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CAG-Expansion Haplotype Analysis in a Population with a Low Prevalence of Huntington's Disease

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Background and Purpose The prevalence of Huntington's disease (HD) among East Asians is less than one-tenth of that among Caucasians. Such a low prevalence may be attributable to a lack of carriers of specific predisposing haplogroups associated with the high instability of the Huntingtin gene (*HTT*). The aim of this study was to evaluate the association between specific *HTT* haplogroups and the occurrence of HD in a Thai population.

Methods CAG-repeat sizes and *HTT* haplotypes were analyzed in 18 Thai HD patients and 215 control subjects. Twenty-two tagging single-nucleotide polymorphisms (tSNPs) were genotyped.

Results Only 18 patients from 15 unrelated families were identified over the last 17 years. Pathological CAG-repeat alleles ranged from 39 to 48 repeats (43.5 ± 3.0 , mean \pm SD), and normal alleles ranged from 9 to 24 repeats (16.49 ± 1.74). Only two of the chromosomes studied comprised intermediate alleles. Unlike the Caucasian data, all but 1 of the 22 tSNPs were not associated with the occurrence of HD. The predisposing haplogroups for Caucasian HD (haplogroups A1 and A2) are very rare in Thai patients (<4%). Both HD and normal chromosomes are commonly haplogroups A5 and C, in contrast to the case for Chinese and Japanese patients, in whom only haplogroup C was common in HD chromosomes. The frequency of CAG-repeat sizes of haplogroup A5 and C were also similarly distributed.

Conclusions HD chromosomes of Thai patients may arise randomly from each haplogroup, with a similar mutation rate. This rate is much lower than the CAG expansions from Caucasian HD haplogroups. These data suggest that the different mechanisms underlie CAG expansion in Thai and Caucasian patients.

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Key Words Huntington's disease, CAG repeat, haplogroup, prevalence, intermediate allele.

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Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by generalized chorea, dementia, and psychiatric disorders. It is caused by an expanded CAG repeat in exon 1 of the Huntingtin gene (*HTT*) on chromosome 4p16.3, which translates to an expanded polyglutamine tract.¹ HD occurs worldwide but with a widely varying

prevalence, from 0.1 to 10 per 100000 population depending on the country.¹ The prevalence of HD is extremely high in certain localized areas, such as around Maracaibo Lake in Venezuela, and Tasmania.² The prevalence of HD ranges from 4 to 9 per 100000 population among most of the Caucasians in Europe and the USA, with the exception of certain countries such as Finland (0.5/100000) and Belgium (1.6/100000).¹⁻³ Recent HD registration data obtained in the United Kingdom suggest that the prevalence of HD is currently underestimated, and that it is likely to be twofold higher than previously reported values.⁴ HD is much less common among East Asians and Africans, in whom the prevalence is reportedly only 0.1-1/100000.⁵⁻⁷ The higher prevalence of HD among Caucasians

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may be associated with specific predisposing haplogroups. These haplogroups may consist of *cis*-elements that are responsible for an increase in CAG instability in *HTT*.⁸

Thai neurologists generally consider HD to be a rare disease in Thailand, similar to those in other East-Asian countries, but there are no epidemiological data to support this viewpoint. In the present study, the frequency distribution of CAG-repeat sizes in *HTT* was analyzed in a large number of control subjects; the frequency of intermediate alleles was also determined. A complete haplotype analysis of *HTT* was also conducted, as in a previous study,⁸ in order to determine the relationship between the various *HTT* haplogroups in HD and normal chromosomes in a Thai population.

Methods

Participants

During the period January 1994 to June 2011, 18 HD patients from 15 unrelated families were identified, and confirmed by positive genetic testing. There was only one sporadic case; the rest were familial-type HD, consistent with an autosomal dominant mode of transmission. There were three families from which DNA samples were collected from two affected family members (i.e., two pairs of siblings and one mother-and-son pair). The control subjects were 215 Thai individuals without any movement disorders. Informed consent to participate was obtained from all of the subjects. The study was approved by the ethical clearance committee on human rights related to research involving human subjects at the Faculty of Medicine, Ramathibodi Hospital, Mahidol University (approval ID 04-51-09).

Genetic analysis

Fluorescently labeled PCR was used to analyze the size of CAG repeats for all 18 HD and 215 control samples.¹ The PCR products were loaded to an ABI 3100 DNA sequencer and analyzed using GeneScan software.

Only 14 DNA samples from 12 unrelated HD patients were available for genotyping. Only the genotype and haplotype data from one affected patient from each family were analyzed

statistically. Twenty-two reported predisposing tagging single-nucleotide polymorphisms (tSNPs) were genotyped on all available HD and control samples using TaqMan SNP Genotyping Assays according to the manufacturer's recommendations. Genotyping was performed on an ABI PRISM7900HT Sequence Detection System. The haplogroup was subsequently inferred from single-nucleotide polymorphism (SNP) genotypes using PHASE software, which implements Bayesian haplotype reconstruction from population data.⁹

Statistics

Comparison of mean and standard deviation values of the frequency of normal CAG-repeat alleles among Thai and other ethnicities (multiple) was achieved using ANOVA, while those of the frequency of normal CAG-repeat alleles between Thai and each ethnic group were compared using multiple comparisons with Bonferroni correction (Table 1). The allele frequency of each tSNP was compared between HD and normal chromosomes using Fisher's exact test (Table 2).

Results

The 18 HD patients included 14 females who all had classical clinical features of adult-onset HD, including generalized chorea, behavioral disorders, and dementia. Only one of the patients had no family history of affected relatives. The age at HD onset was 37.8 ± 8.3 years (mean \pm SD; range, 27–58 years), and the duration from onset to first examination was 3.7 ± 3.4 years (range, 1–10 years). At the time of the last follow-up, 16 of the patients had died; in all cases the cause of death was related to HD. The number of pathological expanded CAG repeats was 43.5 ± 3.0 (range, 39–48).

Frequency distribution of the sizes of normal CAG-repeat alleles

Previous studies have demonstrated a significant correlation between the mean CAG-repeat lengths of normal chromosomes and the prevalence of HD. The mean wild-type triplet repeat size is significantly larger in populations with a higher prevalence of HD.^{10,11} Therefore, the size of the *HTT* CAG re-

Table 1. Comparison of the CAG-repeat sizes of normal and intermediate-allele (IA) chromosomes among Thais and other populations*

CAG-repeat number	Thais	European ⁹	American ¹¹	Finnish ⁹	Black ⁹	Chinese ⁹	Japanese ⁹
Mean	16.5	18.4	19.7	17.1	16.2	16.4	16.6
SD	1.9	3.7	3.2	1.8	2.5	1.5	1.3
Range	8–28*	8–35	11–34	14–23	8–24	8–20	13–23
Number	449	479	545	48	113	90	166
p^{\dagger}		<0.0001*	<0.0001*	0.255	0.55	1	1

*The data of normal alleles and IAs were used for the analysis of the mean CAG-repeat size because previous studies did not use the current cutoff points for IAs. [†]Calculated using Bonferroni correction, * p values were statistically significant (<0.05).

Table 2. Comparison of tSNPs between HD and control chromosomes. Each tSNP is assigned by a specific number as described previously.⁸ Alleles are either major (A) or minor (B). For only 1 out of 22 tSNPs did the allele distribution differ significantly between HD and control chromosomes. The bottom-two rows show the allele distribution of IAs (total numbers of carriers of IAs=2)

tSNPs no.	1	3	11	14	22	55	63	65	69	80	89	95	97	112	119	169	176	178	181	182	185	190
Control																						
A	227	377	228	252	262	424	424	275	414	397	259	424	279	248	277	424	288	278	424	422	424	298
B	197	47	196	172	162	0	0	149	10	17	165	0	145	176	147	0	136	146	0	2	0	126
HD																						
A	1	12	7	7	7	12	12	7	12	12	7	12	7	7	7	12	6	7	12	12	12	11
B	11	0	5	5	5	0	0	5	0	0	5	0	5	5	5	0	6	5	0	0	0	1
<i>p</i> *	0.002†	0.4	0.6	1	0.8	1	1	0.8	1	0.4	1	1	0.6	1	0.8	1	0.2	0.8	1	1	1	0.2
IAs																						
A	0	2	1	1	1	2	2	1	2	2	1	2	1	1	1	2	1	1	2	2	2	1
B	2	0	1	1	1	0	0	1	0	0	1	0	1	1	1	0	1	1	0	0	0	1

**p* values were calculated using Fisher's exact test, †*p* value was statistically significant (<0.05).

HD: Huntington's disease, IAs: intermediate alleles, tSNPs: tagging single-nucleotide polymorphisms.

peat in a large sample of Thai subjects may in turn reflect the prevalence of HD in the Thai population.

The control subjects were aged between 21 and 93 years. The sizes of the normal CAG alleles were analyzed from 430 chromosomes from all of the control subjects and from 15 normal chromosomes from unrelated HD patients. The number of CAG-repeat alleles in normal samples was 16.49 ± 1.74 (range, 9–24). Only 2 out of 445 studied chromosomes consisted of intermediate alleles (27 or 28 CAG repeats). The CAG-repeat size of normal chromosomes is significantly smaller in Thai subjects than in Caucasians in Europe¹⁰ and the USA¹² (ANOVA: F-ratio, 45.17; degrees of freedom, 7; $p < 0.0001$). The mean number of CAG repeats in nonpathological *HTT* alleles in Thai subjects was found to be much smaller than in Caucasians, and was similar to that found in Chinese and Japanese subjects, suggesting that the prevalence of HD among Thai subjects is similar to that of other East Asian populations.

SNP frequency distribution on HD, intermediate, and normal chromosomes

The 22 previously described tSNPs were used to genotype and construct haplotypes for *HTT*.⁸ All HD and control chromosomes were phased according to CAG-repeat size in order to compare the haplotypes of the disease and normal chromosomes. The allele distribution did not differ significantly between HD and control chromosomes for all but 1 of the 22 tSNPs (Table 2).

Haplogroup frequencies on HD, intermediate alleles, and normal chromosomes

Haplogroups were delineated manually into haplogroups A, B, and C. Haplogroup A was subsequently divided into haplogroup variants A1–A5, as reported previously.⁸ The hap-

Table 3. Haplogroup distribution of HD, IAs, and control chromosomes

Haplogroup	Control (%)	HD (%)	IA (%)
A	192 (45)	7 (59)	1 (50)
A1	1 (0.2)	0 (0)	0 (0)
A2	6 (1.4)	0 (0)	0 (0)
A3	14 (3.3)	0 (0)	0 (0)
A4	55 (13)	1 (8)	0 (0)
A5	114 (27)	6 (51)	1 (50)
Other A	2 (0.5)	0 (0)	0 (0)
B	30 (7)	0 (0)	0 (0)
C	156 (37)	4 (33)	1 (50)
Others	46 (11)	1 (8)	0 (0)

HD: Huntington's disease, IAs: intermediate alleles.

logroup frequency distributions of HD and control chromosomes appear to be similar (Table 3). Over 80% of diseased and normal chromosomes belonged to haplogroups A and C. Ten of the 12 HD families carried haplogroups C and variant A5. Comparison of the frequency distribution of CAG-repeat sizes between alleles with haplogroup A5 (17.8 ± 6.4) and C (17.1 ± 4.6) revealed no significant difference (*t*-test: $p = 0.2755$) (Fig. 1).

Discussion

This study provides evidence supporting the contention that Thailand has a low prevalence of HD, similar to the case in other East-Asian populations. First, the means and standard deviations of CAG repeats in normal Thai *HTT* chromosomes reported herein are similar to those of other ethnic populations with a low prevalence of HD, and are significantly lower than those of Caucasians. These associations have been shown consistently in several previous studies.^{10,11,13} Second, the frequen-

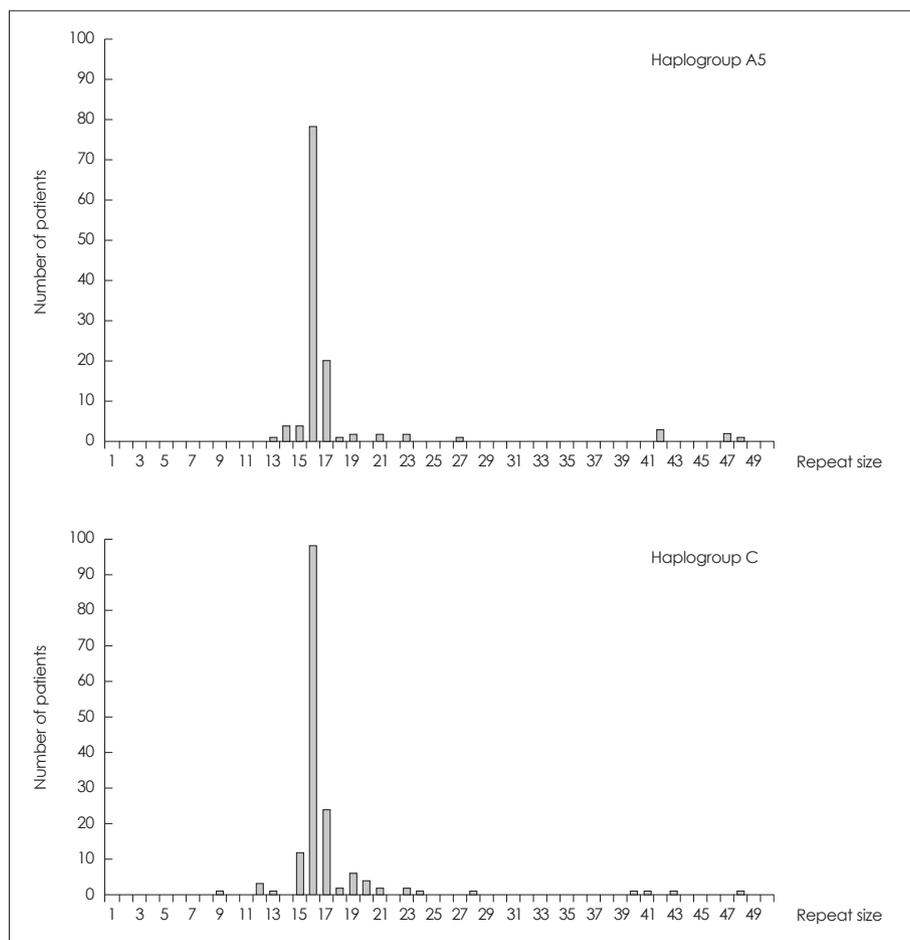


Fig. 1. CAG-repeat sizes of haplogroups A5 and C showed similar distributions ($p=0.2755$).

cy distribution of haplogroups A, B, and C in Thais is similar to those in Chinese and Japanese populations.⁸ Haplogroup variants A1 and A2, which are strongly associated with HD, were extremely rare in the present study. Third, new mutations are likely to originate from intermediate alleles. The frequency of intermediate alleles in the present Thai population (2/445 normal chromosomes; 0.45%) is much lower than that reported for Europeans (2–3%).^{14,15} Therefore, the likelihood of developing a new mutation should be lower in Thais than in Europeans. A single sperm study has demonstrated that the likelihoods of inheriting HD from intermediate HD alleles and in normal families were about 10% and 6%, respectively.¹⁶ These values are consistent with population studies in Caucasians.^{17,18} The high new-mutation rate may be related to the *cis*-element in specific predisposing haplogroups in Caucasians.⁸ The high instability of the CAG repeat in these haplogroups may be an additional factor responsible for the higher prevalence of HD in Caucasians. All of the available evidence supports that HD is uncommon in Thailand.

Warby et al. found that 12 of the 22 studied SNPs were significantly associated with the HD chromosome in Caucasians. These specific SNPs could be clustered into three major hap-

logroups and five haplogroup variants; more than 90% of the HD chromosomes were haplogroups A1, A2, and A3.⁸ Warby et al. therefore hypothesized that the high prevalence of HD associated with haplogroups A1 and A2 was likely to result from *cis*-elements contained within these specific haplogroups. By contrast, haplogroups A1 and A2 are very rare among Africans, Chinese, Japanese, and Thais.^{8,19} Another study of *HTT* haplogroups also demonstrated that Chinese and Japanese patients with HD were commonly associated with haplogroup C.¹⁹ In addition, no association between these specific SNPs and HD chromosomes was identified in the studied Thai population (Table 2), suggesting that there is no genetic factor located on the same region of the DNA molecule that regulates CAG instability.

The frequency of haplogroups in HD and normal chromosomes also share similar distributions (Table 3). Moreover, comparison of the frequency distribution of CAG-repeat sizes between the two most common Thai haplogroups (A5 and C) did not reveal any difference (*t*-test: $p=0.2755$) (Fig. 1). Thus, the CAG-expansion mutation rate in Thai HD patients appears to be equal among all Thai *HTT* haplogroups, and appears to be much lower than that of the common Caucasian HD hap-

logroups. The mutation should randomly occur in multistep expansions without any *cis*-factors as carriers of the predisposing haplogroups.

In summary, the common Caucasian HD haplogroups A1 and A2 were absent in the Thai patients with HD included in this study; haplogroups A5 and C were the most common haplogroups among these Thai HD patients. Since the frequency of haplogroups in HD and normal chromosomes have similar distributions, the HD chromosomes of Thai patients may arise randomly from each haplogroup under a similar mutation rate. This rate is much lower than for CAG expansion from Caucasian HD haplogroups, or A1 and A2. These data and those from other studies^{8,19} suggest that different mechanisms underlie CAG expansion in East Asians and Caucasians.

Conflicts of Interest

The authors have no financial conflicts of interest.

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