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Analysis of CCG Repeats in Huntingtin Gene among HD Patients and Normal Populations in Japan

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Background. Huntington's disease (HD) is a hereditary autosomal dominant neurodegenerative disease characterized by motor, cognitive, and psychiatric symptoms. The molecular basis of the disease is the expansion of the trinucleotide CAG in the first exon of a gene on chromosome four (4p 16.3). There is another triplet sequence, a CCG repeat, immediately 3′ adjacent to the CAG repeat in Huntingtin. This triplet sequence is also polymorphic, alleles of 7 or 10 repeats are predominant in populations, and strong linkage disequilibrium between the CCG (7) allele and HD has been shown in western HD chromosomes, whereas Japanese HD chromosomes strongly associate with an allele of (CCG)10.

Methods. Distribution of CAG and the CCG repeats in Huntingtin in 15 patients with HD living in southern Japan were selected to evaluate the regional difference in the CCG repeat number in Japan.

Results. Among our 15 HD patients, only 4 patients had the (CCG)7 allele, and the (CCG)10 alleles were found in the remaining 11 patients.

Conclusions. In this study, a linkage disequilibrium was found between Japanese HD chromosomes and (CCG)10, whereas western HD chromosomes are strongly associated with (CCG)7. These data suggest that (CCG)10 allele is dominant in southern Japan.

Key Words: Huntington's disease, CCG repeat, CAG repeat, Japanese population.

Introduction

Huntington's disease (HD) belongs to a unique group of autosomal dominant progressive neurodegenerative disorders caused by the expansion of CAG trinucleotide repeats in the coding region of IT15 gene. HD alleles have >36 CAG units in the HD gene, whereas normal individuals have between 10 and 35 CAG units (1,2). HD is characterized by uncontrolled movements (chorea), accompanied by cognitive and/or psychiatric disturbances (3).

Epidemiological studies of HD report a wide variation in the prevalence rates of the disease. In the U.S. and most Western European countries, the prevalence is between 5 and 10 cases/100,000 individuals, whereas in African, Chinese, Japanese, and Finnish populations the disease is less common (4,5). Nakashima et al. reported that the prevalence of HD was 0.65/100,000 in the San-in area of Japan (6).

After cloning of the Huntington's disease mutation, two genetic polymorphisms were identified close to the CAG tract. The first one was a CCG-rich segment downstream to the (CAG)n stretch and the second one was the *f*2642 glutamic acid polymorphism concerning a deletion of three nucleotides at codon positions 2642−2645 (7,8). There is an interesting relationship, which is variable across major human morphological groups, between the CAG repeat numbers and another closely linked CCG repeat locus in the Huntingtin gene (9).

In this respect, we studied CCG polymorphisms among Japanese Huntington patients living in southern Japan and normal populations and evaluated the relationship between

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CAG expansion and CCG polymorphism in Japanese populations.

Materials and Methods

Patient Evaluation

Fifteen patients belonging to 12 unrelated families living in Kagoshima prefecture of Japan were diagnosed as having HD based on their clinical features, family history, and genetic testing, which showed abnormal CAG repeat expansion in Huntingtin gene. All subjects gave informed consent prior to inclusion in the study.

Genetic Analysis

DNA Samples. Blood samples were obtained from the 15 patients. Genomic DNA was extracted from whole blood using standard methods.

CAG and CCG Repeat Analysis

The CAG repeat sequence in Huntingtin was amplified by PCR using the primers HD-1 and HD-3 described by Norremolle et al. (10) and Warner et al. (11). The CCG repeat sequence in Huntingtin was also amplified by PCR using the primers HD-4 and HD-5 described by Rubinsztein et al. (7). PCR products were subcloned using PCR-Script TM Amp Cloning Kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. The plasmids with the inserted fragments were purified using Plasmid Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions, and sequenced using primer M13 (−20) (GTAAAACGACGGCCAGT) and the ABI PRISM 310 DNA sequencer. Statistical analysis was performed using SPSS 8.0 (SPSS Inc., Chicago, IL). Categorical data were analyzed using χ^2 test. Statistical significance for all tests was set at $p \leq 0.05$.

Results

The size of CAG repeats in expanded and wild-type alleles and the size of CCG repeats in the affected alleles in 15 patients are listed in Table 1. The normal range varied from 8 to 31 repeats (mean: 22 repeats) and the HD range is from 36 to 59 (mean: 46 repeats). Frequencies of the CCG repeat alleles in the normal Japanese population reported by Masuda et al. (12) and the repeat number obtained in this study are listed in Table 2. Alleles with 7 and 10 repeats were the majority of the Japanese population. In the 15 HD patients studied, only 4 patients had the 7 repeat alleles. The 10 repeat alleles were found in the remaining 11 patients. The observed allele frequencies for the CCG repeat polymorphism in the 15 HD patients studied were significantly different from those obtained from the normal population ($p = 0.0065$).

Table 1. Size of CAG repeats in expanded and wild alleles and CCG repeats in affected alleles in 15 HD patients

Patient number	(CAG) *n* expanded alleles	(CAG) *n* wild alleles	(CCG) *n*
1	42	31	10
2	42	17	10
3	38	30	10
4	43	18	7
5	41	28	10
6	40	8	10
7	44	21	10
8	57	18	10
9	39	30	10
10	41	25	10
11	48	25	10
12	55	30	7
13	59	15	10
14	36	20	7
15	58	17	7

Discussion

Previous findings have suggested that, apart from the CAG repeat region, which is the primary cause of HD, there could be other genetic factors that may contribute to stability of the CAG repeats as well as to variability of the clinical symptoms and age at onset of the disease. One of the genetic polymorphisms that was identified close to the CAG tract is a CCG repeat polymorphism composed of the two predominant alleles, (CCG)7 and (CCG)10, and some other minor alleles (13).

Variation of the prevalence of HD among different populations has been found to correlate with the frequency of certain polymorphic alleles on normal chromosomes, suggesting different origins for the HD mutation (4,9,14,15). Populations of Western European descent that have a higher prevalence rate of HD have a higher frequency of the (CCG)7 allele, whereas in African Blacks, Japanese, Chinese, and Finnish populations where HD occurs more rarely, the (CCG)10 allele is over-represented (4,9,15). Adachi et al. in their study showed only 36.4% of HD chromosomes in the Chugoku district of Japan had the (CCG)7 polymorphism (16).

The original HD gene is considered to be western European in origin and subsequently spread to Japan by emigration because the prevalence of HD is very low in Japan as

Table 2. Allele frequencies of the CCG repeat polymorphism in the Japanese population* and 15 HD patients

(CCG) *n*	HD no. (Total 15)	HD (%)	Control no. (Total 185)	Control (%)	*p* value
6	0	0.0	1	0.5	
7	4	26.7	115	62.2	
10	11	73.3	69	37.3	0.0065

*Japanese populations are reported by Masuda et al. (12).

compared with western countries. In addition to this hypothesis, it is reasonable to postulate that a new mutational event, expansion in CAG repeat, in the Japanese ancestral population might contribute to the present HD patients in Japan.

To evaluate the relationship between CAG expansion and CCG polymorphism in HD patients living in southern Japan we analyzed the CCG repeat polymorphism in Huntingtin gene in 15 patients affected with HD living in this area. In this study a linkage disequilibrium was found between Japanese HD chromosomes and (CCG)10, whereas western HD chromosomes are strongly associated with (CCG)7 (3,10). A previous study in Japan showed that (CCG)10 repeats were dominant in Japanese patients with HD (12). These data suggest that (CCG)10 allele is also dominant in southern Japan.

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