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SHORT REPORTS

Frequency and Genetic Profile of Compound Heterozygous Friedreich's Ataxia Patients—the Brazilian Experience

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Abstract

Friedreich ataxia (FRDA) is the most common autosomal recessive ataxia in Caucasian populations. It is caused by a homozygous GAA expansion in the first intron of the frataxin gene (*FXN*) (OMIM: 606829) in 96% of the affected individuals. The remaining patients have a GAA expansion in one allele and a point mutation in the other. Little is known about compound heterozygous patients outside Europe and North America. We have thus designed a study to determine the frequency and mutational profile of these patients in Brazil. To accomplish that, we recruited all patients with ataxia and at least one expanded GAA allele at *FXN* from 3 national reference centers. We identified those subjects with a single expansion and proceeded with further genetic testing (Sanger sequencing and CGH arrays) for those. There were 143 unrelated patients (128 families), five of which had a single expanded allele. We identified point mutations in three out of these five (3/128 = 2.34%). Two patients had the c.157delC variant, whereas one individual had the novel variant c.482+1G>T. These results indicate that *FXN* point mutations are rare, but exist in Brazilian patients with FRDA. This has obvious implications for diagnostic testing and genetic counseling.

Keywords Compound heterozygous · Friedreich's ataxia · FRDA mutation · Genetic counseling

Introduction

Friedreich's ataxia (FRDA) is the most common hereditary ataxia overall, with estimated prevalence between 2 and 0.13:100,000 in Caucasians, 0.045:100,000 in Cubans, and even lower figures among Asian populations. FRDA estimated prevalence in South Brazil is 0.20:100,000 and probably portrays the effect of mixed European, African, and Amerindian ancestries for this population [1]. FRDA is characterized by age of onset around puberty in 83% of the cases and late onset in the other 17% [2]; degenerative atrophy of the posterior columns of the spinal cord that leads to progressive ataxia, sensory loss, muscle weakness, and also often observed: scoliosis, foot deformity, cardiac symptoms, and diabetes [3].

The disease is caused by reduced expression of the protein frataxin, caused by a homozygous unstable GAA expansion in the first intron of *FXN* (locus 9q13-21; OMIM: 606829) in 96% of the cases [4]. The other 4% of the patients are compound heterozygous for a

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point/deletion/insertion *FXN* mutation in one allele and the $(GAA)_{>36}$ expansion on the other [5, 6].

There are very few published studies devoted to characterizing compound heterozygous patients [5, 6], and none from Latin America. Here, we present a study aimed to contribute for a better understanding of this disease, looking at a Brazilian cohort with implications on the genetic testing and its counseling.

Methods

Patient's Selection

This study was performed in three reference neurogenetics centers in Brazil: State University of Campinas (UNICAMP), University of São Paulo-Ribeirão Preto (USP-RP), and Federal University of Rio Grande do Sul (UFRGS) between 2007 and 2017. It was approved by the Research Ethics Committee of each center. Each subject signed an informed consent before genetic testing.

We selected all patients followed at these centers with ataxia that had at least one expanded GAA allele at *FXN*. DNA samples of the subjects with a single expanded allele were then submitted to further molecular analyses (described below).

For this study, we excluded patients who had molecular confirmation of other genetic ataxias (or complex heredogenerative diseases with ataxia as one of the signs) or who had no DNA available for additional molecular analyses.

Molecular Analysis

Genomic DNA was extracted from peripheral blood and we sequenced the *FXN* gene for patients who presented GAA expansion in only one of their alleles. The 5 exons of the gene were amplified by the PCR technique and sequenced by the Sanger method through the ABI 3500xl Genetic Analyzer (Applied Biosystems, Foster City, CA) platform. We used previously described primers for exon 1-R and exons 2, 3, 5 [7]. We designed the exon 1-F and exon 4 primers using these sequences: FXN1F 5′-CCAGCGCTGGAGGGCG-3′; FXN4F 5′-GGTGTATTTTGTGTACTTG-3′; FXN4R 5′-GTCACATTTCGGAAGTC-3′.

The electropherograms were analyzed and the identified mutations were searched in international variant databases such as 1000 Genomes [8] and ExAC [9]. We also searched them in two Brazilian variant databases: BIPMed [10] and AbraOM [11]. New variants were submitted to in silico algorithms for pathogenicity evaluations like NNSplice [12] and Mutation Taster [13]; then, we classified these variants according to the American College of Medical Genetics guidelines [14].

For patients in which point/insertion/deletion mutations were not found, we submitted their samples to the chromosomal microarray analysis (CMA) using the Genome-Wide Human SNP Array 6.0 (Affymetrix, Inc. Santa Clara, CA, USA) according to the manufacturer's instructions. The analyses were performed using the Chromosome Analysis Suit—ChAS (Affymetrix, Inc. Santa Clara, CA, USA).

Results

A total of 143 patients (128 unrelated families) were recruited: 68 patients (61 families) were at UNICAMP, 35 patients (29 families) at UFRGS, and 40 patients (38 families) at USP-RP (Table 1). All initial molecular investigation was performed in each center where the samples were collected.

We identified five patients with a single GAA expansion and they had their clinical charts reviewed for confirmation of the disease-related phenotype (Table 2). DNA samples of these individuals were sent to UNICAMP where additional testing was accomplished.

Analyzing the *FXN* sequencing for these five index patients, we managed to identify point/insertion/deletion mutations in three of them—all with European ancestry (Table 2). The patients P1-UNICAMP and P2-UFRGS have the same deletion (c.157delC) although they are from unrelated families and from two different states in Brazil (São Paulo and Rio Grande do Sul respectively). Subject P2-UFRGS was already described [1]. Patient P3-USP-RP had a novel mutation at a canonical splice site near exon 4 of *FXN* (c.482+1G>T) leading to the loss of a splicing donor site.

Table 1 Number of patients, families, homozygous, and compound heterozygous for FRDA at each reference center

Reference center	Families	Patients	Homozygous	Compound heterozygous
UNICAMP	61	68	66	2
UFRGS	29	35	33	2
USP-RP	38	40	39	1
Total	128	143	138	5

Table 2 Phenotypic and genotypic data of compound heterozygous FRDA patients

Patient	Variant found	Age of onset	Clinical phenotype	Cardiomyopathy	Diabetes
P1-UNICAMP	c.157delC	27 years	Mixed ataxia; DTR (+); lower limb spasticity; abnormal sensation	−	−
P2-UFRGS	c.157delC	12 years	Mixed ataxia; DTR (±); abnormal sensation	−	+
P3-USP-RP	c.482+1G>T	10 years	Mixed ataxia; DTR (−); abnormal sensation	+	+
P4-UNICAMP	–	48 years	Ataxia (+); DTR (−); abnormal deep sensation	−	+
P5-UFRGS	–	15 years	Ataxia (+); DTR (+); abnormal deep sensation	−	−

DTR, deep tendon reflexes

Our in silico prediction tests had similar results favoring the pathogenicity of this new variant. We classified this mutation as pathogenic, according to the ACMG guidelines: Variants that result in the loss of a splicing donor site are classified as PVS1—a null variant—and it attends to a moderate pathogenicity criteria—its absence in the overall population according to databases like 1000 genomes [8] and ExAC [9]—as well as a support criteria with computational evidence of its deleterious effect.

The other two patients—P4-UNICAMP and P5-UFRGS—in which we did not find any other variant by means of Sanger sequencing were submitted to the CMA method, but we failed to identify copy number variations (CNV's) within their *FXN* gene.

Discussion

This study of compound heterozygosity in FRDA is the first one in Brazil and we identified a frequency of 3.93% (5/128) considering all the families with clinical findings and confirmation of the expansion in only one of its alleles. If we consider the ones where point/insertion/deletion mutations were identified, then our frequency is 2.34% (3/128). The proportion of possible or confirmed point/insertion/deletion mutations at GAA among the present Brazilian FRDA subjects (3.93%) was similar to the figures found elsewhere, of around 4% among FRDA subjects of European ancestry [5]. The fact that FRDA prevalence is lower in Brazilians than in Caucasians, reflecting a low frequency of expanded GAA alleles in our population, should not impact on proportion of compound heterozygotes among FRDA subjects. However, if only the mutations detected in the present series were taken into consideration, then the proportion of compound heterozygote fell to 3/128 or 2.34%. If compound heterozygosity is less frequent in Brazil than in Europe, or if a larger sample size is needed to clarify this issue, remains to be established.

We observed two patients with the c.157delC mutation that was already reported [1, 5]. This is a frameshift variant that leads to a premature stop codon (p.R53AfsX75). The phenotype in these 2 patients were remarkably different in terms of age at onset, presence of spasticity, and diabetes. Such variability has been already reported in Italian patients with the same variant, where age at onset ranged between 10 and 39 years [5]. In addition, we identified a novel mutation at a canonical splice site: c.482+1G>T. All in silico analyses were concordant and predicted pathogenicity for this variant. This alteration leads to a splice donor site loss and can interfere in protein expression.

Although we did not manage to identify any point/deletion/insertion variants or CNVs in two patients (P4-UNICAMP and P5-UFRGS), the disease-related phenotype makes us suspect that they are compound heterozygous for FRDA. The molecular techniques used in this study have limitations—we could not look at deep intronic alterations or small intronic deletions closer to the exons. Such intronic alterations have been increasingly recognized as disease causing by mechanisms such as disruption of regulatory promotor sites and miRNA binding sites [15]. There is a small chance that these two patients are just carriers for the *FXN* expansion (the carrier prevalence is 1:90 for Caucasian populations and 1:333 in South Brazil [1]), but have other genetically defined ataxia. To find out what really is the case, we would need to perform functional testing or expression analyses, but unfortunately, these patients were lost from follow-up and we do not have tissue samples other than DNA.

These results indicate that *FXN* point mutations are rare but exist in Brazilian patients with FRDA. This has obvious implications for diagnostic testing and genetic counseling. The investigation of potential founder mutations or variants more frequent in specific ethnic subgroups would be important for proper counseling. Further studies with much larger cohorts are needed to reach these goals. Currently, one should consider extending the investigation of specific subjects to include small *FXN* variants in the appropriate clinical setting.

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Compliance with Ethical Standards

This study was approved by the Research Ethics Committee of each center. Each subject signed an informed consent before genetic testing.

Conflict of Interest The authors declare that they have no conflict of interest.

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