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Research article

Mitochondrial DNA haplogroups may influence Fabry disease phenotype

C. Simoncini[a], L. Chico[a], D. Concolino[b], S. Sestito[b], L. Fancellu[c], W. Boadu[c], G.P. Sechi[c], C. Feliciani[d], M. Gnarra[d], A. Zampetti[e], A. Salviati[f], M. Scarpelli[g], D. Orsucci[a], U. Bonuccelli[a], G. Siciliano[a], M. Mancuso[a,*]

[a] Experimental and Clinical Medicine Department, Neurological Clinic, University of Pisa, Italy
[b] Department of Pediatrics University "Magna Graecia" of Catanzaro, Catanzaro, Italy
[c] Department of Clinical and Experimental Medicine, University of Sassari, Italy
[d] Section of Dermatology, Department of Clinical and Experimental Medicine, Parma Medical School, Parma, Italy
[e] Rare diseases and Periodic fevers Research Center, Policlinico A. Gemelli, Rome, Italy
[f] Department of Neurological and Movement Sciences, University of Verona, Italy
[g] Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Italy

HIGHLIGHTS

- Mitochondrial impairment and oxidative stress could be implicated in Fabry disease (FD).
- Haplogroups H and I and haplogroup cluster HV are overexpressed in FD.
- Detection of haplogroup(s) related oxidative stress requires further investigation.

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ABSTRACT

While the genetic origin of Fabry disease (FD) is well known, it is still unclear why the disease presents a wide heterogeneity of clinical presentation and progression, even within the same family. Emerging observations reveal that mitochondrial impairment and oxidative stress may be implicated in the pathogenesis of FD.

To investigate if specific genetic polymorphisms within the mitochondrial genome (mtDNA) could act as susceptibility factors and contribute to the clinical expression of FD, we have genotyped European mtDNA haplogroups in 77 Italian FD patients and 151 healthy controls.

Haplogroups H and I, and haplogroup cluster HV were significantly more frequent in patients than controls. However, no correlation with gender, age of onset, organ involvement was observed.

Our study seems to provide some evidence of a contribution of mitochondrial variation in FD pathogenesis, at least in Italy.

1. Introduction

Fabry Disease (FD) is an X-linked lysosomal storage disorder, affecting both men and women, resulting from deficient activity of the α-galactosidase A enzyme, encoded by the *GLA* gene. As a result, neutral glycosphingolipids, mainly globotriaosylceramide, accumulates in the vascular endothelial and smooth muscle cells, cardiac myocytes, dorsal root ganglion neurons, neurons of the autonomic nervous system, brain and all types of kidney cells [1,2]. This explain the multisystem nature of the disease, and the wide spectrum of clinical signs and symptoms complained by patients, which affect predominantly kidneys, heart, nervous system, skin, ears and eyes [1,2].

The clinical presentation of FD is heterogeneous, ranging from a classic multysistem severe form to oligosymptomatic phenotypes, with a variety of clinical presentations in between and without a definite genotype-phenotype correlation [1].

Abbreviations: mtDNA, mitochondrial genome; FD, fabry disease; ETC, electron transport chain; ROS, reactive oxygen species.

* Corresponding author.
E-mail address: mancusomichelangelo@gmail.com (M. Mancuso).

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To date, it is still unclear why FD presents such a wide heterogeneity of clinical presentation and progression, even within the same family [3]. Environmental, epigenetic, or modifier genes could act as candidates on the basis of their ability to modulate the clinical phenotype of individuals with mono and multigenic diseases. Mitochondrial DNA (mtDNA) could be considered a candidate modifier factor for neurodegenerative disorders, since mitochondrial oxidative stress is thought to be involved in the pathogenesis of these diseases [4–6]. MtDNA is maternally inherited and highly polymorphic. Population specific mtDNA polymorphisms create groups of related mtDNA haplotypes, or haplogroups [7]. Most Europeans are considered to derive from the main mitochondrial haplogroup root called *R*, which has branching subgroups of U (U5, U6, U2, U, U8 and K), V and H, T and J, and B. Several haplogroups have been associated with polymorphic biological features in healthy individuals, and with pathological conditions, modulating disease expression [8].

Aim of this study was to analyze the distributions of mtDNA haplogroups in Italian FD patients, and to evaluate their possible influence on the disease, progression and involvement of different organs.

2. Material and methods

The patients group consisted of 77 Italian genetically proven FD subjects (35 men, 42 females; mean age 42,23 ± 18,12 yrs). 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 of clear Italian origin. Sixty-two cases were unrelated. Five families (fifteen patients) were included in the study, because the mothers—who provided the mtDNA to the offspring- were not the probands (*GLA* normal). Moreover, in case of recurrence of the same mutation in unrelated patients, a detailed family history was again collected to exclude a genetic connection between those pedigrees. Our controls (n = 151, mean age 54 ± 6.6 yrs) were healthy age-matched subjects of Italian origin, unrelated to the patients. Informed consent was obtained from each subject, after the purpose and procedures of the study had been explained. Our local ethic committees approved the study.

Genomic DNA was isolated from whole blood samples. Haplogroups typing has been carried out by restriction analyses of mtDNA according to Torroni and co-workers [9]. In particular, each individual mtDNA region containing the polymorphic restriction sites specific for each European haplogroup (H, I, J, K, T, U, V, W, and X) was amplified with mtDNA specific primers. The nomenclature of the restriction sites is given according to the revised version of the Cambridge reference sequences [10]. The amplified fragments, after digestion with the appropriate restriction enzymes, were loaded on a 2.5% metaphor-agarose gel and stained with ethidium bromide.

We analyzed the frequencies of clusters of phylogenetically related haplogroups, HV, JT, UK and WIX. Rare haplogroups were pooled into a unique category named "other" [9].

Proportions were compared by Fisher's exact test. Bonferroni's correction has been utilized when appropriate. A P value<0.05 was considered as significant. When needed, the numeric data were compared between more than two groups by one-way ANOVA. Data analysis was carried out using MedCalc and SPSS.

3. Results

Table 1 presents the frequency of mtDNA haplogroups among patients and controls. Haplogroups H and I were more frequent in patients than controls ($P < 0.001$ [significance levels after Bonferroni's correction = 0,005]). Moreover, haplogroup cluster HV was more frequent in the patients group than in controls ($P < 0.005$ [significance levels after Bonferroni's correction = 0,01], Table 2).

Table 1
Frequency of mtDNA haplogroups among patients and controls. Haplogroup I and H were more frequent in the patient group than in controls ($P < 0.001$ [significance levels after Bonferroni's correction = 0,005]).

Haplogroups	Controls (n)	%	Patients (n)	%	*P*
H	73	40,4	49	63,6	0,0007
I	3	1,7	10	13,0	0,0004
J	13	7,2	7	9,1	n.s.
K	14	7,7	0	0	n.s.
T	14	7,7	3	3,9	n.s.
U	24	13,3	4	5,2	n.s.
V	5	2,8	1	1,3	n.s.
W	4	2,2	0	0	n.s.
X	6	3,3	0	0	n.s.
OTHERS	25	13,8	3	3,9	n.s.
TOTAL	181		77		

Table 2
Frequency of mtDNA haplogroup clusters among patients and controls. Haplogroup cluster HV was more frequent in the patient group than in controls ($P < 0.005$ [significance levels after Bonferroni's correction = 0,01]).

Haplogroup clusters	Controls (n)	%	Patients (n)	%	*P*
HV	78	43,1	50	64,9	0,0017
WIX	13	7,2	10	13,0	n.s.
JT	27	14,9	10	13,0	n.s.
UK	38	21,0	4	5,2	n.s.
OTHERS	25	13,8	3	3,9	n.s.
Total	181		77		

Table 3
H.C., hypertophic cardiomyopathy.

Haplogroup clusters	H.C.: no (n)	H.C.: yes (n)	*P*
HV	31	19	n.s.
WIX	8	2	n.s.
JT	4	6	n.s.
UK	2	2	n.s.
OTHERS	3	0	n.s
Total	48	29	

We therefore examined the association between haplogroup clusters and clinical features. No association was observed between hypertrophic cardiomyopathy (Table 3), stroke (Table 4), renal failure (Table 5) or other clinical features (such as arrhythmia, myocardial ischemia, acroparesthesias, gastrointestinal involvement) and haplogroup clusters. No association was finally observed between haplogroup clusters and age of onset of organ involvement (data not shown). The heterogeneity of our population does not allow the evaluation of a possible link between mitochondrial haplogroups and progression of organ failure.

4. Discussion

Results obtained during the last years suggest that non-pathological mtDNA variability is potentially responsible for mild differences in OXPHOS activity that may have phenotypic expression in the form of physiological process as longevity and in the penetrance of some diseases in different populations [11]. Moreover, research has also been directed to clarifying the involvement of mtDNA haplogroups in several neurodegenerative and neurogenetic disorders such as Alzheimer [12,13], amyotrophic lateral sclerosis [14], Parkinson [15], Huntinghton [16], and Friedreich diseases [17].

Up to date, no previous studies focusing on the correlation between mitochondrial haplogroups and FD phenotypes have been reported.