Distribution of Huntington's disease Haplogroups in Indian population.

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HTT haplogroup study in Indian population.

Abstract:

Huntington's disease (HD), a rare neurodegenerative disorder, is inherited in an autosomal dominant manner, and caused by a pathological trinucleotide expansion at exon1 of the *HTT* locus. Previous studies have described the haplogroups at the *HTT* locus that can explain the differences in prevalence of HD. We have selected three informative SNPs (rs762855, rs3856973 and rs4690073) to study these haplogroups in an Indian sample. Our results show that the genotype frequencies are significantly different between cases and controls for these SNPs. More than 90% of both cases and controls belong to Haplogroup A which is the predominant European haplogroup.

Key words: Huntington's disease, Haplogroups, CAG repeat, India, HTT, SNP

Introduction:

Huntington's disease (HD) is an autosomal dominant, neurodegenerative disorder, caused by an expansion of an unstable CAG triplet repeat sequence in the Huntingtin (*HTT*) gene. The size of the repeats varies from 17-20, in most individuals. Expansion of this CAG repeat stretch upto 26, is thought not to contribute to disease, while CAG repeats above 36 are considered as disease causing. Correspondingly, CAG repeats from 27 to 35 are considered to be intermediate alleles. The prevalence of HD varies across the world, by both geography (prevalence 5-15/100,000 in Europe; and 1-2/100,000 in sub-Saharan Africa and China); and ethnicity. The number of CAG repeats in the normal allele, and the frequency of intermediate alleles (IAs) perhaps has a bearing on prevalence. One study on the European and American populations showed the intermediate alleles ranged from 0.45% - 8.7% in the general population, 0.05% - 5.1% in the individuals with family history of HD; and was highest in the general Brazilian population (8.7%)[(1)]. Another study in the Northern part of Sweden (SHAPE ;The Swedish Huntingtin Alleles and Phenotype) observed that 6.3% of 7379 individuals from the general population had IAs, and there were even some individuals with very short (<5) with repeat numbers [(2)]. CAG repeat sizing of polyglutamine disorders in the general population showed the HD locus to have a high proportion of IAs; 6% in European (N=13,670) [(3)] and 5.3% in Italian populations (N= 729)[(4)].

Haplotype studies on the *HTT* locus have identified three major haplogroups (A, B and C) which differ across populations [(1, 6)]. Haplogroup A (subgroups A1 &A2), B and C are the major haplogroups of European, African and East Asian HD cases respectively [(5–7)]. There is a similar pattern of haplogroup representation in the IAs, as in HD cases [(8)], suggesting that IAs with the high risk haplogroups are more likely to transmit HD, compared to others. If alleles with certain haplotypes are more susceptible to expansions than others, tracking these may help us understand

the factors that contribute to differences in prevalence, and outcomes. Our study thus attempts to understand the haplogroup structure in persons with Huntington's disease from India.

Materials & Methods:

We have taken HD (N 196; F 74) and healthy controls (N 200; F 88) for the study. All the HD patients were identified in the clinical services, and tested at the Genetic Counseling and Testing (GCAT) clinic of National Institute of Mental Health and Neurosciences (NIMHANS). Healthy controls are volunteers with no neurological or psychiatric illness. Informed consent has been taken for both HD cases and healthy controls. The study was carried out after institutional ethical clearance. Genomic DNA was extracted from peripheral blood and PCR was done for CAG allele determination for both cases and controls with specific primers within the *HTT* gene [(9)]. The three SNPs located at the *HTT* locus on chromosome 4 (rs ID/ gnomAD variant ID: rs762855/4-3074794-A-G, rs3856973/4-3080173-G-A and rs4690073/4-3160150-G-A) were also genotyped. These three SNPs selected from the 22 tag SNPs described in a previous study (3), were used to distinguish between these haplogroups in those detected to have HD and healthy controls. . High resolution melt curve (HRM) analysis was done for rs762855 and PCR-RFLP method for rs3856973 and rs4690073. Validation was done by Sanger sequencing. The primer sequences used for PCR amplification are: rs762855 FP - GCAGTAGCCTCCCTTTTCTTG, RP - TCAAATTCCTGGGTTCAGGT; rs3856973 FP - CAGCAGTGAGCAGACAAAGC, RP - TTCTGGGTTTTGCTGGAAAG and rs4690073 FP - GGGATCAGTTCCCCTGTTGT, RP - CCATGCAGCTTAAAGAGACCT. Haplotype construction was done using homozygous genotypes for the three SNPs (only homozygous genotypes were selected for haplotype construction as heterozygotes could not be assigned to haplotypes unambiguously). The

haplogroups are ascribed based on allele combination of SNPs as Haplogroup A - AGG, Haplogroup B - GGG and Haplogroup C- GAA corresponding to the SNP sequence rs762855, rs3856973 followed by rs4690073. The available phased 1000 genome data of the three SNPs was taken for the south Asian population and haplogroups were constructed (Fig1).

Comparison of genotype frequency between cases and controls was done by Chi-square test.

Results & Discussion:

The CAG repeat allele distribution at the *HTT* locus for HD patients (normal allele - 17.72±2.55 (12-32), expanded allele - 45.64±7.95 (39-113)) and controls (normal allele1 - 16.6±1.35 (10-22), normal allele 2 - 18.8± 2.7 (14-29)) was determined. An intermediate allele was detected in [N= 3, 1.4%] controls; and in [N=3, 1.5%] HD patients with an expanded HD allele, where the other allele was in the intermediate range. The distribution of three SNPs followed Hardy-Weinberg equilibrium, for both cases and controls.

The allele and genotype frequencies of the three selected SNPs were significantly different between HD cases and controls (Table I). The haplotype could be unambiguously ascertained in 203 persons (119 HD; 84 controls); and in these the predominant haplogroup was Haplogroup A (114 cases; 76 controls). On the other hand Haplogroup B (1 each in cases and controls) and C (4 each in cases and controls) were relatively rare. The frequency distribution of haplogroups is shown in Figure I. All the three SNPs were in strong linkage disequilibrium.

We detect that the allele frequencies of three SNPs studied at *HTT* loci differ in subjects with HD, when compared to the background population, as expected. The predominant haplogroup, as defined by these SNPs, however, was the Haplogroup A, in HD patients, as has been reported earlier. However, a few individuals do conform to haplogroup C. This hints that there may be multiple founder events, as well as admixture, that contributes to this diversity in India.

The identified haplogroup broadly overlaps with that of the European population, however it should be borne in mind that variants of Haplogroup A have also been seen in the East Asian and African populations. Since only a subset of samples were homozygous, this description may be valid for only a subset of the individuals. A study with more SNPs/markers around the *HTT* gene locus to aid better classification into haplogroups would help us obtain a better idea about the origin and spread of the disease, and also plan future interventions, such as allele specific therapeutics. Such knowledge could also have a bearing on the underlying mechanisms of other neuro-psychiatric diseases, especially those associated with unstable repeat sequences.

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Conflict of Interest:

The authors have no conflict of interest to report.

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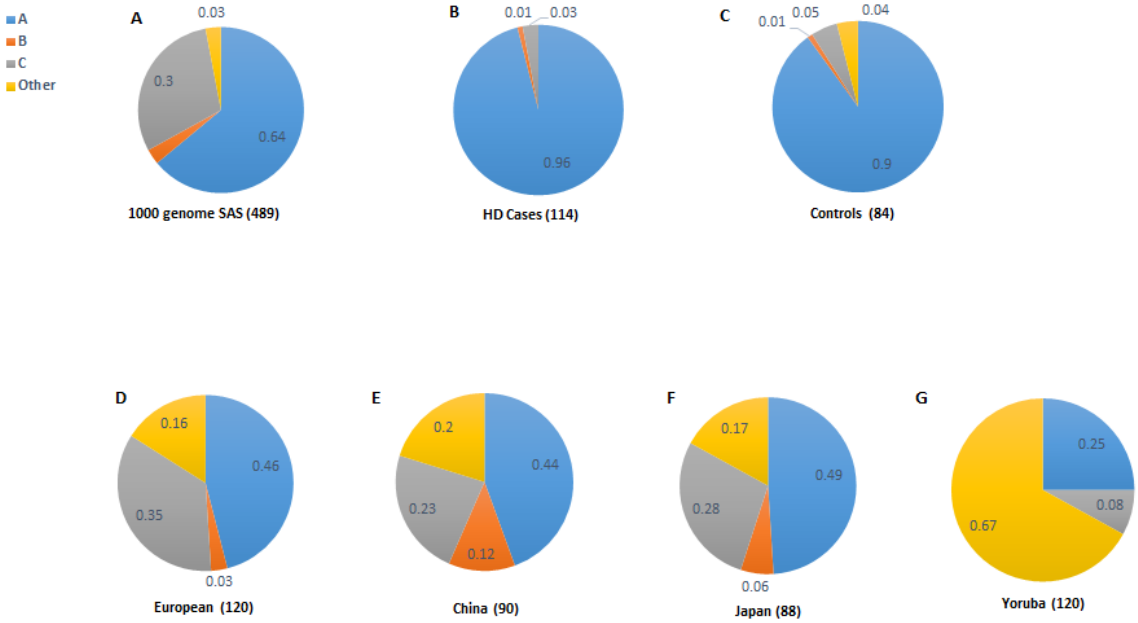
Table I: Allele and genotype frequencies of the 3 studied SNPs

	N	rs762855 (A/G)	rs3856973 (G/A)	rs4690073 (G/A)
HD cases	196	G(0.19)	A(0.17)	A(0.16)
Controls	200	G(0.31)	A(0.31)	A(0.26)
Odds Ratio **P-value**		3.88 <0.01	3.09 <0.01	2.05 0.15
Genotype Frequency (Case / Control)		AA 0.65/0.47 AG 0.33/0.45 GG 0.03/0.09	GG 0.69/0.45 GA 0.28/0.48 AA 0.03/0.07	GG 0.71/0.54 GA 0.26/0.41 AA 0.03/0.05
Genotype frequency (chi -squared statistic) P - value		14.78 <0.01	25.55 <0.01	10.86 <0.01

The odds ratio is significant for rs762855 and rs3856973. The Genotype frequencies are also significantly different between cases and controls ($p < 0.01$).

Fig I: Haplogroup distribution comparison of our study with other populations.



The haplogroup distribution shown in percentage, A - 1000 genome data, B and C (homozygous genotypes were selected for haplogroup construction). - HD cases and controls from our study, D, E, F and G - European, China, Japan and Yoruba general population, adapted from (5)