Analysis of the CAG Repeat Number in a Patient with Huntington’s Disease

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This study was performed to confirm 1) the difference in the trinucleotide CAG repeat number among tissues, 2) somatic mosaicism in each tissue, 3) the correlation of the repeat number with pathological severity in Huntington’s disease. The CAG repeat number was determined by analysis of the polymerase chain reaction (PCR) product in various tissues, including central nervous system (CNS) tissues and non-CNS tissues. We also determined the pathological severity grade in each brain section and compared this with the results of CAG repeat analyses. The patient was a Japanese male with Huntington’s disease who died at 62 years of age. Genomic DNA was extracted from 10 parts of the central nervous system and 6 parts of other tissues from the patient. Each part of the formalin-fixed brain was subjected to gross and microscopic pathological assessment. The main peaks of CAG repeat in all tissues were 22 and 44. In analysis of somatic mosaicism, high degrees of mosaicism were obtained in the caudate nucleus, putamen and cerebral cortex, in which more severe degeneration was observed by pathological examination. These results, although this is a single case study, indicated that pathological severity did not correlate with the CAG repeat number, but it did relate to the degree of somatic mosaicism. Somatic mosaicism might reflect region-specific neuronal degeneration in Huntington’s disease. (Internal Medicine 38: 407-411, 1999)

Key words: triplet repeat disease, somatic mosaicism, neurodegeneration

Introduction

Huntington’s disease (HD) is a hereditary, late onset and progressive neurodegenerative disorder characterized by hyperkinetic motor disturbance, psychiatric manifestation and dementia. It is inherited in an autosomal dominant manner and affects 5 in 100,000 in Europe and 0.5 in 100,000 in Japan (1). The HD gene is localized in the 4p16.3 region (2). In 1993, the mutation was identified as an unstable trinucleotide CAG repeat in the 5’ coding region of the HD gene (3). On normal allele, the CAG repeat number: (CAG)n usually varied between 10 to 36 repeats, whereas on causative allele for HD, the number of (CAG)n expanded to at least 40 repeats or more (3, 4). There was an inverse correlation between the repeat size of the (CAG)n and age of the onset of symptoms (5). In 1994, Telenius et al (6) reported the existence of (CAG)n mosaicism in tissues derived from HD patients. The greatest degrees of mosaicism tended to be seen in the basal ganglia and cerebral cortex in which the most obvious neuropathological changes were also identified. On the other hand, the cerebellar cortex, which was seldom involved, displayed the lowest degree of (CAG)n mosaicism (6). Here, we examined the (CAG)n repeat number and somatic mosaicism in various tissues, including central nervous system (CNS) tissues and non-CNS tissues, taken from a Japanese patient with HD. We also determined the pathological severity grade in each brain section and compared this with the results of CAG repeat analyses.

Materials and Methods

Subject and materials

Postmortem tissues were obtained from a Japanese male patient who had been clinically diagnosed as having HD, did his mother and grandmother. At the age of 48, he was found to have choreiform movement in his shoulder and mouth. Several suicidal actions had ended in failure. He was diagnosed as...
HD at the age of 50. The patient died after respiratory failure caused by bacterial pneumonia at the age of 63.

Ten parts of the brain (frontal cortex, temporal cortex, parietal cortex, occipital cortex, cerebral white matter, caudate nucleus, putamen, thalamus, cerebellar cortex, and cerebellar white matter) and 6 other tissues (from leukocytes, liver, kidney, heart muscle, lung, and testis) were removed within 10 hours of death. All samples were immediately frozen and kept at -80°C until examination. Remaining brain tissues were also kept for pathological examination. An autopsied brain from an age-matched male who had no neurological disorder was also examined as a control.

**Analysis of (CAG)n repeat number**

Tissue homogenates were prepared by the standard liquid nitrogen homogenate method (7). Genomic DNA was extracted from the homogenized samples by two phenol treatments and one phenol/chloroform treatment following the incubation with proteinase K. Polymerase chain reaction (PCR) was performed in 20μl reaction mixture containing 200 ng genomic DNA, 20 pmol of each primer of HD-1 and HD-3 (8), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 mM dNTPs, 10% dimethylsulfoxide, and 0.5 unit Taq polymerase (AmpliTaq, Perkin Elmer, USA). The sequences of the HD-1 and HD-3 primers (8) were 5'-ATG AAC GCC TTC GAG TCC CTC AAG TCC CTC-3' which were fluorescence labeled with Cy5 and 5'-GGC GGT GGC GGC TGT TGC TGC TGC TGC TGC-3', respectively. After heating at 95°C for 3 minutes, 30 cycles of 1 minute at 95°C, 1 minute at 65°C and 1 minute at 72°C were performed with a Perkin Elmer Thermalcycler followed by a 10 minute extension at 72°C. The PCR fragments were separated on 6% denatured polyacrylamide (containing 7 M urea) gels with a DNA sequencer (ALF-read autosequencer, Pharmacia) and each PCR fragment was analyzed with a Pharmacia DNA fragment manager.

**Quantitative analysis of somatic mosaicism**

To evaluate somatic mosaicism in each tissue, we adopted the method described by Telenius et al (6) and calculated the densitometry index (D.I.) (6). The D.I. is one of the means to understand the level of mosaicism and has been applied for the analyses of other triplet repeat disorders (9). For each tissue, the D.I. was obtained by analyzing the ratio between the intensity of the first band immediately above the major size allele to the major band itself. The reproducibility of the index was confirmed by performing PCR at least twice on the same sample.

**Pathological assessment**

The patient’s brain was neuropathologically examined to confirm the clinical diagnosis of HD. Each part of the formalin-fixed brain was subjected to further gross and microscopic pathological assessment. Neuronal loss was graded as -: none, ±: very mild, +: mild, ++: moderate, +++: severe.

### Results

#### Analysis of number of (CAG)n repeat

The number (CAG)n repeat was analyzed with DNA fragment manager (Fig. 1). No difference of the repeat number

![Figure 1. Fluorescent densitometry of PCR fragments of each brain section and other tissues. Lane 1; control (non-HD cerebral cortex), lane 2–17; the HD patient. Lane 2; frontal cortex, lane 3; temporal cortex, lane 4; parietal cortex, lane 5; occipital cortex, lane 6; cerebral white matter, lane 7; caudate nucleus, lane 8; putamen, lane 9; thalamus, lane 10; cerebellar cortex, lane 11; cerebellar white matter, lane 12; leukocytes, lane 13; liver, lane 14; kidney, lane 15; heart muscle, lane 16; lung, and lane 17; testis.](image-url)
obtained from the major peaks of normal and HD allele between the brain and other tissues was observed. The numbers of (CAG)n repeats were 22 (normal allele) and 44 (expanded allele) in the HD patient, and 19 and 22 in the control subject.

**Analysis of somatic mosaicism**

The variability of the numbers of (CAG)n repeat within each tissue showed obvious difference in the analysis of D.I., as shown in Table 1. Higher values of D.I. of expanded alleles were observed in the caudate nucleus (0.71), the putamen (0.69) and the cerebral cortex (frontal: 0.57, parietal: 0.59, temporal: 0.68, and occipital: 0.56). In the patient, the lowest values were shown in the cerebellum; 0.42 in the cerebellar cortex and 0.41 in the cerebellar white matter. Non-CNS tissues tended to have smaller values than CNS tissues.

**Pathological assessment**

The autopsied small-sized brain weighed 1,070 g. In gross findings, it showed severe atrophy of the corpus striatum with dilatation of the lateral ventricles. Atrophy and discoloration of the globus pallidus, subthalamic nucleus, thalamus, and dentate nucleus were also noted. Severe neuronal loss with consequent gliosis in the putamen and caudate nucleus was noticed during microscopic observation. More prominent depletion was seen in the small-sized striatal neurons, while the large-sized striatal neurons were relatively well preserved. These findings were entirely in line with a pathological diagnosis of HD. The grading of the microscopic severity of each brain region is described in Table 1.

**Correlation between D.I. and pathological severity**

The D.I. values for expanded alleles (closed circles) and normal alleles (open circles) were plotted against the semi-quantified pathological severity from the same subjects in Fig.

2. There was an association (p<0.01) in normal alleles (line b). In expanded alleles, a significant correlation (p<0.0001) was obtained (line a).

**Discussion**

HD, like spinocerebellar ataxia 1 (SCA1) (10),

Figure 2. Pathological severity and densitometry index of normal and expanded alleles. Expanded alleles and normal alleles were plotted (x axis: pathological severity, y axis: D.I.). Pathological severity was numerically expressed as −: 0, ±: 0.5, +: 1, ++: 2, +++: 3. Simple regression line a: \( y=0.466+0.081x \), \( r^2=0.863 \), \( p<0.0001 \); simple regression line b: \( y=0.114−0.004x \), \( r^2=0.024 \), \( p<0.01 \).

<table>
<thead>
<tr>
<th>Severity</th>
<th>(CAG)n</th>
<th>Expanded allele</th>
<th>Normal allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>±</td>
<td>44</td>
<td>0.56–0.68</td>
</tr>
<tr>
<td>Cerebral white matter</td>
<td>−</td>
<td>44</td>
<td>0.50</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>+++</td>
<td>44</td>
<td>0.71</td>
</tr>
<tr>
<td>Putamen</td>
<td>+++</td>
<td>44</td>
<td>0.69</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>++</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Thalamus</td>
<td>+</td>
<td>44</td>
<td>0.56</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>++</td>
<td>44</td>
<td>−</td>
</tr>
<tr>
<td>Cerebellar cortex</td>
<td>−</td>
<td>44</td>
<td>0.42</td>
</tr>
<tr>
<td>Cerebellar white matter</td>
<td>−</td>
<td>44</td>
<td>0.41</td>
</tr>
<tr>
<td>Leukocytes</td>
<td></td>
<td>44</td>
<td>0.49</td>
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<tr>
<td>Liver</td>
<td></td>
<td>44</td>
<td>0.50</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td></td>
<td>44</td>
<td>0.41</td>
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<tr>
<td>Testis</td>
<td></td>
<td>44</td>
<td>0.32</td>
</tr>
</tbody>
</table>

−: not examined for (CAG)n and D.I.
dentatorubural pallidoluysian atrophy (DRPLA) (11) and Machado-Joseph disease (MJD) (12) is recognized as a triplet repeat disorder characterized by the expansion of CAG trinucleotide repeat. As with other CAG repeat disorders, triplet CAG repeat expansion in HD has been inversely correlated with the age of onset and the rate of progression. The CAG repeat number is generally within the range of 40–50 in adult onset HD patients. In juvenile onset HD patients, however, the repeat number is larger than adult cases, sometimes exceeding 100 repeats. Telenius et al (6) reported that the (CAG)n repeat numbers in the cerebellum were shorter than those in other brain sections, and that longer expansion affected both the gene instability and the disease severity. They suggested that the more (CAG)n expanded, the more unstable the gene containing trinucleotide would become, thus resulting in the different repeat numbers in different regions of brain or other tissues.

In our experiment, there was no difference in the major peak of (CAG)n repeat numbers among various brain regions and other tissues. In all regions, (CAG)n numbers of non-expanded allele and expanded allele were 22 and 44, respectively. Furtado et al (13) reported that the (CAG)n numbers of the regions commonly affected in HD such as the frontal cortex and caudate nucleus were consistently greater than those of the cerebellum, and this variability seemed to occur only when the repeat number was higher than 44 repeats in cortex or caudate nucleus (13). In our case, the expanded allele showed 44 repeats of (CAG)n, and no difference was seen between the cerebellum and the commonly affected brain region such as the cerebral cortex or caudate nucleus in the number of (CAG)n repeat. This small repeat number may also have reflected the clinical characteristics of this case, such as delayed onset and mild symptoms.

Clear somatic mosaicism, the variety of number of (CAG)n repeat within each tissue, was observed in all tissues examined. In our experiment, the degree of the somatic mosaicism was expressed as a D.I. value; a higher D.I. value is thought to indicate that somatic mosaicism is more prominent (6). The D.I. values of the caudate nucleus, putamen and cerebral cortex were higher than those of other brain regions such as the cerebellum and non-CNS tissues. Although our report is a single case study and has limitations in understanding the somatic mosaicism, these results might indicate the tissue specific somatic variability of (CAG)n repeat.

The expansion of (CAG)n repeat corresponds to sequences of glutamine repeat. Polyglutamine sequences are recognized as the potential sites of protein-protein interactions, and the aberrant expansion of these regions might be conducive to the formation of deleterious protein complexes (14). Somatic heterogeneity is thought to result in the translation of multiple mutant Huntington protein products. Actually, Aronin et al (15) reported the existence of somatic mosaicism of Huntington protein in brains from HD patients, and established that the increased size of mutant Huntington relative to the wild type was highly correlated with CAG repeat expansion. The fact that the mosaicism of the mutant protein was found to be more prominent in more pathologically affected brain regions, and that the mosaicism of the mutant protein was found to be greater in juvenile cases than in adult onset cases, is very much in line with the triplet CAG analysis.

It is not known whether the degree of somatic mosaicism in specific tissues is directly associated with the cause for the region-specific neuronal loss in HD. Nor is it known whether these various aberrant gene products play a role in the pathogenic mechanism in neuronal degeneration of the HD brain. Our results indicate that a 44-repeat of (CAG)n in this HD patient could not give rise to the variation in major (CAG)n repeat numbers among various CNS regions or other tissues, but it could cause the somatic mosaicism in these tissues. Tissue-specific somatic mosaicism might be the result of the alteration of cell population related to selective survival and lost neuronal groups in addition to the glial response in certain CNS regions. It was reported that glial cells clearly manifest a high degree of repeat instability (6, 15). Some authors have hypothesized that neuronal degeneration and subsequent gliosis could give rise to the somatic mosaicism at both the DNA and protein level (15). If this is the case, somatic mosaicism may directly correspond to region-specific neuronal degeneration.

References
