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Fabry disease: Evidence for a regional founder effect of the *GLA* gene mutation 30delG in Brazilian patients

Dayse Oliveira de Alencar [a], Cristina Netto [b], Patricia Ashton-Prolla [b,c], Roberto Giugliani [b,c], Ândrea Ribeiro-dos-Santos [a,e,*], Fernanda Pereira [d], Ursula Matte [c,d], Ney Santos [a,e], Sidney Santos [a,e]

[a] *Laboratório de Genética Humana e Médica, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brazil*
[b] *Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, RS, Brazil*
[c] *Programa de Pós-Graduação em Genética e Biologia Molecular, Departamento de Genética, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*
[d] *Centro de Terapia Gênica, UAMP, CPE, Hospital de Clínicas de Porto Alegre, RS, Brazil*
[e] *Núcleo de Pesquisas em Oncologia, Universidade Federal do Pará, Belém, PA, Brazil*

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ABSTRACT

The Fabry disease is caused by mutations in the gene (*GLA*) that encodes the enzyme α-galactosidase A (α-Gal A). More than 500 pathologic variants of *GLA* have already been described, most of them are family-specific. In southern Brazil, a frequent single-base deletion (*GLA* 30delG) was identified among four families that do not recognize any common ancestral. In order to investigate the history of this mutation (investigate the founder effect, estimate the mutation age and the most likely source), six gene-flanking microsatellite markers of the X chromosome on the mutation carriers and their parents, 150 individuals from the same population and 300 individuals that compose the Brazilian parental populations (Europeans, Africans and Native Americans) were genotyped. A common haplotype to the four families was identified and characterized as founder. The age was estimated with two statistics software (DMLE 2.2 and ESTIAGE) that agreed with 11 to 12 generations old. This result indicates that the mutation *GLA* 30delG was originated from a single event on the X chromosome of a European immigrant, during the southern Brazil colonization between 1710 and 1740.

1. Introduction

Fabry disease (FD) is an X-linked disorder of glycosphingolipid metabolism that results from deficient activity of the lysosomal enzyme α-galactosidase (α-Gal A) and the consequent inability to metabolize globotriaosylceramide (Gb3), leading to the accumulation of this lipid in many organs and tissues. FD is present in all ethnic groups and geographic areas, and the incidence of hemizygotes is generally estimated as 1 in 40,000 to 50,000 males [1,2]. However, results from newborn screening programs [3] suggest that the actual figure might be much higher, especially for atypical cases with milder phenotypes.

In classically affected hemizygous males with less than 1% α-Gal A enzyme activity, disease onset is in childhood or adolescence, with periodic crises of severe pain in the extremities (acroparesthesias), the appearance of vascular cutaneous lesions (angiokeratomas), hypohidrosis, characteristic corneal and lenticular opacities, and proteinuria. Gradual deterioration of renal function to end-stage renal disease (ESRD) usually occurs in men in the third to fifth decades. Despite treatment for renal failure, many affected patients develop cardiovascular and/or cerebrovascular disease, a major cause of morbidity and mortality [4–6]. Although multiple organs are affected by microvascular disease, hormonal function and fertility are preserved in FD males and females and there is no evidence of reduced fitness even in males, regardless of enzyme replacement therapy status [7]. According to the Fabry Registry, life expectancy of males with Fabry disease is 58.2 years, compared with 74.7 years in the general population of the United States [8].

Heterozygous females typically have milder symptoms at a later age, but phenotypic expression in women may range from nearly asymptomatic throughout life to symptoms as severe as those observed in males with the classical phenotype.

Until recently, no specific therapy was available for FD. However, results of clinical studies with the regular administration of recombinant human α-Gal A indicate that enzyme replacement therapy (ERT) provides a specific treatment [9,10]. Evidence is now available showing that ERT for FD is safe and effective in alleviating many signs and symptoms of the disease and can slow or even reverse disease progression [5,11–15].

* Corresponding author at: Laboratório de Genética Humana e Médica, Instituto de Ciências Biológicas, Universidade Federal do Pará, Cidade Universitária Prof. José da Silveira Netto, 01, CEP: 66075-970 Belém, PA, Brazil.
E-mail addresses: akelyufpa@gmail.com, andrea.santos@pq.cnpq.br, akely@ufpa.br (Â. Ribeiro-dos-Santos).

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GLA, the only gene currently known to be associated with FD, spans approximately 13 kb of genomic DNA and contains seven exons, encoding a 429-amino acid polypeptide that includes a 31-amino acid signal peptide. Complete gene sequencing has identified a *GLA* mutation in all disease-affected males. Over 500 pathologic *GLA* allelic variants have been identified, including missense and nonsense mutations, large and small gene rearrangements, and splicing defects [2,13,16–21], http://www.ncbi.nlm.nih.gov/books/NBK1292/-fabry.REF.rodriguezmari.2003.258. Most mutations are family-specific, occurring only in single pedigrees. However, mutations at CpG dinucleotides have been identified in unrelated families of different ethnic or geographic backgrounds. Haplotype analysis of mutant alleles that occur in two or more families revealed that individuals with rare alleles are most likely related, whereas individuals with mutations involving CpG dinucleotide "hot spots" are not. Very few de novo mutations have been detected [22].

In Southern Brazil, 11 probands with FD have been identified, and approximately 50 disease-affected relatives (males and suspected carrier females) have been tested. Among the mutations identified, a recurrent single-base deletion was noted in four apparently unrelated families with the classical phenotype. The *GLA* 30delG mutation results in a frameshift that introduces a premature stop codon at residue 120 of the protein. The mutation was initially described in a large kindred with FD residing in the city of Porto Alegre, Brazil [16]. A few years later, the same mutation was identified in three additional and apparently unrelated families from the same area. Although the mutation has not been described in other countries, detailed interviews with several members of the four mutation-positive families did not identify a possible common ancestor. Thus, the present study was conducted to verify if, in these four families, the mutation was derived from a single event in the past or if repeated, independent mutational events occurred in the same region of the gene as a result of a mutational "hot spot" [19].

2. Materials and methods

2.1. Patients, control population and parental population

DNA samples from nine FD affected males, all carrying the *GLA* 30delG mutation, from four apparently unrelated families were included in this study. Fig. 1 illustrates the pedigree of four families. *GLA* mutation testing was performed after obtaining informed consent and as part of a research project approved by the Institutional Ethics Committee for Research from Hospital de Clínicas de Porto Alegre (GPPG HCPA protocol no. 03-441). All patients and their respective families had resided for at least four generations in the city of Porto Alegre, in Southern Brazil. Additional details on the molecular, clinical, and biochemical profiles of two of these families have been described previously [16,19].

2.2. Genotyping and statistical analyses

To determine whether the 30delG mutation coincided with the same haplotype in all patients studied, we initially analyzed six X-STRs (rs193272630, rs72457140, rs112254359, rs72240121, rs144273731, and rs72101069) distributed along 8 Mbp around the *GLA* gene. Allelic and haplotypic frequencies of the STRs studied were investigated by genotyping 150 healthy male blood donors from the city of Porto Alegre, Brazil (the same geographic area of residence of the patients). In an attempt to identify the most likely geographic origin of the 30delG mutation, we also genotyped a sample of 300 male individuals from parental

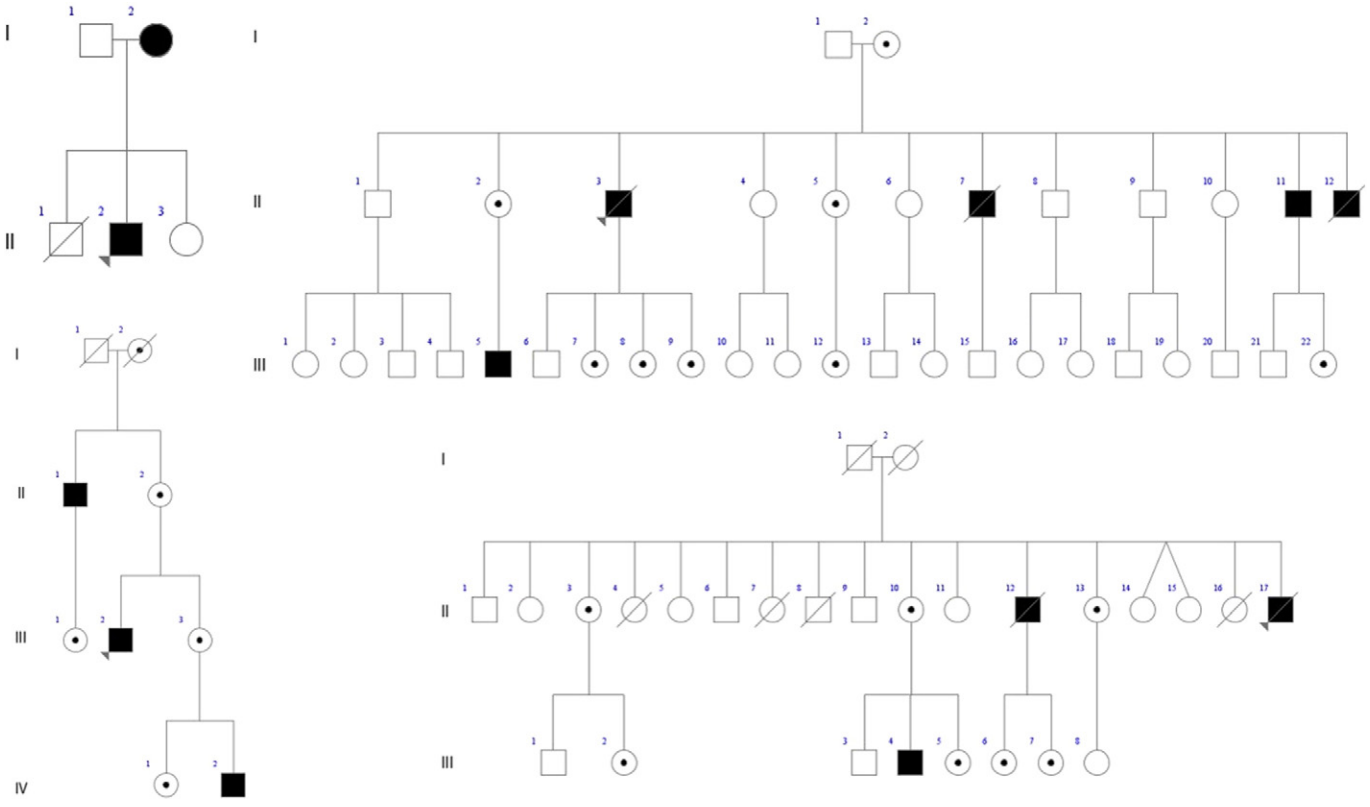

Fig. 1. Pedigrees of the four families harboring the germline 30delG mutation in the *GLA* gene. Probands are indicated by arrows. Blackened symbols indicate individuals clinically diagnosed with Fabry disease. Family I — Proband (MCG) was diagnosed at age 17 years and presented at age 8 years with acroparesthesias, angiokeratomas, hypohidrosis, growth failure, lymphedema. Plasma AGA activity at diagnosis = 0.50 nmol/h/mL. Family II — Proband (RO) was diagnosed at age 29 years and presented at childhood with acroparesthesias, heat intolerance, renal failure, pulmonary complications, impaired heart rate variability, diarrhea. Plasma AGA activity at diagnosis = 0.64 nmol/h/mL. Family III — Proband (RF) was diagnosed at age 44 years and presented at childhood with acroparesthesias, heat intolerance, pain crises, angiokeratomas, diarrhea, renal failure. Plasma AGA activity at diagnosis = 0.03 nmol/h/mL. Family IV — Proband (CADO) was diagnosed at age 39 years and presented at childhood with acroparesthesias, angiokeratomas, stroke, heart problems, and renal failure. Plasma AGA activity at diagnosis = 1.2 nmol/h/mL.

populations responsible for the constitution of the current Brazilian population: 106 Native Americans from different tribes of the Brazilian Amazon, 123 Europeans (all from Portugal), and 71 Africans from different countries of the African continent. The statistical program Arlequin v3.1 [23] was employed to estimate the allelic and haplotypic frequencies in the control population of Porto Alegre and in the parental populations and to estimate the trees of phylogenetic relationships that were subsequently constructed.

2.3. Haplotype analyses

Haplotype analyses were performed, employing six microsatellite markers, localized upstream (rs112254359, rs72240121, rs144273731 and rs72101069) and downstream (rs193272630 and rs72457140) of the *GLA* gene on the X chromosome (Xq21.33-22) and spanning a distance of approximately 13 Mbp (13×10^6 bp; Fig. 2) or 96703 cm using the Rutgers Map Interpolator (http://compgene.rutgers.edu/old/map/-interpolator/). Selection of the markers used in this study was accomplished using the UCSC genome browser (http://genome.ucsc.edubin/cgi/hgGateway) and data from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

2.4. Primers and multiplex PCR

Primers were designed using PRIMER3 and AUTO DIMER CHECK software (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3) and (http://www.cstl.nist.gov/biotech/strbase/AutoDimerHomepage). The sequences of each primer, the concentrations used in the multiplex, marked fluorochromes (dye-labeling), and the size variation of each amplicon are presented in Table 1.

Amplifications were performed in a single multiplex PCR reaction using the Qiagen Multiplex PCR kit (Qiagen, Hilden Germany) at 1× concentration, 0.1 mM of all primers, and 5–20 ng of genomic DNA in a 10 μL final reaction volume. Thermocycling conditions were as follows: initial incubation at 95 °C for 15 min; 10 cycles at 94 °C for 30 s, 60 °C for 90 s, and 72 °C for 60 s; 20 cycles at 94 °C for 30 s, 58 °C for 90 s, and 72 °C for 60 s; and a final extension at 72 °C for 60 min. PCR products were submitted to capillary electrophoresis, while separation and detection of fragments were performed in an ABI PRISM 3130 Genetic Analyzer using the GS-500 LIZ size standard, filter set D, and POP7 polymer (Life Tecnologies, California, USA). Alleles were identified and assigned using GeneMapper ID v3.2 software (Life Technologies California, USA).

2.5. Reconstruction of haplotypes and investigation of the founder effect

Because *GLA* is on the X chromosome and all patients and individuals from the control and parental populations analyzed were males, haplotypes were identified directly. We first centered our analysis on four X-STR markers (rs72457140, rs112254359, rs72240121, and rs144273731) localized in a region very close to *GLA*, spanning a 2.7 Mbp region around the gene. In the second step, we expanded the analysis to include two additional markers (rs193272630 and rs72101069), spanning an ~13 Mbp region around the gene (Fig. 2). To calculate the haplotype frequency (0.00662) in the control population, we employed the formula $1/N + 1$, described in Francez et al. [24]. The 95% upper bound limit haplotype frequency (0.01977) was calculated by applying Nelson's formula $1\text{-}\alpha^{1/N}$ (for $\alpha = 0.05$ and $N = 150$), the same as that previously used by Weir (1992) [25], to calculate the frequency of genotypes not observed in a database of size N.

2.6. Age estimation of the GLA 30delG mutation

GLA 30delG age estimation was performed using ESTIAGE [26] and DMLE v2.3 [27,28] software. ESTIAGE implements a likelihood-based method to estimate the age of the most recent common ancestor (MRCA) of a group of individuals carrying the same mutation using multilocus marker data from these individuals. It is assumed that all individuals descended from a common ancestor, who introduced alleles in healthy controls and the recombination frequencies for the six microsatellite markers. The frequency of recombination between each marker and the *GLA* 30delG mutation was assessed using the linkage-physical map generated by Rutgers map v.2 [29]. Different estimates were made using different mutation types (stepwise and equal) and different mutation rates (0.0001 and 0.00232).

The DMLE methodology is based on the observed linkage disequilibrium between a disease mutation and linked markers in DNA samples of unrelated normal individuals and affected patients. The program uses the Markov chain Monte Carlo algorithm to allow Bayesian estimation of the mutation age, based on the following parameters: haplotypes identified between chromosomes of mutation carriers and controls from the same geographical region (city of Porto Alegre), a distance map between the STR markers used, the expected proportion of chromosomes carrying the mutation, and the average growth rate of the population studied [30]. To estimate the growth rate of the population of Rio Grande do Sul, the Brazilian State from which all of the patients derive, we employed data on its population size between the years 1700 and 2010, as shown in an official publication of the Brazilian

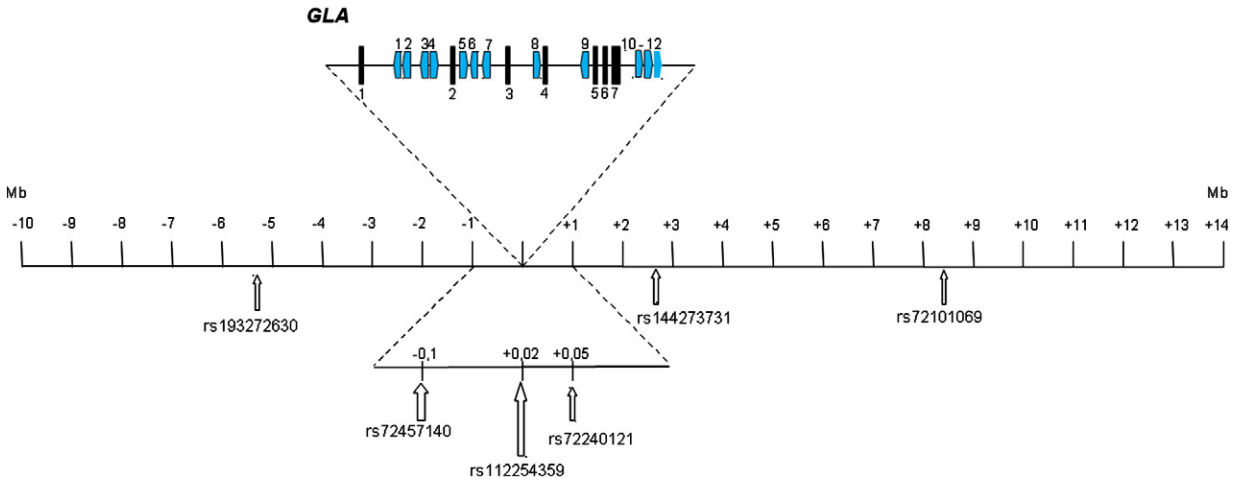

Fig. 2. Position of markers used in the present study and its respective distances in megabases from the *GLA* gene.

Table 1
PCR primers, fluorescent dyes and other parameters for construction of the multiplex genotyping protocol.

X-STR locus	Primer sequence	STR type	Repeat	Fragment size (bp)	Primer (μM)	Dye	Position on the X chromosome
rs193272630	5' GTGGGACCTGATCATGTAA 3' 5' ATCCCTAGAGGGACAGAACCAA 3'	Tetra	GATA	139–187	3	NED	95.255.000
rs72457140	5' AGAGCGAAACTCCATCTCAAAA 3' 5' AGCTGAAGGAGAAATAAGGGAGA 3'	Tetra	TAAA	368–388	8	PET	100.428.597
rs112254359	5' TGATAGCAGCAAACACTAGAGCA 3' 5' AAACACAGGAAGACCCCATCT 3'	Tri	TTA	164–184	2	PET	100.562.665
rs72240121	5' CCTGCTGCCTAGACAGAGATTT 3' 5' CCGAAATCACATCATTGCAC 3'	Tri	TTA	218–244	2	6-FAM	100.594.541
rs144273731	5' TTAGCCATCCAGACTTGTGATCT 3' 5' AGGTTATGGTAAGCCGAGATTG 3'	Tetra	CTTT	205–234	2	PET	103.108.651
rs72101069	5' CCACTTCCAAAAGGGGAAAAA 3' 5' TCCCTAGCTGGTCTGCTT 3'	Tetra	ATAG	272–296	2	6-FAM	108.857.000

Government (Instituto Brasileiro de Geografia e Estatística—IBGE; http://www.ibge.gov.br/home/). The population growth rate (r) was estimated by the equation $T1 = T0 \cdot e^{(gr)}$ [31], in which T1 is the estimated size of the population today (10.7 million of individuals), T0 is the estimated size of the ancestral population (120 thousand individuals), and *g* is the number of generations between these two time points (12.4, assuming 25 years/generation). Accordingly, the population growth rate was estimated to be approximately equal to 0.242. Considering that FD is a rare disease (estimated frequency of 1:40,000–1:60,000 male individuals of the general population) and that the *GLA* 30delG mutation is present in four of 11 families studied in our center, we assumed a proportion of the investigated known chromosomes between 0.3 and 0.4.

2.7. Origin of the mutation

Phylogenetic networks were developed to investigate genetic relationships among haplotypes carrying the *GLA* 30delG mutation and within specific haplotypes using Network 4.6.0.0 software (http://www.fluxus-engineering.com/sharenet.htm), applying the reduced median [32] and the median-joining [33] methods sequentially to

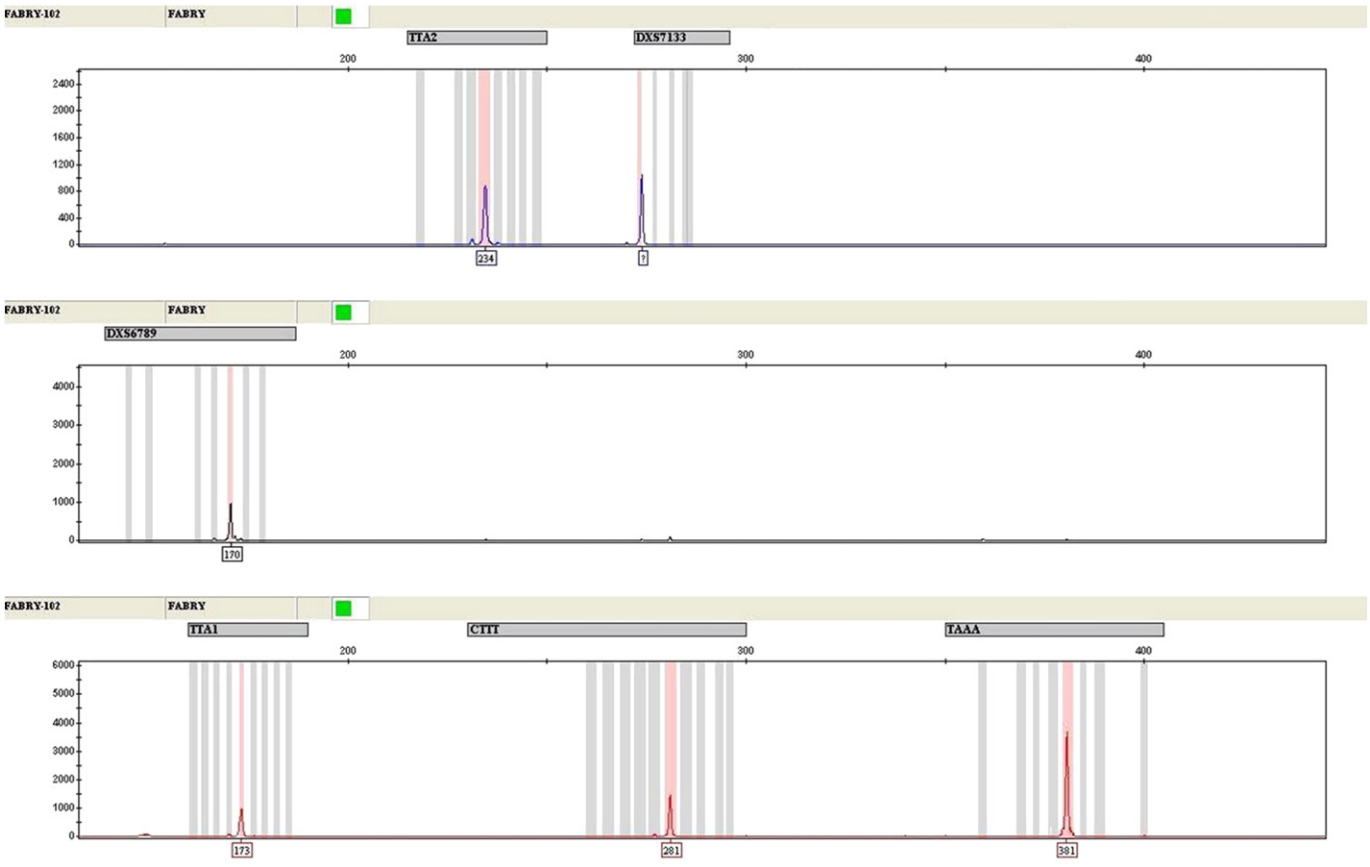

Fig. 3. Electropherogram of the six X-STR plex amplified in sample of individual male.

resolve extensive reticulation. Differential microsatellite weighting was applied to obtain the most parsimonious network. This was performed in accordance with Qamar et al. [34], and the weights for each microsatellite were inversely proportional to their variance.

3. Results

3.1. Multiplex PCR design and optimization

After the multiplex development and optimization, the six markers were successfully amplified in a single PCR reaction, following the final optimum conditions reported in Table 1 and as shown in Fig. 3.

3.2. Founder effect

Genotyping of four X-STRs (rs72457140, rs112254359, rs72240121, and rs144273731) distributed along 2.7 Mbp around *GLA* of the six investigated revealed that all nine 30delG mutation carriers had the same haplotype (10-13-15-26). The same X-STRs were employed to genotype a sample of 150 healthy controls from Porto Alegre, and none of them had the same haplotype shared by the FD patients carrying the *GLA* 30delG mutation.

3.3. Molecular dating

To allow molecular dating of the *GLA* 30delG mutation, analyses were expanded to include two remaining X-STR markers (rs193272630 and rs72101069), resulting in the identification of four different haplotypes among the nine mutation carriers (Table 2). Among these haplotypes, one (21-10-13-15-26-9) was present in four individuals from three of the four families studied. This haplotype, due to its frequency in the mutation-positive families, was considered the most likely ancestral haplotype associated with the *GLA* 30delG mutation Table 3.

Using this haplotype estimated frequency in the control population of 0.0197 (a conservative estimate), we calculated the rate of maximum likelihood between the relationship and the absence of a relationship in the four FD families. The estimated rate of maximum likelihood indicates that the results obtained are ~130,000 times more likely if the four families are related (i.e., there is a founder effect) than assuming that the mutation occurred as four independent events in each of the families on the same haplotypic background.

3.4. Mutation age estimations

The MCRA analysis revealed that haplotypes observed in all 30delG carriers derived from one common ancestral haplotype, introduced in the population 14 generations ago. This analysis also revealed that there were no significant differences between the estimates based on a mutation rate of 0.0001 (N = 14 generations, range 7–24, CI = 95%), as suggested by Lesca et al. [35], or a mutation rate of 0.00232 (N = 11 generations, range 6–21, CI = 95%), as suggested by Nothnagel et al. [36]. There was also no significant difference in the result when employing the stepwise or equal methods of mutation (data not shown).

The disequilibrium linkage analysis estimates varied slightly when altering the proportion of the population sampled, indicating that the *GLA* 30delG mutation could have been introduced in the population 10.6 generations ago (range 7–45; CI = 95%) (Fig. 4).

3.5. Origin of the GLA 30delG mutation

In an attempt to identify the most likely origin of the *GLA* 30delG mutation, we genotyped the six X-STR markers in a sample of 300 individuals from parental populations that contributed to the current admixed Brazilian population, and the results of this analysis aren't presented. Comparative analysis demonstrated that none of the haplotypes identified among the 300 sample individuals of parental populations were identical to the four haplotypes that share the mutation in the nine FD patients. We then attempted to identify the origin of the mutation, analyzing similarities between haplotypes.

Toward this aim, we employed ARLEQUIN v3.1. software [23] to compare the ancestral haplotype with the haplotypes observed in each parental population by using molecular diversity indexes and computing the minimum spanning tree among the haplotypes. The results were visualized as relationship trees of the distances between haplotypes (neighbor joining) using Tree-View 1.6.6 software [37]. From this initial analysis, we selected the 15–20 haplotypes from each parental group with the most similarities in relation to the ancestral haplotype. These haplotypes were then analyzed as a group to identify possible phylogenetic relationships using the Network 4.6.0.0 statistical package. This approach was used to reduce the number of haplotypes included in the analysis by Network to avoid interpretation problems caused by extensive reticulations that are common in the analysis of markers with high mutation rates, such as microsatellites. The results indicated that the ancestral haplotype on which the *GLA* 30delG mutation coincides has the greatest genetic similarity with several haplotypes present in individuals from Portugal.

4. Discussion

Over 500 *GLA* mutations have been described in probands with FD, most of which are “private” or family-specific, with the exception of a few “hotspot” mutations that occur mainly in CpG dinucleotides. In this study, we analyzed nine FD hemizygotes from four apparently

Table 2
Haplotype analysis of nine Fabry disease hemizygotes with the *GLA* 30delG mutation: physical distance of the X-STRs, recombination rate and allelic frequencies.

Patient	rs193272630	rs72457140	*GLA*	rs112254359	rs72240121	rs144273731	rs72101069
F1-63	*20*	10	30delG	13	15	26	9
F1-68	*20*	10	30delG	13	15	26	9
F1-62	*20*	10	30delG	13	15	26	9
F2-1833	***21***	***10***	***30delG***	***13***	***15***	***26***	***9***
F2-1940	***21***	***10***	***30delG***	***13***	***15***	***26***	***9***
F2-3040	21	10	30delG	13	15	26	*11*
F2-4879	*22*	10	30delG	13	15	26	9
F3-5320	***21***	***10***	***30delG***	***13***	***15***	***26***	***9***
F4-8766	***21***	***10***	***30delG***	***13***	***15***	***26***	***9***
Mb (*)	**95.255**	**100.428**	**100.539**	**100.563**	**100.594**	**101.299**	**103.108**
Θ (†)	**0,037625**	**0,000789**	–	**0,000173**	**0,000393**	**0,018293**	**0,059222**
Freq (‡)	**0,242991**	**0,186916**	–	**0,7103**	**0,17757**	**0,046729**	**0,429907**

Mb(*): Physical distance in Mbp according to www.ncbi.nlm.nih.gov. The distance between rs193272630 and rs72101069 is 96303 cm according to Rutgers Map Interpolator (http://compgen.rutgers.edu/old/map-interpolator/).
Θ(†): Recombination rate between the *GLA* 30delG mutation and the STR analyzed.
Freq(‡): Frequency of the alleles shared by the patients (in italic), estimated in 150 unrelated healthy individuals from the city of Porto Alegre.

Table 3
Haplotype analysis of nine Fabry disease hemizygotes with the *GLA* 30delG mutation and identification of the most recent common ancestral haplotype (depicted in bold and italic).

Patient	rs193272630	rs72457140	rs112254359	rs72240121	rs144273731	rs72101069
Haplotype 1						
F1-63	20	10	13	15	26	9
F1-68	20	10	13	15	26	9
F1-62	20	10	13	15	26	9
Haplotype 2 — most recent common ancestral						
F2-1833	***21***	***10***	***13***	***15***	***26***	***9***
F2-1940	***21***	***10***	***13***	***15***	***26***	***9***
F3-5320	***21***	***10***	***13***	***15***	***26***	***9***
F4-8766	***21***	***10***	***13***	***15***	***26***	***9***
Haplotype 3						
F2-3040	21	10	13	15	26	11
Haplotype 4						
F2-4879	22	10	13	15	26	9

unrelated families carrying a frameshift mutation, 30delG. Our goal was to verify if the mutation derived from a single event or from repeated, independent mutational events. To answer this question, we developed a multiplex system for simultaneous genotyping of six X-chromosome STRs around and at distances up to 8 Mbp from the *GLA* gene. The results from this analysis indicated that all 30delG carriers share the same haplotype of four STRs localized within a 2.7-Mbp region around *GLA*. Furthermore, the genotyping of 150 controls did not identify any haplotype that was similar to the one shared by the patients. Further, our estimates indicate that the four families are ~130,000 times more likely to be related (i.e., there is a founder effect). Thus, all results point to the conclusion that the 30delG mutation occurred as a single event in the past and that dispersion in different families led to non-recognition of an existing biological relationship among the family members interviewed. We then decided to estimate the most likely date of occurrence of the mutation. To avoid possible distortions, these estimates were made by employing two very different strategies, which resulted in estimates that were not particularly distant. Using ESTIAGE software, the occurrence of the mutation was estimated at 11 and 14 generations before the present (depending on the mutation rate used, 0.0023 or 0.0001, respectively). Using DMLE v.2.3 software, the occurrence was estimated at 10.6 and 11.7 generations before the present (depending on the proportion of the sampled population used, 0.40 or 0.30, respectively). The small variations between the two different estimates are due to factors that cannot be measured precisely, such as identification of the true mutation rate of each X chromosome STR marker and of the true proportion between haplotypes assessed in this sample of individuals and the real distribution of haplotypes occurring in the population. In the interpretation of the results, we preferred to follow the approach presented by Winbo et al. [38], who proposed that the true estimation of the age of a mutation corresponds to the range of overlap of the confidence intervals between the

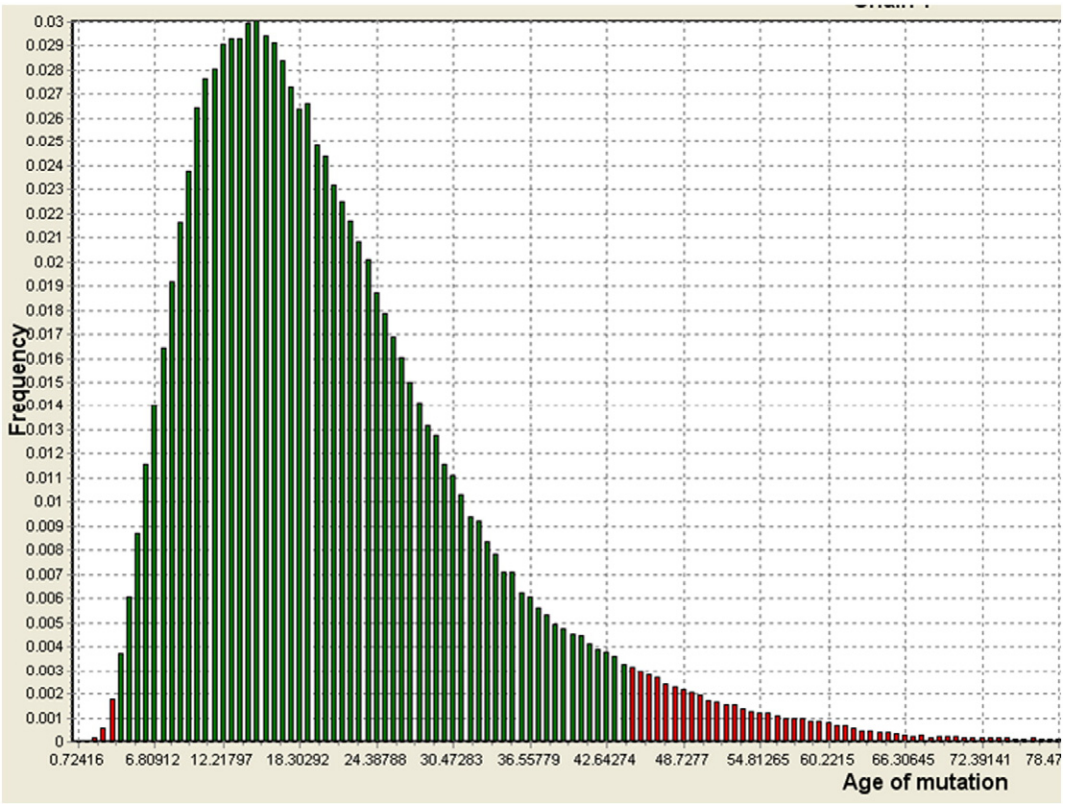

Fig. 4. Histogram generated by the software DMLE2.3, regarding the estimated age of the 30delG mutation. Note that the highest frequency is between 7 and 24 generations.

estimates obtained using ESTIAGE and DMLE softwares. Following this approach, we can estimate that the *GLA* 30delG mutation occurred between 11 and 12 generations before the present, with a confidence interval between seven and 24 generations. If we consider an average of 25 years per generation, it is likely that the mutation occurred between the years of 1710 and 1740 (between 1410 and 1840, considering a C.I. of 95%). If we consider an average of 20 years per generation, it is likely that the mutation occurred between the years of 1770 and 1790 (between 1530 and 1870, considering a C.I. of 95%), a period of rapid population expansion in Southern Brazil due to planned colonization. As the Portuguese and Spanish crowns disputed sovereignty over this region in the beginning of the 18th Century, the King of Portugal encouraged the immigration of approximately 6000 settlers from the Azores Islands to establish Portuguese domination over Southern Brazil.

4.1. Origin of the GLA 30delG mutation

Genotyping of the patients and healthy controls from the city of Porto Alegre clearly indicated that the 30delG mutation occurred at a relatively recent time on a relatively rare haplotypic background. Considering that the Brazilian population is admixed and that the mutation dating points to occurrence of the mutation at a time when colonization of the Brazilian territory had already begun, we tried to investigate the most likely origin of the mutation by comparing haplotypes that share the 30delG mutation with the haplotypes identified among individuals from the different parental populations that contributed to the current admixed Brazilian population. In this analysis, none of the mutation-associated haplotypes was encountered in the parental populations; however, phylogenetic relationship analyses demonstrated that the ancestral haplotype on which the mutation coincides is most similar to haplotypes encountered in European populations. Thus, it is likely that the *GLA* 30delG mutation occurred on an X chromosome from European immigrants during the initial colonization period of Southern Brazil.

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Conflict of interest disclosure

The authors have declared that no competing interests exist.

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References

[1] P.J. Meikle, J.J. Hopwood, A.E. Clague, W.F. Carey, Prevalence of lysosomal storage disorders, JAMA 281 (1999) 249–254.
[2] R.J. Desnick, Y.A. I, C.M. Eng, Alpha-galactosidase A deficiency: Fabry disease, in: C.R. Scriver, al Beaudet, W.S. Sly, D. Valle, K.E. Kinzler, B. Vogelstein (Eds.), The Metabolic and Molecular Bases of Inherited Disease, McGraw-Hill, New York, 2001, p. 8e.
[3] M. Spada, S. Pagliardini, M. Yasuda, T. Tukel, G. Thiagarajan, H. Sakuraba, A. Ponzone, R.J. Desnick, High incidence of later-onset Fabry disease revealed by newborn screening, Am. J. Hum. Genet. 79 (2006) 31–40.
[4] A. Ortiz, J.P. Oliveira, S. Waldek, D.G. Warnock, B. Cianciaruso, C. Wanner, Nephropathy in males and females with Fabry disease: cross-sectional description of patients before treatment with enzyme replacement therapy, Nephrol. Dial. Transplant. 23 (5) (2008) 1600–1607.
[5] A. Mehta, M. Beck, F. Eyskens, C. Feliciani, I. Kantola, U. Ramaswami, A. Rolfs, A. Rivera, S. Waldek, D.P. Germain, Fabry disease: a review of current management strategies, Q. J. Med. 103 (9) (2010) 641–659.
[6] U. Ramaswami, Update on role agalsidase beta during pregnancy in Fabry disease, Drug Des. Dev. Ther. 5 (2011) 155–173.
[7] A.C1. Hauser, A. Gessl, F. Harm, M. Wiesholzer, J. Kleinert, M. Wallner, T. Voigtländer, C. Bieglmayer, G. Sunder-Plassmann, Hormonal profile and fertility in patients with Anderson–Fabry disease, Int. J. Clin. Pract. 59 (9) (Sep 2005) 1025–1028.
[8] S. Waldek, M.R. Patel, M. Banikazemi, R. Lemay, P. Lee, Life expectancy and cause of death in males and females with Fabry disease: findings from the Fabry Registry, Genet. Med. 11 (2009) 790–796.
[9] C.M. Eng, M. Banikazemi, R.E. Gordon, M. Goldman, R. Phelps, L. Kim, A. Gass, J. Winston, S. Dikman, J.T. Fallon, S. Brodie, C.B. Stacy, et al., A phase ½ clinical trial of enzyme replacement in Fabry disease: pharmacokinetic, substrate clearance, and safety studies, Am. J. Hum. Genet. 68 (3) (2001) 711–722.
[10] R. Schiffmann, J.B. Kopp, H.A. Austin, 3rd Enzyme replacement therapy in Fabry disease: a randomized controlled trial, JAMA 285 (21) (2001) 2743–2749.
[11] M. Beck, R. Ricci, U. Widmer, F. Dehout, A.G. de Lorenzo, C. Kampmann, A. Linhart, G. Sunder-Plassmann, G. Houge, U. Ramaswami, A. Gal, A. Mehta, Fabry disease: overall effects of agalsidase alfa treatment, Eur. J. Clin. Investig. 34 (12) (2004) 838–844.
[12] P. Schiffmann, P. Hauer, B. Freeman, M. Ries, L.J. Scott, M. Polydefkis, R.O. Brady, J.C. McArthur, K. Wagner, Enzyme replacement therapy and intraepidermal innervation density in Fabry disease, Muscle Nerve 34 (1) (2006) 53–56.
[13] D.P. Germain, Fabry disease, Orphanet J. Rare Dis. 5 (2010) 30.
[14] A. Pisani, B. Visciano, G.D. Roux, M. Sabbatini, C. Porto, G. Parenti, M. Imbriaco, Enzyme replacement therapy in patients with Fabry disease: state of the art and review of the literature, Mol. Genet. Metab. 107 (3) (2012) 267–275.
[15] R.P. El Dib, P. Nascimento, G.M. Pastores, Enzyme replacement therapy for Anderson–Fabry disease, Cochrane Database Syst. Rev. 28 (2) (2013) CD006663.
[16] P. Ashton-Prolla, G.A. Ashley, R. Giugliani, R.F. Pires, R.J. e Desnick, C.M. Eng, Fabry disease: comparison of enzymatic, linkage, and mutation analysis for carrier detection in a family with a novel mutation (30delG), Am. J. Med. Genet. 84 (1999) 420–424.
[17] J. Shabbeer, M. Yasuda, E. Luca, R.J. Desnick, Fabry disease: 45 novel mutations in the alpha-galactosidase A gene causing the classical phenotype, Mol. Genet. Metab. 76 (2002) 23–30.
[18] E. Schäfer, K. Baron, U. Widmer, P. Deegan, H.P. Neumann, G. Sunder-Plassmann, J.O. Johansson, C. Whybra, M. Ries, G.M. Pastores, A. Mehta, M. Beck, et al., Thirty-four novel mutations of the GLA gene in 121 patients with Fabry disease, Hum. Mutat. 25 (4) (2005) 412.
[19] F.S. Pereira, L.B. Jardim, C.B. Netto, M.G. Burin, C. Cecchin, R. Giugliani, U.S. Matte, Genomic analysis of Brazilian patients with Fabry disease, Braz. J. Med. Biol. Res. 40 (12) (2007) 1599–1604.
[20] A. Gal, Molecular Genetics of Fabry Disease and Genotype–Phenotype Correlation, in: D. Elstein, G. Altarescu, M. Beck (Eds.), Springer, Dordrecht, Heidelberg, London, New-York, 2010, pp. 3–19.
[21] L.T. Turaça, J.G. Pessoa, F.L. Motta, M.V. Muñoz Rojas, K.B. Müller, C.M. Lourenço, W. Junior Marques, V. D'Almeida, A.M. Martins, J.B. Pesquero, New mutations in the GLA gene in Brazilian families with Fabry disease, J. Hum. Genet. 57 (6) (2012) 347–351.
[22] P. Ashton-Prolla, B. Tong, J. Shabbeer, K.H. Astrin, C.M. Eng, R.J. Desnick, Fabry disease: twenty-two novel mutations in the alpha-galactosidase A gene and genotype/phenotype correlations in severely and mildly affected hemizygotes and heterozygotes, J. Investig. Med 48 (4) (Jul 2000) 227–235.
[23] L. Excoffier, G. Laval, S. Schneider, Arlequin (version 3.0): an integrated software package for population genetics data analysis, Evol. Bioinformatics Online 1 (2005) 47–50.
[24] P.A.C. Francez, E.M.R. Rodrigues, A.M. Velasco, S.E.B. Santos, Insertion–deletion polymorphisms-utilization on forensic analysis, Int. J. Legal Med. 126 (4) (2012) 491–496.
[25] B.S. Weir, Independence of VNTR alleles defined as fixed bins, Genetics 130 (4) (1992) 873–887.
[26] E. Génin, A. Tullio-Pelet, F. Begeot, S. Lyonnet, L. Abel, Estimating the age of rare disease mutation: the example of triple-A syndrome, J. Med. Genet. 41 (6) (2004) 445–449.
[27] B. Rannala, G. Bertorelle, Using linked markers to infer the age of a mutation, Hum. Mutat. 18 (2) (2001) 87–100.
[28] B. Rannala, P.J. Reeve, DMLE+: Bayesian linkage disequilibrium gene mapping, Bioinformatics 18 (6) (2002) 894–895.
[29] T.C. Matise, F. Chen, W. Chen, F.M. de la Vega, M. Hansen, C. He, F.C.L. Hyland, K.X. Kong, S.S. Murray, J.S. Ziegle, W.C.L. Stewart, S. Buyske, A second-generation combined linkage physical map of the human genome, Genome Res. 17 (12) (2007) 1783–1786.
[30] B. Borroni, C. Bonvicini, D. Galimberti, L. Tremolizzo, A. Papetti, S. Archetti, M. Turla, A. Alberici, C. Agosti, E. Premi, I. Appollonio, I. Rainero, C. Ferrarese, M. Gennarelli, E. Scarpini, A. Padovani, Founder effect and estimation of the age of the Progranulin Thr272fs mutation in 14 Italian pedigrees with frontotemporal lobar degeneration, Neurobiol. Aging 32 (3) (2011) 555.e1–555.e8.
[31] R. Russo, A. Gambale, M.R. Esposito, MIL Serra, A. Troiano, I. DeMaggio, M. Capasso, L. Luzzatto, J. Delaunay, H. Tamry, A. Iolascon, Two founder mutations in the SEC23B gene account for the relatively high frequency of CDA II in the Italian population, Am. J. Hematol. 86 (2011) 727–732.
[32] H.J. Bandelt, P. Forster, B.C. Sykes, M.B. Richards, Mitochondrial portraits of human populations using median networks, Genetics 141 (1995) 743–753.
[33] H.J. Bandelt, P. Forster, A. Rohl, Median-joining networks for inferring intraspecific phylogenies, Mol. Biol. Evol. 16 (1999) 37–48.

[34] R. Qamar, Q. Ayub, A. Mohyuddin, A. Helgason, K. Mazhar, A. Mansoor, T. Zerjal, C. Tyler-Smith, S.Q. Mehdi, Y-chromosomal DNA variation in Pakistan, Am. J. Hum. Genet. 70 (2002) 1107–1124.
[35] G. Lesca, E. Genin, C. Blachier, C. Olivieri, F. Coulet, G. Brunet, S. Dupuis-Girod, E. Buscarini, F. Soubrier, A. Calender, C. Danesino, S. Giraud, et al., Hereditary hemorrhagic telangiectasia: evidence for regional founder effects of ACVRL1 mutations in French and Italian patients, Eur. J. Hum. Genet. 16 (6) (2008) 742–749.
[36] M. Nothnagel, R. Szibor, O. Vollrath, C. Augustin, J. Edelmann, M. Geppert, C. Alves, L. Gusmão, M. Vennemann, Y. Hou, U.D. Immel, S. Inturri, et al., Collaborative genetic mapping of 12 forensic short tandem repeat (STR) loci on the human X chromosome, Forensic Sci. Int. Genet. 6 (6) (2012) 778–784.
[37] R.D.M. Page, TREEVIEW: an application to display phylogenetic trees on personal computers, Comput. Appl. Biosci. 12 (1996) 357–358.
[38] A. Winbo, U.B. Diamant, A. Rydberg, J. Persson, S.M. Jensen, E.L. Stattin, Origin of the Swedish long QT syndrome Y111C/KCNQ1 founder mutation, Heart Rhythm. 8 (4) (2011) 541–547.