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Molecular pathology of dentatorubral–pallidoluysian atrophy

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Dentatorubral–pallidoluysian atrophy (DRPLA) is an autosomal dominant disorder characterized clinically by myoclonus, epilepsy, cerebellar ataxia, choreoathetosis and dementia. Cardinal pathological features of DRPLA are a combined degeneration of both the dentatorubral and the pallidoluysian systems. Although the early sporadic cases were reported by Western neuropathologists, a strong heritability and an age of onset-dependent variability of the clinical features were carefully deduced by Japanese clinicians. The disease is fairly common in Japan, but extremely rare in Caucasians. Since the gene was identified in 1994, DRPLA is known as one of the CAG repeat expansion diseases, in which the responsible gene is located on chromosome 12p and its product is called atrophin 1. DRPLA shows prominent 'anticipation', which is genetically clearly explained by a marked instability of the expanded CAG repeat length during spermatogenesis. Moreover, the instability of the CAG repeat length also seems to occur in the somatic cells, resulting in 'somatic mosaicism'. Possible mechanism(s) underlying the neuronal cell death in DRPLA are discussed in terms of molecular pathological points of view.

Keywords: dentate nucleus; external segment of pallidum; anticipation; CAG repeat disease; atrophin 1; intranuclear inclusion body

1. INTRODUCTION

Dentatorubral–pallidoluysian atrophy (DRPLA) is an autosomal dominant neurodegenerative disorder characterized clinically by myoclonus, epileptic seizures, cerebellar ataxia, choreoathetotic movements, personality change and dementia. The cardinal clinical features depend on the age of onset. The dentate nucleus of the cerebellum is the most severely affected site followed by the pallidum. Recently, the causative gene of DRPLA was assigned to the short arm of chromosome 12, and the underlying abnormality was identified as an expansion of a CAG repeat within its coding region. The function of the gene product, atrophin 1, has not yet been determined. Among the CAG repeat diseases, DRPLA exhibits a most prominent instability in the number of CAG repeats; they expand significantly in following generations. Furthermore, DRPLA shows a strong ethnic predilection for Asian, particularly Japanese populations. A short history of DRPLA, its clinical and pathological features, classical and molecular genetics, and molecular pathology will be summarized here in order to contribute to a better understanding of DRPLA.

2. HOW DRPLA WAS FOUND

The original presumed case of hereditary DRPLA was reported by Titica & Van Bogaert (1946), describing unique pathological features of the pallidoluysian atrophy combined with an atrophy of the dentatorubral system. This is an important contribution, which is revisited later. Naito *et al.* (1972) made a clinical report on two families who suffered from progressive myoclonus epilepsy with autosomal dominant transmission, pathological studies of which were performed later suggesting the combined degeneration of the pallidal and dentatal systems. Responding to these findings, Oyanagi (1978) examined 15 autopsied brains based 'purely on pathological criteria', i.e. the presence of degenerative changes in efferent systems of both the pallidum and the dentate. Surprisingly, the clinical diagnosis of these cases fell into three groups: (i) progressive myoclonus epilepsy; (ii) Huntington's disease; and (iii) not carrying a specific diagnosis (combination of myoclonus, cerebellar ataxia and choreoathetosis). In addition to this, Naito & Oyanagi (1982) noticed a strong heritability of DRPLA with the pattern of an autosomal dominant trait. Although the first report of DRPLA appeared in a Western country, the establishment of this disease was achieved mostly in Japan.

3. CLINICO-PATHOLOGICAL FEATURES OF DRPLA

Overall, in DRPLA patients of every stage cerebellar ataxia and dementia appeared in 100%, choreoathetoid movements in 74%, epileptic seizures in 65% and myoclonus in 56% (Komure *et al.* 1995). The clear relationship between the clinical picture and the age of onset was noted. Indeed, the juvenile type of DRPLA (age of onset of less than 20 years old) predominantly exhibits myoclonus and epileptic seizures and is often diagnosed as progressive myoclonus epilepsy, whereas the late adult type (age of onset of more than 40 years old) exhibits choreoathetosis, cerebellar ataxia and dementia with no myoclonus or epileptic seizures (Naito 1990). Almost all patients of the adult type show personality

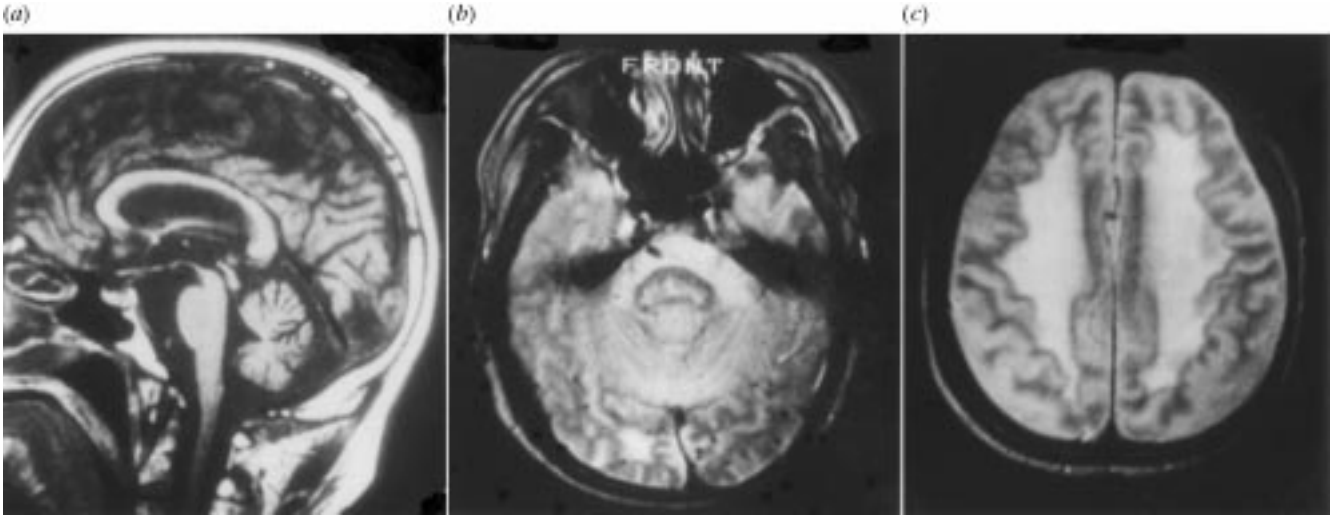

Figure 1. MRI (1.5 T) findings in an adult onset DRPLA patient (54-year-old male). (*a*) The T1-weighted (TR/TE 400/15) midsagittal MRI image reveals proportional but 'small in size' brainstem and cerebellum with VIth ventricular dilatation. (*b*) The T2-weighted (TR/TE 3000/45) axial MRI image reveals diffuse high-intensity signals in the middle pontine region. (*c*) The T2-weighted (TR/TE 3000/45) axial MRI image reveals symmetrical, diffuse high-intensity signals in the periventricular white matter.

changes, i.e. mood changes swinging from euphoria to anger, childish behaviour and severe attention deficit. This personality change is extremely important for the clinical diagnosis.

Electroencephalograph abnormalities are frequently observed, especially in early onset DRPLA patients.

Abnormalities revealed by brain magnetic resonance imaging (MRI) are more clear than those found in computed tomography scans. A cerebellar 'atrophy' with VIth ventricular dilatation (figure 1*a*), and tegmental atrophy of the midbrain with aqueductal dilatation are the cardinal features of brain imaging. A T2-weighted MRI image of late adult onset DRPLA patients frequently demonstrates diffuse high intensity in the cerebral white matter (figure 1*b*). In addition to this, high-signal lesions in the T2-weighted MRI image are found in the pons (figure 1*c*).

Macroscopic pathology revealed that the cerebellum and the brainstem are 'just small in size' or 'hypoplastic', rather than atrophic, because the contours of the cerebellum and pons are well-preserved. Microscopically, combined degeneration of the dentatorubral (dentatofugal) and pallidoluysian (pallidofugal) systems is a characteristic feature of this disease. Neurons in the dentate nucleus are constantly, sometimes most severely, affected (figure 2) (Takahashi *et al.* 1995). The red nucleus neurons are least frequently affected with mild gliosis, if any. On the other hand, neuronal loss in the pallidum is almost always present. It is noteworthy that the neuronal loss in the external segments of the globus pallidus is usually more pronounced than in the internal segments (Takahata *et al.* 1978). Gliosis is clearly demonstrated by the Holzer staining in the external segments (figure 3).

Besides the lesions mentioned above, there is another characteristic feature of DRPLA in the cerebral white matter in which myelin is diffusely lost, corresponding to the high-intensity area in T2-weighted MRI images and being regarded as the lesion responsible for the dementia associated with DRPLA (Yagishita & Inoue 1997).

4. CLASSICAL AND MOLECULAR GENETICS OF DRPLA

Autosomal dominant inheritance of DRPLA has been repeatedly reported (Naito *et al.* 1972; Takahata *et al.* 1978). Although seemingly isolated sporadic cases have sometimes been reported in the literature based on clinical and/or pathological findings, most of those patients were revealed later to have an abnormality in the gene. DRPLA families clearly show anticipation, where in later generations, symptoms began earlier than in preceding generations. Indeed, the age of onset is 10–30 years earlier in the following generation (Sano *et al.* 1994).

Using the CTG-B37 clone reported by Li *et al.* (1993), two Japanese groups (Koide *et al.* 1994; Nagafuchi *et al.* 1994) independently showed that CAG repeats in the gene were expanded exclusively in patients with DRPLA and the DRPLA gene was assigned to 12q13.31. The consensus DRPLA cDNA sequence was revealed to encode 1185 amino acids.

Polymerase chain reaction using a primer set reported by Li *et al.* (1993) amplified products encompassing 7–34 CAG repeats in normal DNA samples and 53–88 CAG repeats in DRPLA patients (Komure *et al.* 1995; Ikeuchi *et al.* 1995). No overlap was found in the number of CAG repeats between normal and DRPLA chromosomes (figure 4). When the relationship between DRPLA gene CAG repeat length on the DRPLA chromosomes and the age of onset of disease in the patients is examined, there is a statistically significant negative correlation between the two; the longer the CAG repeat length, the earlier the age of onset (figure 5).

The acceleration of the age of onset based on a larger increase of the expanded CAG repeats is prominent when the disease is paternally transmitted compared with maternal transmission. Instability of the CAG repeat is considered to result from 'slippage', especially in paternal transmission, i.e. during spermatogenesis. Concerning the correlation between the numbers of

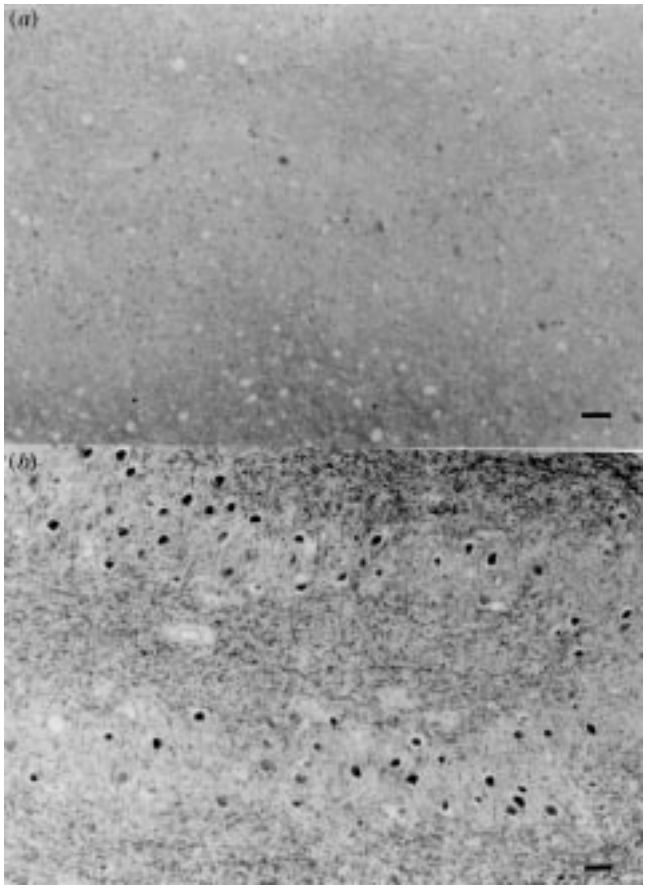

Figure 2. Neuropathological features of the dentate nucleus. (*a*) The normal control dentate nucleus (Klüver–Barrera stain; scale bar, 100 μm). (*b*) Severe neuronal loss of the dentate nucleus (Klüver–Barrera stain; scale bar, 100 μm).

CAG repeats and disease phenotype, clinical features are strongly influenced by the age of onset of DRPLA. Since the age of onset is directly related to CAG repeat length, clinical features should correlate with the number of CAG repeats. Indeed, the mean value of the CAG repeat lengths was much longer for the juvenile type (<20 years old before onset) and shorter for the adult type (>20 years old before onset). Moreover, the age of manifestation of each clinical symptom such as myoclonus, epilepsy, ataxia, choreoathetosis and dementia strongly correlates with the length of the CAG repeat (Ikeuchi *et al.* 1995).

The frequency in normal populations of alleles possessing more than 18 or 19 CAG repeats in the DRPLA gene was clearly higher in Asians (Japanese, Korean and Chinese) than in Caucasians. Therefore, these populations representing longer CAG repeat alleles would provide the basis for a high frequency of DRPLA in Asian populations (Yanagisawa *et al.* 1996).

Haplotypes associated with DRPLA have been extensively analysed (Yanagisawa *et al.* 1996). In normal individuals from Asian populations, the frequency of A1-B1 haplotype (base 1010 is adenine and base 1885 is thymine) is the highest. On the other hand, in Caucasians the frequency of this allele is the lowest. All Japanese DRPLA patients were found to have a haplotype of A1-B1. This would be the reason why DRPLA is more frequent in Asians than Caucasians.

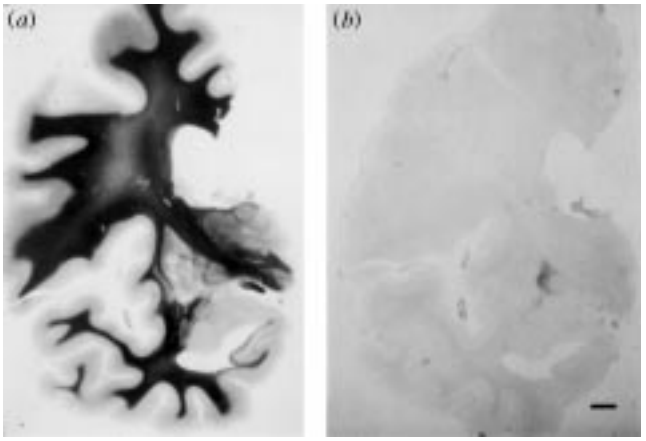

Figure 3. Neuropathological features of the globus pallidus (bar = 1 cm). (*a*) Myelin stain. Note a slight atrophic change of the globus pallidus especially of the external segment of the globus pallidus. (*b*) Holzer stain. Note a strong gliosis restricted to the external segment.

5. MOLECULAR PATHOLOGY OF DRPLA

(a) *Somatic instability or mosaicism*

Gonadal mosaicism in CAG repeat diseases was first investigated in HD. However, somatic cell mosaicism was not prominent in HD (Duyao *et al.* 1993). On the other hand, in the DRPLA cerebellum the expanded allele is constantly smaller than in other tissues (Takano *et al.* 1996; Hashida *et al.* 1997). Interestingly, the degree of relative reduction of expansion of the CAG repeat in the cerebellum is positively correlated with the age of onset: individuals with an onset of disease at a younger age show smaller differences (figure 6). Since DNA in the cerebellar tissue largely comes from granule cells, it is reasonable to suppose that post-mitotic neuronal cells show a smaller degree of expansion of the CAG repeat compared with mitotic cells such as glia, endothelial cells or other non-neural tissues.

(b) *Expression of mRNA and protein, atrophin 1*

Northern blot analyses of mRNA isolated from various tissues revealed that the DRPLA gene product, atrophin 1, is ubiquitously expressed in all normal human tissues examined. However, preferential expression of atrophin 1 mRNA in the cerebellum was also demonstrated using *in situ* hybridization methods (Margolis *et al.* 1996; Nishiyama *et al.* 1997) and antisense riboprobes, especially in the granular cell layer. Moreover, the gene was predominantly found to be expressed in neurons. It is noted, however, that atrophin 1 expressions in DRPLA brain were not different from that of normal brain (Nishiyama *et al.* 1997).

We raised rabbit polyclonal antisera against the C-terminal peptide in atrophin 1 (Yazawa *et al.* 1995). Employing Western blot analysis of human brain tissues using the antisera, the DRPLA gene product in normal brain was identified as an approximately 190 kDa protein. In DRPLA brain, an additional protein of *ca.* 205 kDa was found, corresponding to an expanded CAG repeat allele exclusively present in the DRPLA tissues (figure 7).

Using C-terminal antisera and newly raised rabbit polyclonal antisera against the N-terminal peptide of

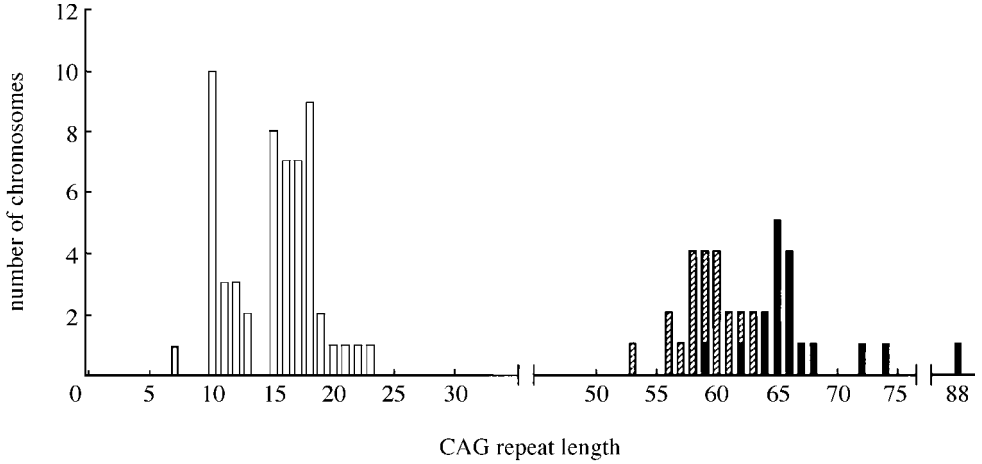

Figure 4. Distribution of CAG repeat length in normal chromosomes (open columns) and the larger of the two alleles (black or shaded columns) in DRPLA patients. Black columns indicate DRPLA chromosomes of juvenile-onset patients and shaded columns those of adult-onset patients (from Komure *et al.* 1995).

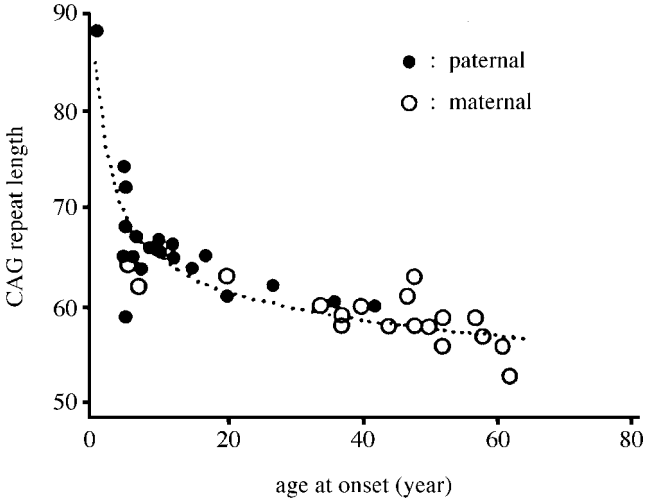

Figure 5. Relationship between CAG repeat length in DRPLA chromosomes and age of onset. Open circles represent alleles of maternal origin, and filled circles alleles of paternal origin. Note the longer the repeat, the earlier the onset (from Komure *et al.* 1995).

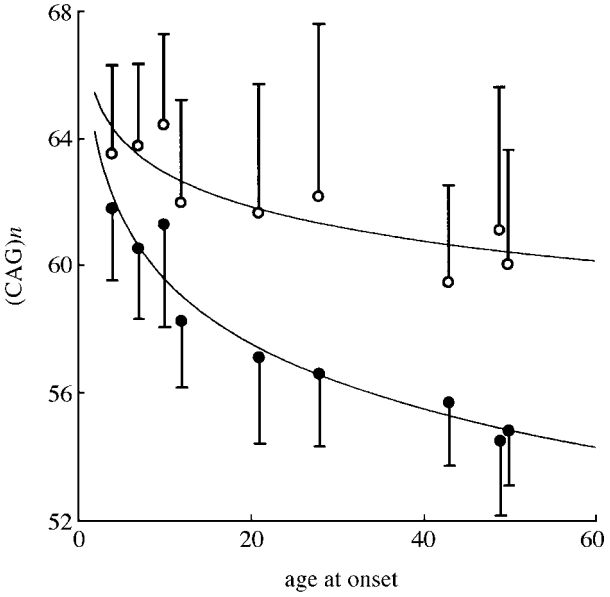

Figure 6. Relationship between the age of onset of DRPLA in patients and the mean size of the expanded allele in frontal cortex (open circles) and cerebellar cortex (filled circles). Bar, 1 s.d. Note the smaller expansion in the cerebellar cortex compared with those in the cerebral cortex (from Hashida *et al.* 1997).

atrophin 1, we confirmed that the atrophin 1 is untruncated in tissues (Yazawa *et al.* 1998). In addition, we found that under non-reducing conditions human atrophin 1 tends to aggregate and to form a large molecular complex (>250 kDa) at the top of the stacking gel (Yazawa *et al.* 1998). Since DRPLA is transmitted in an autosomal dominant fashion, the underlying mechanism for neuronal cell death should be the result of a 'gain of function' due to the presence of protein(s) with abnormal structures and toxic properties.

(c) *Intranuclear inclusion bodies*

Perutz *et al.* (1994) proposed a possible mechanism for neuronal cell death caused by polyglutamine stretches, demonstrating that these molecules can form polar zippers based on their β-sheet structure, which could form the possible intracellular precipitation of such aggregated protein and could lead neurons to die. In fact, Bates and her colleagues (Mangiarini *et al.* 1996; Davies *et al.* 1997) succeeded in revealing intranuclear inclusions in neurons of transgenic mice expressing exon 1 of the HD gene and carrying extremely expanded CAG repeats. Although obvious neuronal cell death was absent, the inclusions are thought to bring about cellular dysfunction.

DRPLA patients also exhibit neuronal inclusions (Igarashi *et al.* 1998). Intranuclear inclusions in neurons of affected patients are located near the nucleolus with their size ranging from 1 to 3 μm. These inclusions can be immunostained with antibodies raised against the expanded polyglutamine stretch or against ubiquitin. It is worth noting that transgenic mice exhibit neurological phenotypes. It is possible, therefore, to suppose that the intranuclear inclusions are somehow related to the pathogenesis of expanding CAG repeat diseases. Neuronal cell loss in DRPLA is prominent in the dentate nucleus, globus pallidus and other regions. Therefore, one should

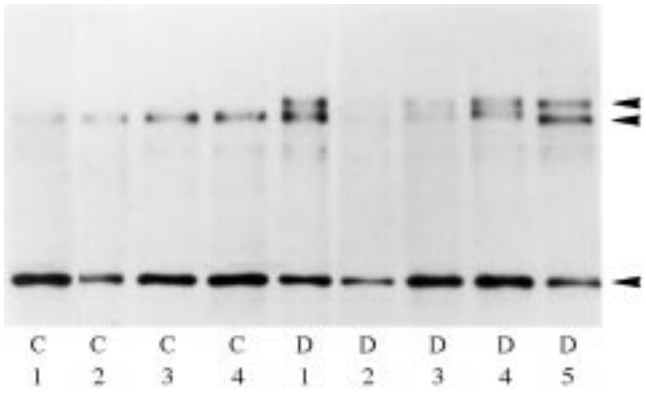

Figure 7. Western blot of DRPLA protein, atrophin 1 in brain tissues, using specific anti-atrophin 1 antiserum detecting the C-terminus peptides. C, control brains (1–4); D, DRPLA brains (1–5). Arrow heads correspond to molecular masses of 205, 190 and 100 kDa, respectively. The bands of 205 kDa indicate the protein containing an abnormally long polyglutamine stretch (from Yazawa *et al*. 1995).

attempt to elucidate the mechanism underlying this selective neuronal cell death.

Recently, using a DRPLA gene expressed in COS cells, Igarashi *et al*. (1998) provided some evidence on the *in vitro* formation of intranuclear inclusions and the induction of apoptosis. They found that only truncated DRPLA protein containing an expanded polyglutamine tract forms perinuclear and/or intranuclear aggregates when expressed in cultured COS-7 cells, which subsequently undergo apoptosis.

Although the above mentioned 'intranuclear inclusion body hypothesis' is most plausible for explaining neuronal cell death at the moment, a problem yet to be solved is that we cannot fully explain the mechanism(s) for the 'selectivity' of neuronal cell death, since atrophin 1 and other abnormally long polyglutamine stretch-containing proteins are expressed widely throughout the nervous system, i.e. there is no evidence for their predominant presence in specific neuronal populations.

6. CONCLUSION

Clinical diagnoses of DRPLA patients are sometimes difficult due to the diversity of the clinical features. However, it is not impossible to reach the correct diagnosis, if (i) the clear relationship between the age of onset and the clinical types, (ii) autosomal dominant inheritance with clear anticipation, and (iii) MRI imaging showing small-sized cerebellum and brainstem, and a diffuse high-intensity lesion in T2-weighted condition in adult patients, are taken into consideration. After the identification of the responsible gene for DRPLA, the above concept is strongly supported by the clear correlation between the length of the CAG repeat and the age of onset. As one of the members of the CAG repeat diseases, neurons in the nervous system of DRPLA patients contain inclusion bodies, which provide the most plausible marker of neuronal cell death at present.

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