that of wild type DRPLAP may result in a decrease in GAPDH activity and energy failure in cells expressing mutant DRPLAP (Burke *et al.*, 1996).

Recently Ikeda *et al.* reported that COS cells transfected with truncated MJD cDNA with an expanded CAG repeat undergo apoptosis (Ikeda *et al.*, 1996). They proposed that the expanded polyglutamine tract, but not normal polyglutamine tract, exhibits marked toxicity to COS cells, and suggested that a difference in processing of the gene products among cells of various lineages may be the reason for the difference in distribution of affected regions. Since only the central nervous system is involved in the diseases such as DRPLA and MJD, it is unclear whether such marked toxicity demonstrated in COS cells underlies the mechanisms of neurodegeneration in these diseases.

Recently, it has been suggested that huntingtin, the product of the gene for HD, may be the substrate for apopain (Goldberg *et al.*, 1996). Apopain is the human counterpart of the nematode cysteine protease death-gene product, CED-3

(Goldberg *et al.*, 1996) and was proposed to be involved in the proteolytic processing of huntingtin. They reported that the rate of cleavage of huntingtin increases with the length of the polyglutamine tract. It is intriguing to hypothesize that thus truncated protein product containing the polyglutamine tract is toxic to neuronal cells. As mentioned above the toxicity of expanded polyglutamine tracts to neuronal cells has recently been demonstrated in a transgenic mouse and a cell culture system using truncated MJD cDNA (Ikeda *et al.*, 1996). Taken together, the findings described above suggest that processing of gene products with an expanded polyglutamine tract and the toxicity of the processed products of the proteins play key roles in the pathogenesis of neurodegeneration.

Conclusion

As discussed in the present review, many challenging issues regarding the molecular mechanisms of neurodegeneration are associated with investigation of triplet repeat diseases. Since the molecular structures of the causative genes for triplet repeat diseases have been fully elucidated, triplet repeat diseases no doubt offer the best model systems to investigate the pathophysiology of neurodegenerative diseases, and it is strongly expected that creation of animal models of human diseases and development of cell culture systems for investigation of neuronal toxicity caused by triplet repeat expansions will be a crucial step for development of therapeutic measures for triplet repeat diseases.

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