Neuroscience Letters 444 (2008) 83–86

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Mitochondrial DNA haplogroups do not influence the Huntington's disease phenotype

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ARTICLE INFO

Article history:
Received 23 June 2008
Received in revised form 24 July 2008
Accepted 6 August 2008

Keywords:
Haplogroups
Huntington's disease
mtDNA
Neurodegeneration

ABSTRACT

Various lines of evidence demonstrate the involvement of mitochondrial dysfunction in the pathogenesis of Huntington's disease (HD). However, the precise role of mitochondria in the neurodegenerative cascade leading to HD is still unclear. Mitochondrial DNA (mtDNA) haplogroups-specific polymorphisms were previously related to several neurodegenerative diseases. The length of CAG repeat seems to be related to the clinical features of HD, such as age of onset and progression of motor impairment. The basis for the impaired cognitive functions and for the mood changes is less clear. Aim of this study was to determine whether mtDNA polymorphism(s) play the role of "modifier gene(s)" in this disease. In this work we have genotyped predefined European mtDNA haplogroups in 51 patients with HD and 181 matched controls. The frequency of the haplogroups and haplogroup clusters did not differ between the two groups, and no correlation with gender, age of onset and disease status was observed. No significant difference was observed between different haplogroups and haplogroup clusters in the cognitive or motor progression of the disease. Our study does not support any association between mtDNA haplogroups and HD.

Huntington's disease (HD) is a genetic disease characterized by psychiatric disturbances, progressive cognitive impairment, choreiform movements, and death 15–20 years after the onset of symptoms [31]. Psychiatric and behavioural symptoms do not show stepwise progression with disease severity [7]. In most patients the onset of the disease occurs in the fourth to fifth decade of life. The pathological hallmark is the selective degeneration of medium spiny GABAergic neurons in the striatum, which results in the progressive atrophy of the caudate nucleus, putamen and globus pallidus [31]. HD is inherited as an autosomal dominant disorder, and the mutation is highly penetrant. It is one of the diseases that involve trinucleotide expansion. In particular, HD is caused by an expansion of a CAG trinucleotide in the *IT15* gene on chromosome 4. This gene encodes a large cytoplasmatic protein called huntingtin, the function of which is as yet unknown [27]. The CAG expansion results in abnormally long stretches of polyglutamine (poly-Q) in the protein.

The length of CAG repeat seems to be related to the clinical features, such as age of onset [3] and progression of motor impairment [22]. The basis for the impaired cognitive functions and for the mood changes is less clear [12,14,21]. Other genetic or environmental factors might influence such parameters. Modifier genes are defined on the basis of their ability to modulate the clinical phenotype of individuals with monogenic and multigenic diseases [11].

Various lines of evidence demonstrate the involvement of mitochondrial dysfunction in the pathogenesis of HD [17]. Polymorphisms in mitochondrial DNA (mtDNA) may cause subtle differences in the encoded proteins and, thus, minimum changes in mitochondrial activity and free radical overproduction. This could predispose an individual, or a population sharing the same mtDNA genotype, to an earlier onset of apoptotic processes, such as accumulation of somatic mtDNA mutations and impairment of respiratory chain activities. The opposite could be true for different polymorphism(s), which could be beneficial in increasing mitochondrial respiration and/or reducing reactive oxygen species production. In mice, mtDNA polymorphism seems to be involved in cognitive functioning [23]. For these reasons, polymorphisms in mtDNA could represent modifier factors in HD. In humans, common mtDNA polymorphisms determine classes of continent-specific genotypes, haplogroups, which can be detected by restriction fragment length polymorphism (RFLP) analysis. In Europe, nine

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doi:10.1016/j.neulet.2008.08.013

different mitochondrial haplogroups have been identified (H, I, J, K, T, U, V, W, X), accounting for more than 90% of all mitochondrial genomes in the population [28]. MtDNA haplogroups have been investigated in several neurodegenerative diseases such as Alzheimer's disease [30,18], amyotrophic lateral sclerosis [16], and Parkinson's disease [10]. MtDNA haplogroups seem also to influence the phenotype of another disease involving trinucleotide expansion such as Friedreich's ataxia [11].

Aim of this study was to analyze the distributions of mtDNA haplogroups and haplogroup clusters in Italian HD patients, and to evaluate an eventual influence in the progression of the disease and in clinical features (i.e. dementia, motor involvement, etc.).

The patients group consisted of 51 unrelated Italian subjects (28 men, 23 females; mean age 60.3 ± 14.3, range 39–73). The clinical diagnosis of HD was confirmed by genetic testing. Among HD patients, only those with a similar number of triplets (mean number 49.3 ± 5.3, range 45–60) were enrolled in the study, in order to reduce the bias due to the length of triplets in the progression of the disease. Our controls (n = 181, 90 men, 91 females, mean age 66.1 ± 6.6, range 60–92) were healthy age-matched subjects of Italian origin. They were not related to the patients and had no history or family history of dementia and neurodegenerative disorders. In addition a mini mental state examination (MMSE) has been performed to the controls, to exclude present cognitive impairment [8]. To minimize the risk of "genetic contamination", which could lead to false associations between gene markers and disease, we were careful to enrol in the study only patients and controls of clear Italian origin (patients with at least three generations of Italian-born relatives). Informed consent was obtained from each subject or from a relative, after the purpose and procedures of the study had been explained.

HD patients have been followed for a mean period of 9.3 years. Over the last 3 years, patients have undergone prospective evaluations, with a control every 6 months. A movement disorder neurologist performed the motor part of the Unified Huntington's Disease Rating Scale (UHDRS) part III [26] at the baseline and at the follow-up controls. The cognitive decline was measured by MMSE. Moreover, the HD Activities of Daily Living (ADL) scale [5] was included as a functional measure.

Genomic DNA was isolated from whole blood samples using standard protocols [24]. Haplogroups typing has been carried out by restriction analyses of mtDNA according to Torroni et al. [28]. For each individual, mtDNA regions containing the polymorphic restriction sites specific for each European haplogroup (H, I, J, K, T, U, V, W, and X) were amplified with mtDNA specific primers. The nomenclature of the restriction sites is given according to the revised version of the Cambridge reference sequences [1]. The amplified fragments, after digestion with the appropriate restriction enzymes, were loaded on a 2.5% agarose gel and stained with ethidium bromide.

Mitochondrial haplogroup and haplogroup clusters frequencies were compared between cases and controls using χ^2 tests and logistic regression analysis. Tests for statistical significance were two-sided with $\alpha = 0.05$. Effect size for the association was measured as odds ratio (OR) with 95% confidence intervals (CI). STATA 9.0 (College Station, TX, USA) statistical software was used for all statistical analyses.

Since haplogroup status was categorical independent variable with more than two categories, there were multiple ways to assign the reference group. We analyzed the frequencies of clusters of phylogenetically related haplogroups, HV, JT, UK and WIX, choosing the cluster HV as the reference group since it was found at the highest frequency. Rare haplogroups were pooled into a unique category named "other" [28].

All major European haplogroups were observed in our cohort of patients and controls (Table 1). The most common haplogroup observed in both the control and patient groups was the haplogroup H (52.9% in HD and 40.4% in controls). A similar percentage of individuals (7.8% in HD cases and 13.8% in controls) did not fit into the nine predefined haplogroups and were classified as "other". These subjects could either belong to rare haplogroups or reflect a recent mutation in a restriction site, preventing its identification.

The frequency of haplogroups H, J, K, T, U, V, W, I and X did not differ between patients and healthy controls (χ^2 = 3.21, 4 d.f., p = 0.52). The frequency of haplogroup clusters WIX, JT and UK did not differ between HD subjects and controls, either (Table 2). Further, there were no significant differences between genders for mtDNA haplogroup distribution in both HD patients and control groups.

We examined the association of the different haplogroup clusters with quantitative measures of HD progression, such as MMSE for cognitive progression and UHDRS (part III) for movement aspects. Each evaluation was made at baseline (visit 0) and every 6 months for 3 years (visits 1–5). No significant difference was observed between different haplogroup clusters in the age of onset and in the baseline (visit 0) cognitive or motor involvement (Table 3). Only two patients with haplogroup H did not complete the 3-year follow up, one for sudden death, one because of poor compliance. No significant difference was observed between different haplogroup clusters in the cognitive or motor progression (Table 3).

Table 1
Frequency of mtDNA haplogroups among HD patients and controls

Haplogroups	Controls (n)	%	Patients (n)	%
H	73	40.4	27	52.9
I	3	1.7	1	1.9
J	13	7.2	2	3.9
K	14	7.7	7	13.7
T	14	7.7	3	5.8
U	24	13.3	4	7.8
V	5	2.8	1	1.9
W	4	2.2	1	1.9
X	6	3.3	1	1.9
Other	25	13.8	4	7.8
Total	181		51	

Table 2
Frequency of mtDNA haplogroup clusters among HD patients and controls

Haplogroup clusters	Controls (n)	%	Patients (n)	%	OR	95% CI lower–upper	p
HV	78	43.1	28	54.9	Ref.		
WIX	13	7.2	3	5.9	0.64	0.17–2.43	0.51
JT	27	14.9	5	9.8	0.51	0.18–1.47	0.21
UK	38	21.0	11	21.6	0.81	0.36–1.79	0.60
Other	25	13.8	4	7.8	0.44	0.14–1.39	0.16
Total	181		51				

OR, odds ratio; CI, confidence interval.