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Somatic Mosaicism of Expanded CAG Repeats in Brains of Patients with Dentatorubral-Pallidoluysian Atrophy: Cellular Population-Dependent Dynamics of Mitotic Instability

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Summary

Dentatorubral-pallidoluysian atrophy (DRPLA) is an autosomal dominant neurodegenerative disease caused by unstable expansion of a CAG repeat in the DRPLA gene. We performed detailed quantitative analysis of the size and the size distribution (range) of the expanded CAG repeats in various regions of the CNS of eight autopsied patients with DRPLA. Expanded alleles (AE) showed considerable variations in size, as well as in range, depending on the region of the CNS, whereas normal alleles did not show such variations, which indicates the occurrence of somatic mosaicism of AE in the CNS. The AE in the cerebellar cortex were consistently smaller by two to five repeat units than those in the cerebellar white matter. Moreover, the AE in the cerebral cortex were smaller by one to four repeat units than those in the cerebral white matter. These results suggest that the smaller AE in the cerebellar and cerebral cortices represent those of neuronal cells. The ranges of the AE in the cerebral cortex, cerebral white matter, and cerebellar white matter showed considerable variation ranging from 9 to 23 repeat units, whereas those in the cerebellar cortex showed little variance and were ~7 repeat units. The ranges of the AE in the cerebral cortex, cerebral white matter, and cerebellar white matter were much broader in patients with higher ages at death than they were in patients with lower ages at death, raising the possibility that the range of AE increases with time, as the result of mitotic instability of AE.

Introduction

Dentatorubral-pallidoluysian atrophy (DRPLA; MIM 125370) is an autosomal dominant neurodegenerative disease that has been reported to occur predominantly in Japanese (Oyanagi et al. 1976; Naito et al. 1977; Takahata et al. 1978; Naito and Oyanagi 1982; Naito 1990). DRPLA is characterized by a broad range of age at onset, from the 1st to the 7th decade, and by considerable heterogeneity of clinical phenotypes depending on the age at onset. Patients with onset at age <20 years tend to have a phenotype of progressive myoclonus epilepsy (PME phenotype), whereas adult-onset patients exhibit cerebellar ataxia, choreoathetosis, and dementia (non-PME phenotype) (Takahata et al. 1978; Naito and Oyanagi 1982; Takahashi et al. 1988; Ikeuchi et al. 1995). Characteristic features of DRPLA are accelerated age at onset and enhanced severity of the disease in successive generations, which are more prominent in paternal than in maternal transmission (Takahata et al. 1978; Ikeuchi et al. 1995; Komure et al. 1995).

Speculating that these characteristics of DRPLA are a result of unstable expansion of a CAG repeat, we and others have identified the DRPLA gene that contains a CAG repeat in the coding region (Koide et al. 1994; Nagafuchi et al. 1994*b*). The number of CAG repeat units of normal alleles (AN) in the DRPLA gene ranges from 7 to 35, whereas that of expanded alleles (AE) ranges from 49 to 88 (Koide et al. 1994; Nagafuchi et al. 1994*b*; Ikeuchi et al. 1995; Komure et al. 1995). There is an inverse correlation between the age at onset and the size of AE, and the intergenerational increase in the size of AE has been demonstrated to result in the anticipation (Koide et al. 1994; Nagafuchi et al. 1994*b*; Ikeuchi et al. 1995; Komure et al. 1995).

Unstable expansions of CAG repeats have been identified as the causative mutations in four other neurodegenerative diseases—namely, spinal and bulbar muscular atrophy (SBMA), Huntington disease (HD), spinocerebellar ataxia type 1 (SCA1), and Machado-Joseph disease (MJD) (La Spada et al. 1991; Huntington's Disease Collaborative Research Group 1993; Orr et al. 1993; Kawaguchi et al. 1994). All these diseases are characterized neuropathologically by neuronal degeneration in selected regions of the CNS, with a characteristic distri-

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bution for each disease. In DRPLA, prominent neuronal degeneration is observed in the dentatorubral and pallidoluysian systems (Takahata et al. 1978; Naito and Oyanagi 1982; Takahashi et al. 1988). Another important finding of autopsied DRPLA brains is that they are often unusually small, weighing <1,000 g, which is more frequently observed in patients with higher ages at death (Oyanagi et al. 1976; Takeda et al. 1992; Iwabuchi et al. 1993).

The structures of the gene for DRPLA, as well as that of those for HD, SCA1, and MJD, have been elucidated, and the CAG repeats have been found to encode polyglutamine tracts (Banfi et al. 1944; Huntington's Disease Collaborative Research Group 1993; Kawaguchi et al. 1994; Nagafuchi et al. 1994*a;* Onodera et al. 1995). It has been demonstrated that mRNAs and the proteins encoded by the DRPLA gene, as well as those encoded by the HD and SCA1 genes, are expressed ubiquitously without predilection for the regions with severe neuronal degeneration in the CNS (Hoogeveen et al. 1993; Li et al. 1993; Strong et al. 1993; Nagafuchi et al. 1994*a;* Landwehrmeyer et al. 1995; Onodera et al. 1995; Stine et al. 1995; Yazawa et al. 1995). Recently, the presence of mutant gene products with expanded polyglutamine tracts has been detected in brains of patients with DRPLA, SCA1, and HD (Aronin et al. 1995; Schilling et al. 1995; Servadio et al. 1995; Sharp et al. 1995; Trottier et al. 1995; Yazawa et al. 1995). A role in vesicle trafficking has been suggested for the HD gene product (DiFiglia et al. 1995; Gutekunst et al. 1995). Although a number of hypotheses as to the functions of the polyglutamine tract have been proposed—including hypotheses of transcriptional regulation (Gerber et al. 1994), a polar zipper joining specific transcriptional factors (Perutz et al. 1994), or a substrate for transglutamination (Green 1993)—it remains unknown how the presence of the expanded CAG repeats leads to neuronal degeneration in selected regions of the CNS.

Telenius et al. (1994) analyzed the size distributions of AE in various regions of the autopsied brains of patients with HD and found that the AE in the cerebellum were smaller than those in other regions of the CNS. On the basis of this observation, they suggested that the smaller size of the AE in the cerebellum may account for the fact that the cerebellum is not affected in HD (Telenius et al. 1994). However, it recently has been reported that the AE in the cerebellum are also smaller than those in the cerebrum in SCA1 (Chong et al. 1995) and DRPLA (Ueno et al. 1995). Since the cerebellum is affected in SCA1 and DRPLA, the smaller size of the AE is not necessarily associated with lack of neuronal degeneration. Therefore, the significance of the somatic mosaicism of AE in the CNS in these diseases is unclear.

Since DRPLA showed the largest intergenerational changes in the size of AE, as well as the most prominent anticipation, compared with the other diseases caused by expansions of CAG repeats (Ikeuchi et al. 1995), we considered DRPLA to be quite suitable for investigating phenomena associated with the instabilities of the CAG repeats. Thus, we have undertaken detailed quantitative analysis of the somatic mosaicism in various regions of autopsied DRPLA brains, to elucidate the roles of somatic mosaicism in neuronal degeneration in DRPLA.

Patients, Material, and Methods

Patients and Samples

We analyzed various tissues from eight autopsied DRPLA patients whose profiles are summarized in table 1. The neuropathological findings of all of the patients except N20-91 have been described elsewhere (Naito and Oyanagi 1982; Takahashi et al. 1988; Takeda et al. 1992). For analysis of various regions of the CNS, cerebral cortices from all eight patients, white matter from seven, cerebella from six, thalami from two, striata from two, spinal cords from two, dentate nucleus from one, and dorsal root ganglion from one were analyzed. Livers from four patients, hearts from three, kidney from one, and skeletal muscle from one also were analyzed in four patients with PME phenotype. These tissues were immediately frozen at autopsy and were kept at −70°C until the analysis. For detailed comparison, cortices and white matter of cerebra and cerebella from five patients (N17-81, N20-91, KK, N17-88, and N20-86) were precisely dissected under a stereomicroscope. Lymphoblastoid cell lines established from peripheral lymphocytes of the 5 DRPLA patients were analyzed and were compared with peripheral white blood cells of the same 5 patients, as well as with those of 16 other DRPLA patients. High-molecular-weight genomic DNAs were extracted from these samples by using a standard method (Maniatis et al. 1989).

Analysis of the CAG Repeat

The genomic DNA segment containing the CAG repeat of the DRPLA gene was amplified by PCR using a method described elsewhere (Koide et al. 1994), with minor modifications as follows: PCR was performed in a total volume of 25 μl containing 10 mM Tris HCl (pH 8.4), 50 mM KCl, 2.0 mM $MgCl_2$, 2.0 M N,N,N-trimethylglycine (Reeves et al. 1994), 250 μM each dNTP, 6.25 pmol of each primer, with the one primer labeled at the 5′ end (150 kBq), and 200 ng of genomic DNA; and PCR products were electrophoresed through denaturing 5% polyacrylamide gels at 53–55°C and were autoradiographed to Fuji RX films by using an intensifying screen.

The numbers of CAG repeat units were determined by using, as size markers, the PCR products from the plasmid DNAs containing various numbers of the CAG

Table 1

Profiles of Autopsied DRPLA Patients—and Somatic Mosaicism of AE in Various Tissues

	N17-81	N66-73	N20-91	N17-76	N44-75	KK	N17-88	N20-86
Age at onset (years)	8	10	10	13	30	33	61	62
Age at death (years)	18	24	28	26	37	48	76	79
Disease duration (years)	10	14	18	13	7	15	15	17
Phenotype	PME	PME	PME	PME	Non-PME	Non-PME	Non-PME	Non-PME
Brain weight (g)	920	700	950	1,010	1,020	980	960	870
Size of AN (repeat units)	24	17	18	16	9	18	11	9
Size [size of range; range[a]] of AE (repeat units):								
Cerebral cortex[b]	68 [10; 64–73]	67 [11; 63–73]	66 [12; 62–73]	66 [14; 61–74]	64 [15; 57–71]	63 [16; 57–72]	60 [19; 53–71]	60 [20; 53–72]
Cerebral white matter[c]	69 [9; 66–74]	68 [12; 64–75]	68 [12; 64–75]		67 [15; 60–74]	66 [16; 60–75]	62 [19; 57–75]	64 [19; 57–76]
Cerebellum	66 [7; 63–69]		64 [7; 61–67]	64 [9; 60–68]		61 [7; 58–64]	55 [7; 52–58]	55 [7; 51–57]
Cerebellar cortex	66 [7; 63–69]		64 [7; 61–67]			61 [7; 58–64]	55 [7; 52–58]	55 [7; 53–59]
Cerebellar white matter	69 [11; 65–75]		67 [12; 63–74]			66 [16; 60–75]	61 [23; 52–74]	55 [19; 53–71]
Other CNS regions[d]	67 [10; 63–72] (DRG)		67 [11; 63–73] (SP)		65 [15; 58–72] (ST)	64 [16; 58–73] (T)	55 [6; 53–58] (ST) 60 [19; 52–70] (DN) 60 [19; 52–70] (SP)	60 [19; 54–72] (T)
Nonneural tissues[e]	67 [7; 65–71] (H) 66 [17; 65–81] (L)	66 [7; 64–70] (H) 66 [18; 64–81] (K) 69 [18; 64–81] (L)	65 [7; 62–68] (M) 65 [21; 62–82] (L)	65 [10; 61–70] (H) 64 [21; 61–81] (L)				

[a] Shows smallest and largest AE showing ≥10% of signal intensity of major band.
[b] Frontal cortices were analyzed in all the patients. In N17-88, temporal and occipital cortices were also analyzed, and there was no difference in size and range of AE, compared with those for the frontal lobe.
[c] Frontal white matter was analyzed in all patients. In N17-88, occipital white matter was also analyzed, and there was no difference in size and range of AE.
[d] DRG = dorsal root ganglion; SP = spinal cord; ST = striatum; T = thalamus; and DN = dentate nucleus.
[e] H = heart; M = skeletal muscle; L = liver; and K = kidney.

repeat units (Koide et al. 1994). For quantitative analysis of the sizes and the size distributions (ranges) of AE, the autoradiograms were subjected to densitometric scanning using a Shimadzu scanning densitometer (CS-9300PC). We defined as the major band the band that gave the most intense signal, and we defined as the size of the AE the number of the CAG repeat units of the major band. To determine the degree of somatic mosaicism, the range of AE was defined as the number of bands giving $\geq 10\%$ of the signal intensity of the major band.

Statistical Analysis

Pearson's correlation test was used to determine both the correlation between the size of AE and the age at death and the correlation between the range of AE and either the age at death or the size of AE. A P value $<.05$ was considered statistically significant. We performed these statistical analyses by using SPSS version 6.01 for Windows.

Results

Somatic Mosaicism of AE in DRPLA

Representative autoradiograms of PCR products from genomic DNAs of various tissues from N17-76 and N17-81 are shown in figure 1*A*. PCR products of the AN showed a single intense band and a few less-intense bands, similar to the PCR products obtained by using the plasmid DNA containing 62 CAG repeat units as the template. In contrast, PCR products of the AE showed characteristic ladder patterns consisting of multiple bands of similar intensities. For the quantitative analysis of the sizes and the ranges of the AE, the autoradiograms were subjected to densitometric scanning. Representative densitometric tracings of the lanes of the autoradiogram of N17-76 are shown in figure 1*B*. The sizes and the ranges of the AE showed considerable variations among different regions of the CNS, as well as among different nonneural tissues in the same patients (table 1). These results strongly indicate the occurrence of somatic mosaicism of the AE in various tissues of DRPLA patients.

In each tissue, the size of the AE was found to be inversely correlated with the age at death (fig. 2). Significant correlation between the size of the AE and the age at death was seen for the cerebral cortex ($r = -.985$, $P < .05$), cerebral white matter ($r = -.963$, $P < .05$), cerebellar cortex ($r = -.998$, $P < .05$), and cerebellar white matter ($r = -.917$, $P < .05$). Similar inverse correlation was observed between the size of the AE and the age at onset (data not shown).

AE in the Cerebellum: Smaller than Those in Other CNS Regions

The AE in the cerebellum were consistently smaller by two to five repeat units than those in other CNS regions of the same patients (table 1). Furthermore, the ranges of AE in cerebellum were seven or nine, much narrower than those in other regions of the CNS of the same patients (table 1).

The cerebella of N17-88 (Takeda et al. 1992) and N20-86 (Takahashi et al. 1988) were more severely affected, showing severer Purkinje cell loss, than those of the other patients. Even in these two patients, the AE in the cerebella were smaller by five repeat units than those in other regions (table 1). Therefore, these findings do not support the interpretation that the regions with smaller AE are less affected than those with larger AE. Among the severely affected regions in DRPLA, the dentate nucleus was analyzed in only one patient (N17-88), and the size and the range of the AE were similar to those in the cerebral cortex of the same patient (table 1).

AE in Cortex: Smaller than Those in White Matter

The cerebellum showed the smallest AE and the narrowest range of AE, among all tissues examined in each patient (table 1). We considered that the small AE in the cerebellum may reflect the characteristic cellular populations of the CNS regions. We then dissected the cerebellum precisely, into the cortex and white matter, under a stereomicroscope, and analyzed the AE of the five patients (fig. 3*A* and *B*). We found characteristic differences between the cerebellar cortex and white matter. The sizes and the ranges of the AE in the cerebellar cortex were similar to those in the cerebellum in each patient (table 1). In contrast, the sizes and the ranges of the AE in the cerebellar white matter were found to be much larger and broader, respectively, than those in the cerebellar cortex (fig. 3 and table 1). In the cerebellar white matter of N20-86, the AE showed a ladder pattern with an extremely broad range (53–71 repeat units) and a bimodal distribution with peaks at 55 and 60 repeat units (fig. 3*B*). Although the size of the most intense band in the cerebellar white matter of this patient was the same, by definition, as that in cerebellar cortex (See Patients, Material, and Methods), the size of the second-most-intense band (60 repeat units) was larger by 5 repeat units than that in the cerebellar cortex (fig. 3*B*). The bimodal distribution of AE in the cerebellar white matter of N20-86 might reflect a dissection artifact with inclusion of some cortical tissue in the white matter preparation.

Given the fact that the AE in the cerebellar cortex were smaller than those in the cerebellar white matter, we speculated that such significances might be characteristic in cortices containing neuronal cells. We then dissected the cerebrum into the cortex and white matter, under a stereomicroscope. We found that the AE in the cerebral cortex were consistently smaller by one to four repeat units than those in the cerebral white matter,

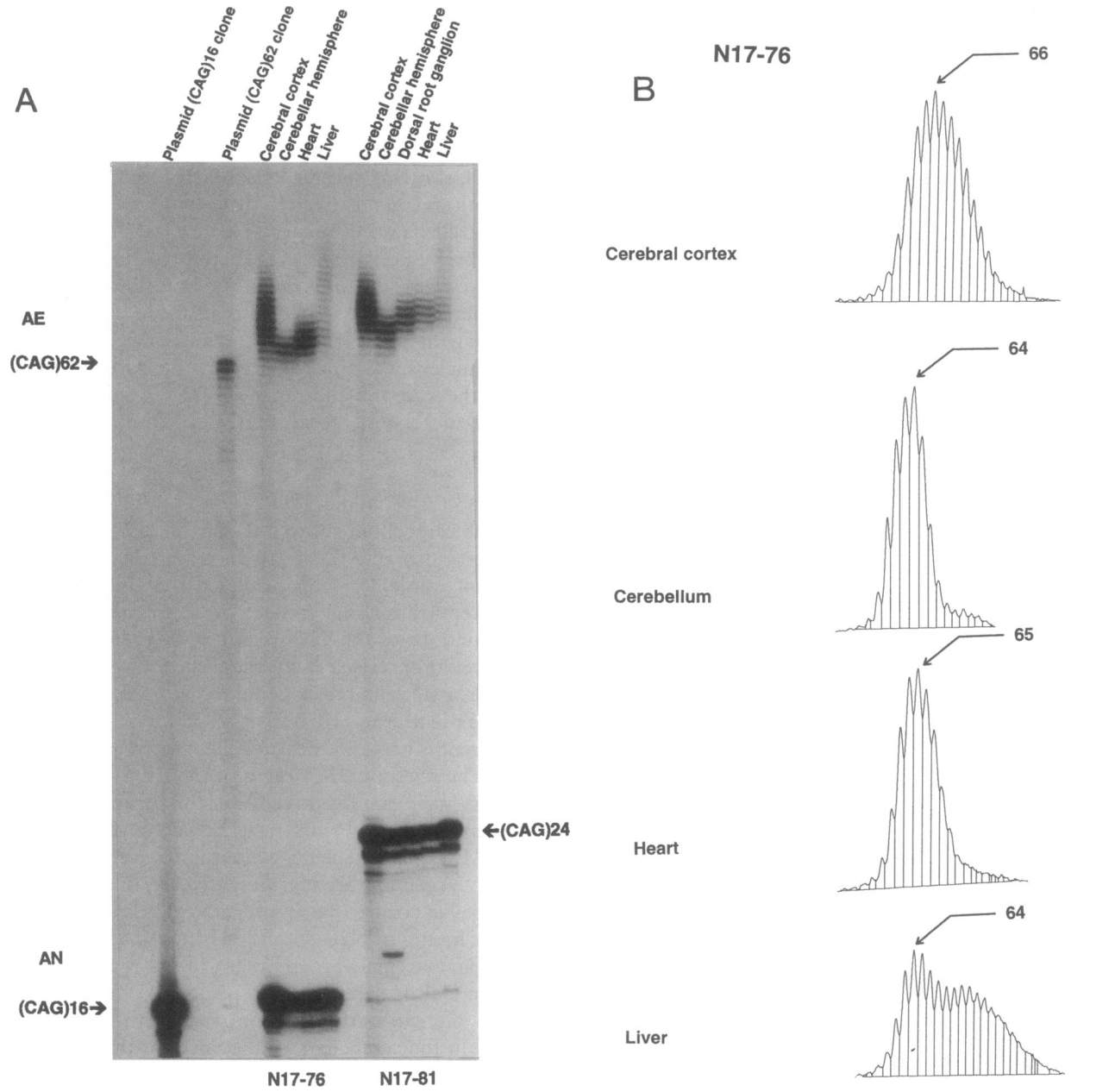

Figure 1 *A*, Representative autoradiogram of the PCR products obtained from 2 patients (N17-76 and N17-81). The genomic DNA segment containing the CAG repeat of the DRPLA gene was amplified by PCR and was electrophoresed through a 5% denaturing polyacrylamide gel, along with PCR products obtained by using, as a template, plasmid DNA containing 62 CAG repeat units. PCR products derived from AN showed a very intense band and a few less-intense bands. PCR products from AE showed a ladder pattern consisting of multiple bands of similar intensities. *B*, Representative densitometric tracings of the N17-76 lanes on the autoradiogram. The major band was designated as the band showing the most intense signal. The size of the AE was defined as the number of CAG repeat units of the major band. The range of the AE was defined as the number of bands giving ≥10% of the signal intensity of the major band.

although the ranges of the AE in the cerebral cortex were as broad as those in the cerebral and cerebellar white matters (fig. 3 and table 1).

As shown in the photomicrograph of the cerebellar cortex of one DRPLA patient (N20-86; fig. 4*A*), granule cells were preserved in DRPLA, although Purkingie cells were reduced in number. There were numerous granule cells, which are neuronal cells, in the granular layer of the cerebellar cortex. Therefore, the smaller AE in the cerebellar cortex are likely to represent mostly, if not entirely, those of granule cells. In contrast, the cerebral cortex showed a heterogeneous cellular population with

Figure 2 Relationship between size of AE and age at death. A significant correlation between the size of the AE and the age at death was seen for the cerebral cortex ($r = -.985$, $P < .05$), cerebral white matter ($r = -.963$, $P < .05$), cerebellar cortex ($r = -.998$, $P < .05$), and cerebellar white matter ($r = -.917$, $P < .05$).

mild reduction in neuronal cells and astroglial proliferation in DRPLA (fig. 4*B*), which suggests that the AE of larger size and broader range in the cerebral cortex, cerebral white matter, and cerebellar white matter largely represent those of nonneuronal cells.

Ranges of AE: Broader in Patients with Higher Ages at Death and Smaller AE

Whereas the ranges of the AE in the cerebellar cortex were constant at approximately seven repeat units, irrespective of the age at death, those in the cerebral cortex, cerebral white matter, and cerebellar white matter were broader in patients with higher ages at death and smaller AE. There was a significant correlation between the age at death and the range of AE in the cerebral cortex ($r = .967$, $P < .05$), cerebral white matter ($r = .969$, $P < .05$), and cerebellar white matter ($r = .946$, $P < .05$) (fig. 5*A*), which raises the possibility that the range of AE gets broader with age. There was a significant inverse correlation between the range and the size of AE in the cerebral cortex ($r = -.995$, $P < .05$) and cerebral white matter ($r = -.928$, $P < .05$), but not in cerebellar white matter ($r = -.755$, $P < .14$) (fig. 5*B*).

Comparison of Somatic Mosaicism of AE of Lymphoblastoid Cell Lines and Peripheral White Blood Cells

We compared the sizes and the ranges of AE of lymphoblastoid cell lines established from five patients at the 10th passage with those of AE of peripheral blood cells from the corresponding patients. Although the sizes of the AE of the lymphoblastoid cell lines were similar to those of the AE of the peripheral white blood cells of the same patients, the AE of the lymphoblastoid cell line of one patient (Kb) were larger by two repeat units than those of the peripheral white blood cells of the same patient (fig. 6). The ranges of the AE of the lymphoblastoid cell lines were narrower by one or two repeat units than those of the peripheral white blood cells. We also analyzed the AE of peripheral white blood cells from 21 patients, including these 5 patients, of various ages and sizes of AE, to determine whether there was a correlation between the range of AE and either the patients' age or the size of AE. The ranges of the AE in the peripheral white blood cells were almost constant between 7 and 11 repeat units, irrespective of either the age of the patient or the size of AE (data not shown).

Discussion

As shown in figure 1*A*, the PCR products of the AE were visualized as 7–23 bands of similar intensity on autoradiograms, which is in strong contrast to the case of the AN, where a single intense band and a few less-intense bands were observed, as was observed for the case of the PCR products derived from the plasmid DNA containing 62 CAG repeat units. The presence of a few less-intense bands in addition to the most intense major band of the PCR products derived from the plasmid DNA containing 62 CAG repeat units, however, indicates that PCR artifacts were present to some extent. Although some corrections might be required if contributions from such PCR artifacts are to be avoided, the correction for the PCR products derived from genomic DNA containing multiple copies with different CAG repeat lengths can be complex. Furthermore, the striking difference, in ladder patterns, between the PCR products derived from AE and those derived from AN suggests that our analyses mostly reflect the presence of AE with variable CAG repeat lengths. The presence of multiple bands derived from AE, therefore, reflects length heterogeneity in the expanded CAG repeat in somatic cells—that is, somatic mosaicism. It should be emphasized that there were no differences, in size or range of the AN, among different tissues even in the same patient. These results strongly indicate that there is a striking difference between the mitotic instability of AE and that of AN.

For the quantitative analysis of the somatic mosaicism, we performed densitometric scanning of autoradiograms, and we determined the size and the range of the AE, as defined in Patients, Material, and Methods (fig. 1*B*). Although there was variation in the size and the range of the AE in different regions of the CNS, our results did not indicate that regions with smaller AE or narrower ranges of AE showed less neuronal degeneration than those with larger AE or broader ranges of AE. Another recent study on somatic mosaicism in DRPLA

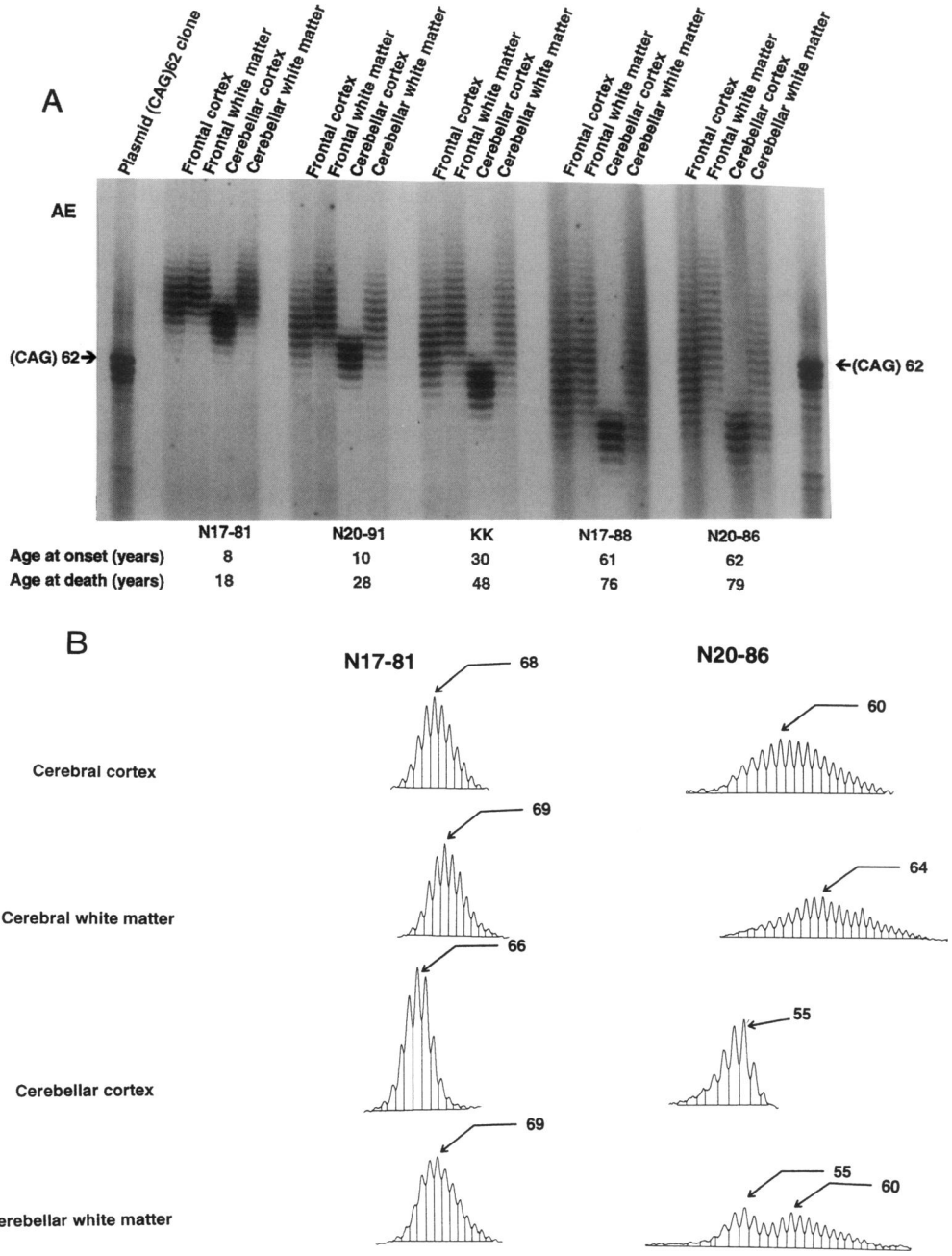

Figure 3 *A*, Comparison of AE among the cerebral cortex, cerebral white matter, cerebellar cortex, and cerebellar white matter. The cerebellar cortex showed the smallest AE in each patient. In contrast, the cerebellar white matter contained AE that were much larger in size and broader in range than those in the cerebellar cortex. The AE in the cerebral cortex were consistently smaller by one to four repeat units than those in the cerebral white matter. Whereas the ranges of AE in the cerebellar cortex were constant at approximately seven repeat units, those in the cerebral cortex, cerebral white matter, and cerebellar white matter were broader in patients with higher ages at death than in those with lower ages at death. *B*, Densitometric-tracing data for two patients (N17-81 and N20-86). These data clearly show that the ranges of AE in the cerebral cortex, cerebral white matter, and cerebellar white matter were markedly broader in the patient with the higher age at death (N20-86; age at death, 79 years) than in the patient with the lower age at death (N17-81; age at death, 18 years).

(Ueno et al. 1995) has revealed that, in the brains of six individuals, the AE in the dentate nucleus were similar in size to those in the cerebral cortices in three patients and were smaller in three other patients. These observations also do not support the hypothesis that larger AE are associated with severer neurodegeneration than are smaller AE. However, it remains possible that most of the neuronal cells in the dentate nucleus were lost and

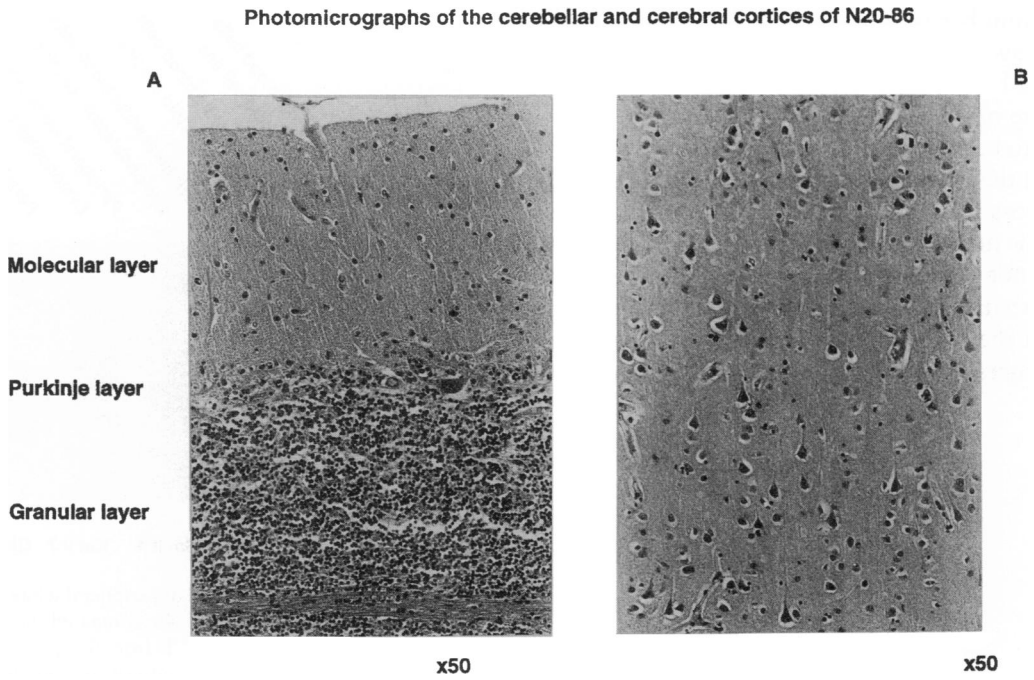

Figure 4 Photomicrographs of cerebellar cortex (*A*) and cerebral cortex (*B*) of DRPLA patient N20-86. In the cerebellar cortex, Purkinje cells were reduced in number, and the molecular layer was reduced in width, with a mild increase of astrocytic nuclei. However, granular cells, which are neuronal cells, were preserved. In the cerebral cortex, there were heterogeneous cellular populations, with reduction in neuronal cells and proliferation of astroglia.

that the AE do not represent those of neuronal cells in the dentate nucleus.

The presence of smaller AE in the cerebellum than in other regions of the CNS has been reported not only in cases of DRPLA (Ueno et al. 1995) but also in cases of HD and SCA1 (Telenius et al. 1994; Chong et al. 1995). Although the cerebellum is not affected in HD, it is the major region showing neuronal degeneration in SCA1 (Genis et al. 1995). These findings indicate that the smaller AE in the cerebellum than in other regions of the CNS is a phenomenon common to these diseases caused by expansions of CAG repeats. An interesting discovery in the present study is that the smaller AE in the cerebellum represent those of the cerebellar cortex. A similar finding was obtained in the case of the cerebral cortex, although the difference, in the size of the AE, between the cerebral cortex and cerebral white matter is much smaller than that between the cerebellar cortex and cerebellar white matter. Thus, we assume that the AE of neuronal cells are smaller in size and narrower in range than those of glial cells. The AE in the dorsal root ganglion (N17-81), which is also rich in neuronal cells, were smaller than those in the cerebral cortex (fig. 1*A* and table 1), which further supports the hypothesis that neuronal cells have smaller AE than do glial cells. Since we did not analyze the AE in individual neuronal cells or glial cells in the present study, the interpretation that neuronal cells contain smaller AE than do glial cells awaits confirmation by the analysis of single cells.

The second-smallest AE in the neural, as well as non-neural, tissues examined were observed in heart and skeletal muscle cells (table 1). Since heart and skeletal muscle cells are also postmitotic cells, the smaller AE may be a feature common to postmitotic cells. As shown in figure 6, some of the lymphoblastoid cell lines at the 10th passage showed AE that were smaller by one or two repeat units than those of peripheral white blood cells of the corresponding patients. This may reflect the clonal nature of the lymphoblastoid cell lines. On the other hand, the livers of the four patients examined showed considerably broad ranges of AE (table 1 and fig. 1). These findings indicate that the degree of mitotic instability varies among mitotic cells of different lineages.

The most interesting finding in the present study is that the ranges of AE in the cerebral cortex, cerebral white matter, and cerebellar white matter are broader in patients with higher ages at death (fig. 5) than in patients with lower ages at death. In addition, there was also an inverse correlation between the range of AE and the size of AE. Thus the range of AE is correlated with the age at death and with the size of AE. Although the results raise the possibility that the range of AE increases with age, detailed analysis of somatic mosaicism by us-

ing a larger number of autopsied brains from patients with various ages at death, as well as with various sizes of AE, would be required to determine how the age at death and size of AE contribute to the range of AE.

It remains to be elucidated how the somatic mosaicism is involved in the neuronal degeneration in DRPLA. Although it is possible that increased somatic mosaicism is involved in the neurodegenerative processes, the present findings do not provide data that support this contention. Alternatively, there may be no direct relationship between the somatic mosaicism and the neurodegenerative processes. The somatic mosaicism may even be protective for neuronal cells, since the range of AE is larger in patients with higher ages at death.

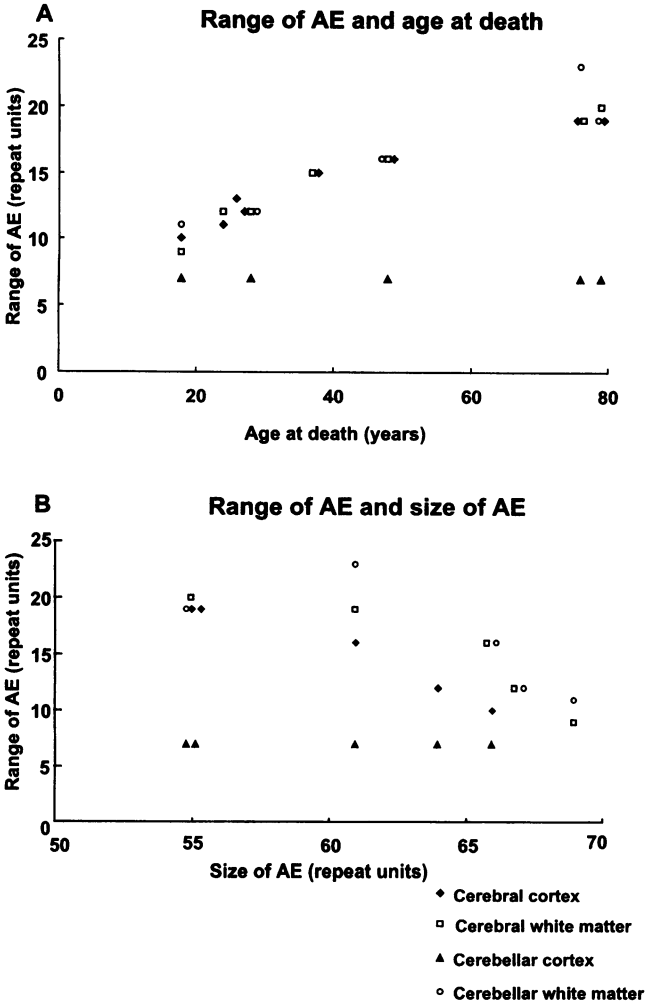

Figure 5 *A*, Relationship between range of AE and age at death. A significant correlation between the range of AE and the age at death was seen for the cerebral cortex ($r = .967$, $P < .05$), cerebral white matter ($r = .969$, $P < .05$), and cerebellar white matter ($r = .946$, $P < .05$). *B*, Relationship between range of AE and size of AE. An inverse correlation between the range of AE and the size of AE was seen for the cerebral cortex ($r = -.995$, $P < .05$) and cerebral white matter ($r = -.928$, $P < .05$). This relationship was not significant in the cerebellar white matter ($r = -.755$, $P < .14$).

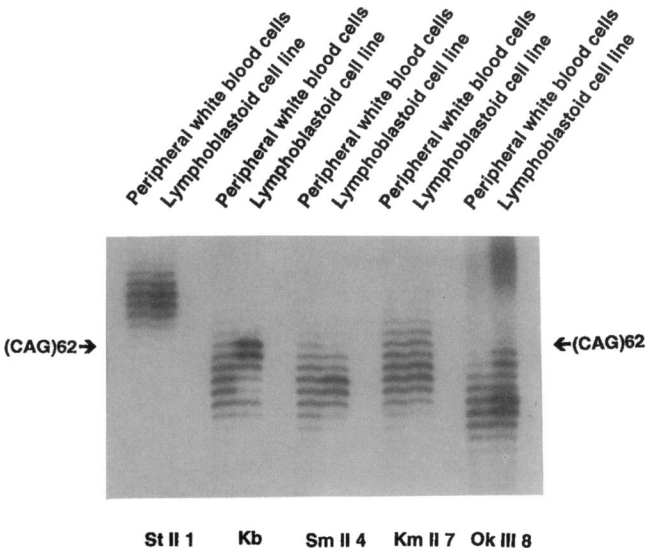

Figure 6 Comparison of AE of peripheral white blood cells with those of lymphoblastoid cell lines. We compared the sizes and ranges of AE of peripheral white blood cells from five patients with those of AE of corresponding lymphoblastoid cell lines at the 10th passage. Although the AE of the lymphoblastoid cell lines were similar in size to those of the peripheral white blood cells of the same patients, the ranges of the AE of the peripheral white blood cells were narrower by one or two repeat units.

As shown in Results, there seem to be considerable heterogeneities of the size and the range of AE among various regions of the CNS. Given such heterogeneities, we need to analyze the size and the range of AE of individual neuronal and glial cells of patients with various ages at death, to investigate whether the unique distribution of neuronal loss is correlated with the instability of the CAG repeat.

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