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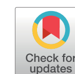
Fabry disease caused by the *GLA* p.Phe113Leu (p.F113L) variant: Natural history in males



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ABSTRACT

Background, aims and methods: The α-galactosidase gene (*GLA*) c.337T > C/p.Phe113Leu variant was originally described in patients with late-onset cardiac forms of Fabry disease (FD), who had residual α-galactosidase activity. It has since emerged as the most commonly reported *GLA* variant in Portuguese subjects diagnosed with FD but is also prevalent in the Italian population, where two boys carrying the *GLA* Leu113 allele were identified in a large-scale newborn screening program, the variant allele segregating in both cases with the same surrounding haplotype. To further delineate the genotype-phenotype correlations of this *GLA* variant, we have reviewed the natural history and clinical phenotypes of 11 symptomatic Portuguese males, from 10 unrelated families originating from several different areas in mainland Portugal and Madeira Island, who were diagnosed with FD associated with the *GLA* Leu113 allele in a diversity of clinical and screening settings. Nine of the patients were the probands of their respective families. To test whether the *GLA* Leu113 allele inherited by the 10 Portuguese and the two Italian families resulted from independent mutational events, we have additionally performed a haplotype analysis with 5 highly polymorphic, closely linked microsatellite markers surrounding the *GLA* gene.

Results and conclusions: Hemizygosity for the *GLA* Leu113 variant allele is associated with a late-onset form of FD, invariably presenting with severe cardiac involvement. Clinically relevant cerebrovascular and kidney involvement may also occur in some patients but the pathogenic relationship between the incomplete α-galactosidase deficiency and the risks of stroke and of chronic kidney disease is not straightforward. The observation that the Leu113 allele segregated within the same *GLA* microsatellite haplotype in both the Portuguese and Italian families suggests its inheritance from a common ancestor.

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1. Introduction

Fabry disease (FD; OMIM#301500) is an X-linked lysosomal storage disorder caused by deficient activity of lysosomal α-galactosidase leading to progressive, multi-systemic accumulation of globotriaosylceramide (GL-3 or Gb_3) and other undegraded neutral glycosphingolipids (Germain, 2010). The pathogenesis of FD is incompletely understood (Schiffmann, 2009; Weidemann et al., 2013), but the involvement of vascular endothelial and smooth muscle cells, particularly in small vessels, of cardiomyocytes, as well as of glomerular and tubular epithelial cells in the kidney, are critical for the development of the cerebrovascular, cardiac and renal complications that constitute the major causes of debilitating morbidity and early mortality of adult FD patients (Germain, 2010; Tuttolomondo et al., 2013; Weidemann et al., 2013; Hagège et al., 2019).

According to the usual age at clinical presentation and to the extent and severity of organ involvement, the natural history of FD in the males can be broadly classified into two major forms (Desnick et al., 2003; Germain, 2010; Oliveira and Ferreira, 2019): (*i*) the rare "classic" phenotype, with childhood- or adolescent-onset of distinctive neuropathic (e.g., acroparesthesias), dermatologic (e.g., angiokeratomas, hypohidrosis) and ocular (e.g., cornea *verticillata*) manifestations, which is due to absent or severe deficiency of α-galactosidase activity (< 2–5%); and (*ii*) the more frequent later-onset attenuated phenotypes, with manifestations confined to a single or only a few organ systems, which are associated with variable degrees of residual enzyme activity (REA). The later-onset forms of FD are most often diagnosed in patients presenting with left ventricular hypertrophy (LVH) or hypertrophic cardiomyopathy (HCM), usually in mid-to late-adulthood (Nakao et al., 1995; Sachdev et al., 2002), at ages when patients with classic FD would be severely affected or would have died (Senechal and Germain, 2003). A minority of such patients additionally exhibit mild proteinuria and may develop renal impairment later in life (Germain et al., 2018; Lavalle et al., 2018). Likely as a consequence of X-chromosome inactivation (Echevarria et al., 2016), the clinical spectrum of FD in the heterozygous females vary widely (Germain, 2010; Wilcox et al., 2008), leading to greater ambiguity of their phenotypic characterization. The diagnosis of FD can be established either by assaying the α-galactosidase activity, usually in plasma or (preferably) in leukocytes, or by identifying a pathogenic variant in the α-galactosidase gene (*GLA*; OMIM*300644), by molecular genetic testing (Desnick et al., 2003; Germain, 2010); although the enzyme assays perform well for the diagnosis of FD in males, genotyping is mandatory in females.

The Human Gene Mutation Database (HGMD®, 2019) currently reports over 900 pathogenic *GLA* variants, most of which are point mutations and associated with the classic phenotype. The majority of the pathogenic *GLA* variants identified so far are private to single families, and most of the recurrent point mutations identified in genetically unrelated families occur at CpG dinucleotides (Gal, 2010). Mutations that compromise the active site of α-galactosidase lead to complete loss of enzyme activity, but the largest number of the disease-causing *GLA* variants disrupt the hydrophobic core of the protein, reducing its stability and leading to folding defects (Guce and Garman, 2010), with diverse consequences upon enzyme activity. Many of the mutant α-galactosidase forms retaining residual catalytic activity may be clinically amenable to salvage therapy with pharmacological chaperones, such as 1-deoxygalactonojirimycin (DGJ) (Benjamin et al., 2017; Ishii et al., 2007), which is an orally absorbed galactose analog iminosugar. Pharmacological chaperones are small molecules that bind to the active site of the enzyme at neutral pH in the endoplasmic reticulum (ER), rescuing misfolded mutant proteins from ER-associated degradation (ERAD), thereby allowing their trafficking to lysosomes where, at the low pH, the chaperone dissociates and the enzyme can catabolize its substrates (Frustaci et al., 2001; Germain and Fan, 2009; Ishii et al., 2007).

The thymine-to-cytosine transition of the first nucleotide of the *GLA* codon 113 (Sequence Ontology term NM_000169.2:c.337T > C, Single Nucleotide Polymorphism Database of Short Genetic Variations; National Center for Biotechnology Information, U.S. National Library of Medicine; Bethesda MD, USA), that leads to the replacement of phenylalanine by leucine (p.Phe113Leu; HGMD® Accession Number: CM972770) in the α-galactosidase polypeptide, has been consistently associated with an attenuated, predominantly cardiac phenotype (Eng et al., 1997; Hagège et al., 2011; Park et al., 2009; Spada et al., 2006). Because the *in vitro* activity of the Leu113 α-galactosidase can be partially restored by DGJ (Benjamin et al., 2017; Ishii et al., 2007; Park et al., 2009; Spada et al., 2006), patients with FD caused by this *GLA* variant are candidates to treatment with oral pharmacological chaperones, hence avoiding the risk of immunogenicity, the psychosocial and economic burden posed by lifelong intravenous enzyme replacement therapy (ERT) as well as its inability to cross the blood-brain barrier (Germain et al., 2016). Therefore, comprehensive description of the genotype–to–phenotype correlations observed in the natural course of FD in patients carrying *GLA* variants amenable to pharmacological chaperone therapy provides critical information to support therapeutic decisions and to prospectively assess its therapeutic effect.

Since the Leu113 allele has recently been recognized as the most common *GLA* variant identified in Portuguese patients with FD (Soares et al., 2016) but detailed descriptions of the corresponding phenotype are still lacking, the major purpose of this study was to describe the natural history of FD associated with hemizygosity for *GLA* Leu113 allele in clinically symptomatic males of Portuguese ancestry.

1.1. Ethical aspects and patient data

This study was conducted in accordance with the Ethical Principles for Medical Research Involving Human Subjects adopted by the World Medical Association; the applicable Portuguese, French and Swiss laws regulating genetic testing; and the regulatory and ethical standards of the participant institutions. The research protocol was reviewed and approved (Deliberation nº 19/2016, May 13, 2016) by the Health Ethics Committee of the São João University Hospital Center (CHUSJ)/Faculty of Medicine of the University of Porto (FMUP), Portugal. According to CHUSJ institutional norms, approval for reuse of archive clinical data, including of deceased patients and patients lost to follow-up, was obtained from the Data Access Office.

1.2. Study design and definitions

The study design was strictly observational, and all the relevant clinical data were obtained as part of standard care for FD patients. A spreadsheet compiling de-identified data on patient demographics, age at and circumstances of FD diagnosis, clinical features, family history, comorbidities and diagnostic and therapeutic management, was designed for capturing each patient's medical history in as much detail and standardized manner as possible. Descriptions of clinical features and intervening events, as well as diagnostic terms were mostly transcribed into the spreadsheet as entered in the patients' medical records and reports of medical tests and diagnostic procedures. Heart failure was graded according to the New York Heart Association functional classification (Ponikowski et al., 2016). When discrepancies were found on the evaluation of cardiac function by different methods, magnetic resonance imaging (MRI) findings were given priority over transthoracic echocardiographic measurements (Hindieh et al., 2017), and these over radionuclide scan findings. LVH was defined as a septal thickness ≥11 mm (Lang et al., 2005); HCM was defined by a wall thickness ≥15 mm in one or more left ventricular myocardial segments, as measured by echocardiography, MRI or computed tomography, not explained solely by loading conditions (Authors/Task Force members et al., 2014). Chronic kidney disease (CKD) was defined as abnormalities of kidney structure or function, present for > 3 months, and staged according to the "Kidney Disease: Improving Global Outcomes

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(KDIGO)" clinical practice guidelines (Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group, 2013). Regarding tobacco smoking, patients were classified as never smokers; past smokers – individuals who quit smoking ≥3 months prior to baseline assessment); and current smokers – individuals who currently smoked or quit < 3 months prior to the baseline assessment (Nakanishi et al., 2015). Excessive alcohol consumption was defined as ≥15 standard drinks per week (US Department of Health and Human Services and US Department of Agriculture, 2015).

Eligible cases were either the affected males who first led to the recognition of FD in their respective families (hereinafter referred to as "probands") or any symptomatic male relative(s) of theirs, undergoing diagnostic investigation or clinical follow-up for medical conditions in the phenotypic spectrum of FD (Oliveira and Ferreira, 2019) at the time the diagnosis was established in the proband. "Natural history" was defined as the course of FD from its inception until the beginning of ERT or patient's demise; for patients who were alive and not receiving ERT at study termination (December 31, 2018), censoring occurred at the time of the last available clinical assessment.

1.3. Patient ascertainment and sources of clinical data

Among all patients carrying the *GLA* c.337T > C variant diagnosed at the FMUP Human Genetics Laboratory during the last 20 years, 7 were male probands of apparently unrelated Portuguese families, who had been referred for *GLA* genotyping either as first-tier diagnostic approach to FD, or after a standard diagnostic or screening enzyme assay had shown deficient α-galactosidase activities. All but one of the patients were under periodic clinical follow-up at the CHUSJ cardiology and/or nephrology and/or adult inherited metabolic diseases clinics, and four were referred to the CHUSJ medical genetics clinic for genetic counselling and family screening, having eventually consented to participating in the ongoing «Fabry Registry» international observational program [*ClinicalTrials.gov* Identifier: NCT00196742]. Three additional unrelated Portuguese families with FD segregating with the same *GLA* variant were identified through male probands immigrated in France and Switzerland, and several relatives of theirs have been subsequently referred to the CHUSJ medical genetics clinic, either for FD screening or temporary clinical follow-up during vacations in Portugal. In two of the 10 unrelated families, cascade family screening eventually led to establishing the diagnosis of FD in two patients with HCM, whose etiology had still not been recognized.

The critical clinical data for this study were provided by the physicians who were caring for the patients at the time of FD diagnosis and, when deemed necessary, by any other physicians who were, had been or eventually became involved in their care. When discordant diagnoses or disease staging were reported by different physicians, the relevant specialist judgement was assumed. For patients enrolled in the «Fabry Registry», the registry records were used as the data source for this study.

2. Methods

2.1. Molecular genetic analyses

Genomic DNA – and, in most cases, also total cellular RNA –, from at least an affected male of each of the 10 unrelated families, were extracted from peripheral blood leukocytes using standard laboratory methods and commercial reagents. In nine cases, the DNA/RNA study samples were prepared at the FMUP human genetics laboratory, while the human genetics laboratory of the University of Versailles provided a DNA sample from the proband of the family immigrated in France. *GLA* genotyping was carried out by direct Sanger sequencing of polymerase chain reaction (PCR) or reverse transcription-PCR products, as more convenient in each case, using an automated fluorescent DNA sequencing method. The laboratory protocols used at the FMUP human genetics laboratory for *GLA* genotyping are described in detail elsewhere (Oliveira et al., 2008).

2.2. Microsatellite haplotyping studies

When the second apparently unrelated Portuguese family with FD segregating with the *GLA* c.337T > C variant was identified at the FMUP Human Genetics Laboratory, *GLA* gene haplotyping was added to the standard molecular testing for such cases. The DXS8020, DXS8034, DXS8089, DXS8063 and DXS8096 microsatellite markers, which surround the *GLA* locus in close genetic linkage, encompassing about 3.2 Mb of genomic DNA, were selected for the haplotype analyses. The haplotyping was carried out as described elsewhere (Spada et al., 2006), with minor adaptions. Genomic DNA samples from the two boys carrying the Leu113 allele who were identified in the Italian newborn screening were used as controls. Microsatellites were analyzed with an ABI Prism 3500 Genetic Analyzer (Applied Biosystems, Foster City CA, U.S.A.), using the dedicated GeneMapper® Analysis Software (Version 4.1; Applied Biosystems).

2.3. Nomenclature and human genomic reference sources

For the description of *GLA* sequence variants we have used the nomenclature recommended by the Human Genome Variation Society (den Dunnen et al., 2016) and the cDNA Reference Sequence NM_000169.2 (National Center for Biotechnology Information – NCBI, National Library of Medicine, Bethesda MD, U.S.A.; http://www.ncbi.nlm.nih.gov/nuccore/125661058).

3. Results

The relevant demographic, clinical and family history data of the 9 probands and two independently ascertained affected relatives are summarized in Table 1. All patients were hemizygous for the *GLA* c.337T > C variant and none carried any other sequence variants in the *GLA* gene coding sequence, the exon/intron boundaries, or the 5′-untranslated region. In three of the probands, the *GLA* variant was originally identified in multigene panel testing for HCM, by targeted next generation DNA sequencing. The geographic origins of 8 of the 10 families could be traced to a few neighboring municipalities in the Northwest of Portugal, while the remaining two were respectively from a Southern town in mainland Portugal and from Madeira Island. Following the confirmation of FD diagnosis by mutational testing, all patients were offered comprehensive medical investigation to screen for manifestations of FD in different organ systems and as baseline assessment in consideration for ERT.

Median age at genetic diagnosis of FD was 61 years (range: 41–71). Three of the probands were diagnosed in case-finding studies of high-risk populations, namely of patients with end-stage renal failure (ESRF) on chronic hemodialysis treatment in Portugal (Mignani et al., 2010), as well as patients with HCM, in France (Hagège et al., 2011) and in Switzerland; all other patients were screened for FD on the differential diagnosis of LVH/HCM. Median prospective natural history follow-up was two years (range: 1–5).

3.1. Cardiac and cerebrovascular phenotype

All patients manifested structural, ischemic or electrophysiological cardiac disorders attributable to FD. LVH was invariably present (Fig. 1A and B), but conduction disturbances and/or clinically significant arrhythmias were also highly prevalent; 5 patients eventually needed implantation of a permanent pacemaker and/or cardioverter-defibrillator, at a median age of 62 years (range: 50–69). History of non-ST-segment elevation myocardial infarction (NSTEMI), occurring as early as in the late thirties, was reported by three patients at baseline evaluation, while during the follow-up a further patient was diagnosed

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Table 1

	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5	Patient #6	Patient #7	Patient #8	Patient #9	Patient #10	Patient #11
Age at Fabry disease diagnosis	66	53	63	71	45	71	61	60	41	61	58
Age at last available natural history follow-up	69	55	66	75	46	76	63	60	41	64	58
Indication for testing	HD[scr]	LVH	HCM	LVH	LVH	LVH	LVH	HCM[scr]	HCM[scr]	HCM^FHx	HCM^FHx
α-Galactosidase assay {biological sample: result}	DBS: BDL	DBS: BDL	DBS: 0.84 pmol/h/punch	DBS: 0.75 pmol/h/punch	ND	DBS: 0.2 pmol/h/punch	P: 0 nmol/h/ml	L: 4.3% (of normal control)	L: 5.6 nmol/h/mg	DBS: BDL	L: 3.5 nmol/h/mg
Cardiac phenotype											
Clinical syndromes											
Syncope						69		50			
Angina pectoris			58							≈38	56
Myocardial infartion/[type]			60/[NSTEMI]	71/[NSTEMI]		75/[NSTEMI]				≈38/[NSTEMI]	
Congestive heart failure /[NYHA functional classification]	62/[?]			72/[II]		75/[II-III]	59/[I-II]	60/[III]		62/[I-II]	
Acute pulmonary edema					44					61	58
Heart block and conduction abnormalities											
Bifascicular block (RBBB + LAFB)	59			71		69	61			59	
1st degree atrioventricular block										61	
2nd degree atrioventricular block/[type]										62/[2:1, Mobitz II]	
3rd degree atrioventricular block			65			69		50			
Arrhythmias											
Sinus bradycardia				75							58
Atrial fibrillation | flutter		53				69 | 69	61 |				
Paroxysmal supraventricular tachycardia				71							
Non-sustained ventricular tachycardia								57			
Morphological abnormalities											
Left atrial enlargement/[degree]	59/[mild]	53	58/[mild]	71	44/[mild]	75/[mild]	62/[mild]			61/[severe]	58
Right atrial enlargement/[degree]		53				75/[moderate]				61/[mild]	
Aortic root dilation/[degree]	59/[mild]				44/[mild]			58/[mild]			
Left ventricular hypertrophy/[type]		53/[concentric]	58/[concentric]			69/[concentric]	59/[concen-tric]		41/[septal]	44/[concentric]	58/[concen-tric]
Hypertrophic cardiomyopathy {≥15 mm}	59		63	71	44			58		56	58
{ECG voltage criteria for LVH}	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes
Right ventricular hypertrophy				72	44	69	61			61	58
Degenerative valve disease				71		69				61	
{MRI} Late myocardial enhancement location/fibrosis [%]		53: IL(midB)		71: D			61: IL(B)/ < 25%		41: IL(B)/4%		58: D/8%
Functional abnormalities											
Systolic dysfunction, left ventricular /[degree]						75/[mild]				62	
		53	63/[mild]	71	44/[mild]					61	58/[mild]

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Table 1 (*continued*)

	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5	Patient #6	Patient #7	Patient #8	Patient #9	Patient #10	Patient #11
Diastolic dysfunction, left ventricular /[degree]											
Systolic dysfunction, right ventricular /[degree]						75/[mild]					
Systolic anterior motion of the mitral valve			63								
Aortic regurgitation/[degree]				71/[mild]	44/[mild]						
Mitral regurgitation/[degree]			65/[severe]	71/[mild]	44/[mild]	75/[mild]	59/ [mild]			61/[mild]	58/[mild]
Tricuspid regurgitation/[degree]						75/ [moderate-severe]					
Segmental left ventricular hypokinesis /[location]			65/[apical]								
*Diagnostic and therapeutic procedures**											
Holter monitoring, 24-h			66	72					41: normal	56, 61	58: normal
Coronary angiography			65: no significant CAD	71: no significant CAD						≈38/56: no significant CAD	
Myocardial scintigraphy {with adenosine stress}		53: no perfusion defects			44: no perfusion defects		62: no perfusion defects				
Endomyocardial biopsy										62	
Pace-maker insertion	62		65			69		50		62	
Cardioverter-defibrillator implantation								57			
Cerebrovascular phenotype											
Clinical syndromes											
Stroke/[type]		53/[LACS]			44/[ICH]					64: lacunar	
Transient ischemic attack				75							
Neuroimaging											
Modality [age, if normal result]		MRI	CT [63]	CT	CT/MRI	CT	CT/MRI	CT [60]	(ND)	CT [62]/MRI	(ND)
Multiple cerebral infarcts		53		75							
Multiple old lacunes				75			61				
Intracerebral hematoma					44						
Leukoaraiosis/[location]		53/[SC]		72/[PV]	44/[SC]	75/[P]	61/[PV, CR]			64/[F,P]	
Renal phenotype											
Clinical syndromes											
Chronic kidney disease, stage 3		54		64		67					
Chronic kidney disease, stage 4	≈50			72	44	72					
Chronic kidney disease, stage 5				72		76					
*Urinary abnormalities***											
Microalbuminuria {UACR: 30–300 mg/g}				64					41		
Pathologic, non-nephrotic proteinuria {UACR: > 300 mg/g}	≈45	53			44	67					
Nephrotic proteinuria {UPE > 3.0 g/day}					44						
Hematuria	≈45	54			44		60				

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Table 1 (*continued*)

	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5	Patient #6	Patient #7	Patient #8	Patient #9	Patient #10	Patient #11
*Kidney ultrasound abnormalities***											
Multiple cysts/[location]	59 /[parenchymal]		63 /[cortical]			68 /[para-pelvic]					58 /[cortical]
Diagnostic procedures											
Kidney biopsy/evaluation method: pathology diagnoses	58/LM: FSGF	54/LM: FN, HTN			44/LM,EM: MPGN, HTN						
Renal replacement therapy											
Hemodialysis	58			75	44	76					
Ophthalmologic phenotype											
Slit-lamp ophthalmoscopy: cornea verticillata	ND	55: no	64: no	72: incipient (unilateral)	59: no	ND	63: no	60: no	41: no	59: no	ND
Otologic phenotype											
Hearing impaiment, self-reporetd /[type]	No	No	60 [SNHL; presbyacusis]	72 [SNHL]	No	≈60/ [uncertain]	62/ [SNHL]	No	No	No	No
Tinnitus, persistent						≈60 (unilateral)					
Major cardiovascular and other confounding comorbidites											
Systemic hypertension	42	≈30	40s	50s	43	50s	59	58		≈40	
Diabetes mellitus, type 2				≈55						59	
Hypercholesterolemia				60s		70	59	58	Yes	≈50	Yes
Obesity (BMI≥30)		Yes							Yes	Yes	Yes
Tobacco smoking	(past smoker; ceased > 2 years)	Yes: current	(past smoker; ceased > 2 years)		Yes: current	(past smoker; ceased < 2 years)			(past smoker; ceased > 2 years)	(past smoker; ceased > 2 years)	(past smoker; ceased > 2 years)
Excessive alcohol consumption		Yes			Yes						
Other	69: Hemorrhagic esophagitis. 69: Cholelithiasis. 69: Cirrhosis → HCC.		61: severe anemia < > chronic GI bleeding.		11-40: drug addiction (cocaine, heroin). 43: HCV hepatitis < > cirrhosis. 44: Septic shock → ARDS, AKI. 44: Cryoglobuli-nemia < > DVT. 44: Cholelithiasis.	48: Kidney cancer → right ne-phrectomy. 61: Rectal cancer → colostomy. 60s: COPD. 70: BPH.		58: high ferritin level.		51: OSA → CPAP. 57: BPH.	58: MGUS, IgG-K.
Family history											
Family proband?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Relevant family history data	Unaware of cardiac disease in the mother. No daughters. At-risk first-degree relatives refused genetic screening.	Mother: HT, stroke; †72. Unaware of FHx of cardiac disorders.	Unaware of cardiac disease in the mother. A brother had SCD.	Mother: †57, ovarian cancer; no overt evidence of FD. Two asymptomatic daughters. Several sibs diagnosed with LVH and/or severe arrhythmia.	Mother: syncope (78) → PM; Hx HT, renal disease; †83. Several sibs diagnosed with LVH and/or severe arrhythmia.	Mother: †≈70, SCD. None of three sisters aware of cardiac disease; no brothers. The only daughter lives abroad.	Mother: †76, pneu-monia; T2DM; no overt evidence of FD. Sister: †72, SCD. No daugh-ters.	Mother: †87; Hx of ill-defined cardiac symptoms. Sister: †72, SCD.	Mother: †46, gastic cancer; no overt evidence of FD. One daughter. All 6 brothers dignosed with FD, one with a PM; one of two sisters diagnosed with FD.	Brother of patient #5.	Mother: 85; no overt evidence of FD. Two asympto-matic daughters. Two brothers and 2 sisters with FD; brother

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Table 1 (*continued*)

	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5	Patient #6	Patient #7	Patient #8	Patient #9	Patient #10	Patient #11
											received a KTx in France, at age 44.
Enzyme replacement therapy											
Age at initiation		55	66				63	60	41		58
Death											
Age/cause of death	69/Cirrhosis, HCC			75/ CHF < > CRS	46/SCD						

Numbers are ages at presentation/diagnosis/first occurrence/demise; when only the approximate age could be estimated, the number is preceded by the sign "≈"; when only the decade of life could be estimated, the number indicates the age at the beggining of the life decade and is followed by the letter “s”. “?": relevant clinical data not available or uncertain. “ < > ": related to. “→": leading to. “^": links a condition previously diagnosed when family history information led to screening for Fabry disease. “†": age of death.
“FHx”: family history of; “Hx”: personal history of. “ND”: not done. [scr], high-risk patient population screening.
* all patients had at least an electrocardiogram and an echocardiogram available for review at baseline; ** all patients had at least a urinalysis and a kidney ultrasound examination report available for review at baseline.
Abbreviations and normal ranges for α-galactosidase assays: DBS, dried blood spot [8.75–15.6]; L: leukocyte protein extract [19.2–39.4]; P: plasma [6.0–19.0]; BDL, below the pre-defined limit for genotyping.
Abbreviations for clinical conditions, histological methods, imaging techniques and therapeutic interventions: AKI, acute kidney injury; APE, acute pulmonary edema; ARDS, acute respiratory distress syndrome; BMI, body mass index; BPH, benign prostatic hyperplasia; CAD, coronary artery disease; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CPAP, continuous positive airway pressure therapy; CRS, cardiorenal syndrome; CT, computed tomography scan; DVT, deep venous thrombosis; ECG, electrocardiographic; EM, electron microscopy; FD, Fabry disease; FN, Fabry disease nephropathy; FSGS, focal and segmental glomerulosclerosis; GI, gastrointestinal; HCC, hepatocellular carcionoma; HCM, hypertrophic cardiomyopathy; HCV, hepatitis C virus; HD, hemodialysis; HT, arterial hypertension; HTN, hypertensive nephrosclerosis; ICH, intracerebral hemorrhage; KTx, kidney transplant; LACS, lacunar stroke; LAFB, left anterior fascicular block; LM, light microscopy; LVH, left ventricular hypertophy; MGUS, monoclonal gammopathy of undetermined significance; MPGN, membranoproliferative glomerulonephritis; MRI, magnetic resonance imaging; NSTEMI, non-ST segment elevation myocardial infarction; NYHA, New York Heart Association; OSA, obstructive sleep apnea syndrome; PM, pacemaker implantation; RBBB, right bundle branch block; SCD, sudden cardiac death; SNHL, sensorineural high-frequency hearing loss; T2DM, type II diabetes mellitus.
Abbreviations for myocardial fibrosis locations: D, diffuse; IL, inferolateral; B, basal.
Abbreviations for white matter lesions locations: CR, corona radiata; F, frontal; P, parietal; PV, periventricular; SC, subcortical.

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with NSTEMI, at age 75; remarkably, none of the three patients who underwent a coronary angiogram had signs of coronary artery disease. The patients' heart problems had been first recognized at a median age of 56 years (range: 38–71) and the median delay of FD diagnosis was 2 years, but it ranged up to more than 20 years. In a patient where the diagnosis of FD cardiomyopathy was histologically confirmed, light microscopy (LM) and electron microscopy (EM) examination of an endomyocardial biopsy specimen showed GL-3 storage only in cardiomyocytes, sparing the microvascular endothelium (Fig. 2).

History of long-standing arterial hypertension (HT) was reported by 6 patients, one of whom also had type 2 diabetes mellitus (T2DM). Two patients had suffered acute stroke events shortly before baseline evaluation, respectively at ages 53 and 44 years, which was of lacunar type and associated with atrial fibrillation, in the former case, and a parenchymal hematoma supervening to cryoglobulinemic vasculitis, in the latter; during follow-up, a lacunar stroke occurred in a third patient, at age 64.

3.2. Renal phenotype

At baseline evaluation, two patients had already reached ESRD, having initiated hemodialysis respectively at ages 58 and 45 years. Three additional patients had moderate to severe proteinuric progressive CKD, which was associated with one or more major risk factors for CKD initiation or progression (Levey et al., 2003), and two of them eventually required starting chronic hemodialysis treatment, respectively at the ages of 75 and 76 years. Diagnostic kidney biopsies had been obtained in three of the five probands, two of which were available for review (Fig. 3A and B); however, in only one case the kidney biopsy was assessed by EM, in addition to standard LM examination. The proband identified in the hemodialysis screening had been biopsied at age 50 for progressive CKD associated with nephrotic-range proteinuria and microscopic hematuria, and his final histopathological diagnosis was of focal and segmental glomerulosclerosis (FSGS). The proband who presented at age 53 with a lacunar stroke had multiple major cardiovascular risk factors, including a protracted history of severe, poorly controlled HT, obesity, and heavy tobacco smoking and alcohol drinking habits; he was biopsied at age 54, for progressive CKD associated with non-nephrotic proteinuria and microscopic hematuria, having been diagnosed with FD nephropathy and hypertensive nephrosclerosis (Fig. 3A). The patient with chronic hepatitis C virus infection and liver cirrhosis was biopsied at age 44, for evaluation of rapidly progressive nephritic syndrome associated with mixed cryoglobulinemic vasculitis. He had a long-term history of regular cocaine and heroin consumption and had been taking antihypertensive medication for about one year. He had recently been hospitalized for pneumonia complicated by septic shock and multiple organ dysfunction syndrome and had needed temporary hemodialysis for treatment of acute kidney injury. The patient's kidney biopsy (Fig. 3B) was diagnostic of membranoproliferative glomerulonephritis and hypertensive nephrosclerosis, but did not show any GL-3 deposits typical of FD, either on LM examination of semi-thin sections or in the EM study (Warnock et al., 2010).

3.3. Miscellaneous phenotypic feature

None of the patients recalled history of acroparesthesias/dysesthesias in the hands or feet, of sweating problems or heat intolerance and, on careful skin examination, none presented multiple angiokeratomas typical of classic FD. The corneas appeared normal in 7 of 8 patients who underwent slit-lamp ophthalmological examination.

3.4. Family history

The family histories were relevant for a high prevalence of LVH and severe arrhythmias among matrilineal relatives; sudden cardiac death had occurred in first degree relatives of 4 of the probands, including three females in the early 8th decade of life. Four of the obligate carrier mothers of patients in the studied cohort had lived up to the 8th and 9th decade of life without manifesting any health problems attributable to FD. Of note, the mother of the proband immigrated in France had died at age 87 years, despite compound heterozygosity for both the *GLA* p.Phe113Leu and p.Arg118Cys variants. Only one of the patients reported family history of ESRF in a first–degree affected relative. None of the patients reported a paternal history of LVH/HCM or was aware of any cardiac disorders segregating in paternal lineage relatives.

3.5. Clinical findings in patients ascertained on prospective family screening

Although individuals identified on cascade genetic screening who had not yet sought medical attention for health problems attributable to FD at the time of their diagnosis were not eligible for the unbiased characterization of the natural clinical history of the *GLA* p.Phe113Leu variant, some medically relevant data were obtained from systematic assessment of 6 such young adult subjects: (*i*) most remained clinically

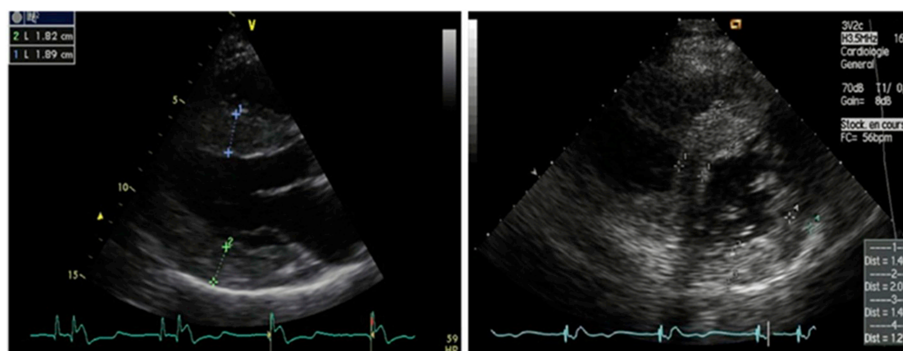

Fig. 1. A – Severe left ventricular hypertrophy on transthoracic echocardiogram
(images above) Transthoracic echocardiogram of the 60-year-old patient identified in a prospective screening for Fabry disease among patients diagnosed with hypertrophic cardiomyopathy (HCM), carried out in France: the left ventricular mass index was estimated as 312 g/m^2 (reference range: 49–115). In our patient cohort, the left ventricular hypertrophy (LVH) appeared concentric in every patient aged ≥ 44 years, while it was predominantly septal in the youngest patient in the series, diagnosed at age 41 years. Despite the imaging evidence of LVH/HCM, three (27%) out of the 11 patients did not exhibit electrocardiographic voltage criteria for LVH;
B – Severe left ventricular hypertrophy with late enhancement on cardiac magnetic resonance imaging
(figures on the top of next page) Four-chamber long-axis (left) and mid short-axis (right) cardiac magnetic resonance imaging (MRI) of the 58-year-old patient showing severe, concentric left ventricular hypertrophy and late gadolinium enhancement (LGE; white arrow) throughout the left ventricle, representing myocardial fibrosis. In our cohort, all five patients with available cardiac MRI data exhibited LGE: in three (aged 41, 53 and 61 years) this typically involved the inferolateral basal or mid-basal segments and spared the subendocardium while in the other two (aged 58 and 71 years) it appeared more diffuse.

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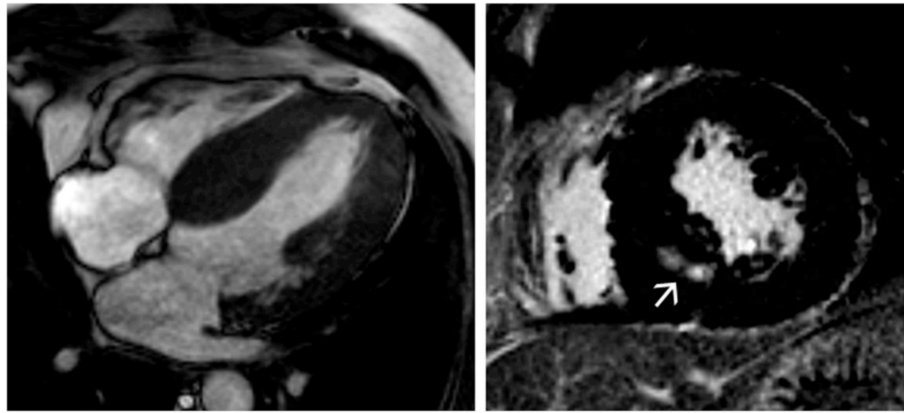

Fig. 1. (*continued*)

asymptomatic well into the 4th decade of life; (*ii*) echocardiographic evidence of LVH was not observed in any case until the mid-thirties; (*iii*) proteinuria may be the inaugural clinical manifestation of FD, appearing as early as in the mid-twenties; (*iv*) GL-3 storage in podocytes may be histologically demonstrated early in 3rd decade of life, even in non-albuminuric patients with normal renal function (Fig. 3C).

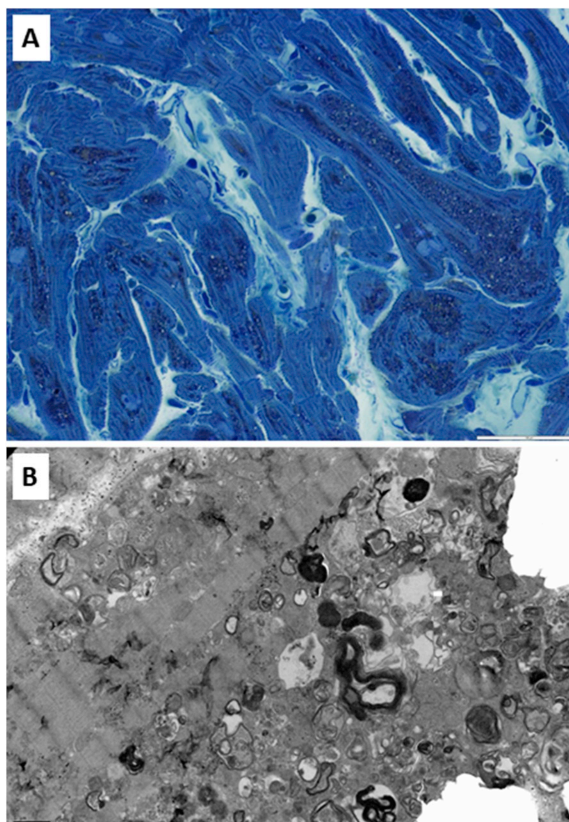

Fig. 2. – Myocardial pathology in a 62-year-old patient with severe left ventricular hypertrophy and ischemic heart disease
A. Extensive GL-3 storage within cardiomyocytes, visible as clusters of cytoplasmic dark blue-gray granular inclusions; in contrast, no GL-3 accumulation was found in interstitial capillary endothelial cells. [Light microscopy, semithin section of plastic embedded tissue: toluidine blue stain, magnification x600; white bar, lower right corner = 100 μm].
B. Electron photomicrograph of a cardiomyocyte showing the typical multilamellated membrane-bound GL-3 inclusions, localized between contractile elements. [Electron microscopy: magnification x 12000; black bar, lower left corner = 1 μm]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

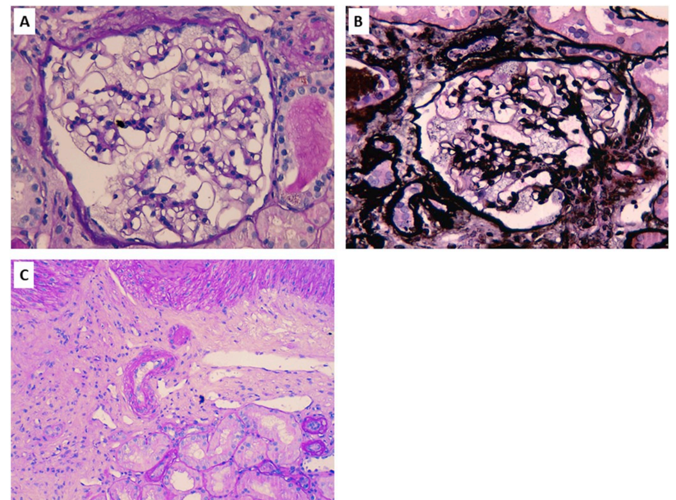

Fig. 3. A – Kidney pathology in a 54-year-old patient with stage 3 chronic kidney disease, and overt proteinuria
(images above) A. Glomerulus showing prominent cytoplasmic podocyte vacuolation and adhesions of the glomerular tuft to the Bowman's capsule. [Light microscopy: periodic acid-Schiff stain, magnification x400].
B. Argyrophilic granules remaining within podocyte vacuoles in paraffin-embedded tissue sections are a characteristic feature of GL-3 inclusions. [Light microscopy: Jones' methenamine silver stain, magnification x 400].
C. Arteriole showing medial hypertrophy with hyaline deposits, and intimal proliferation with luminal narrowing, close to an area of interstitial fibrosis and tubular atrophy; the tubular epithelial cells are not vacuolated. [Light microscopy: periodic acid-Schiff stain, magnification x200].
B – Kidney pathology in a 44-year-old patient with rapidly progressive cryoglobulinemic glomerulonephritis
(figures on next page, upper left corner) A. Medium-sized muscular artery showing luminal narrowing and prominent fibrointimal proliferation. There is no vacuolation of endothelial cells. [Light microscopy: trichrome stain, magnification x100].
B. Glomerulus with no evidence of GL-3 inclusions. [Light microscopy, semithin section of plastic embedded tissue: toluidine blue stain; magnification, x400].
C. Ultrastructural detail of a glomerulus, with no evidence of GL-3 deposits. [Electron microscopy: magnification x4000].
C – Kidney pathology in a 22-year-old asymptomatic subject with normal kidney function and normal urinary albumin excretion
(figure appearing two pages further down) A podocyte showing multiple, relatively small, mostly amorphous GL-3 inclusions. There was no evidence of GL-3 inclusions in any other kidney cell types. [Electron microscopy: magnification x5200]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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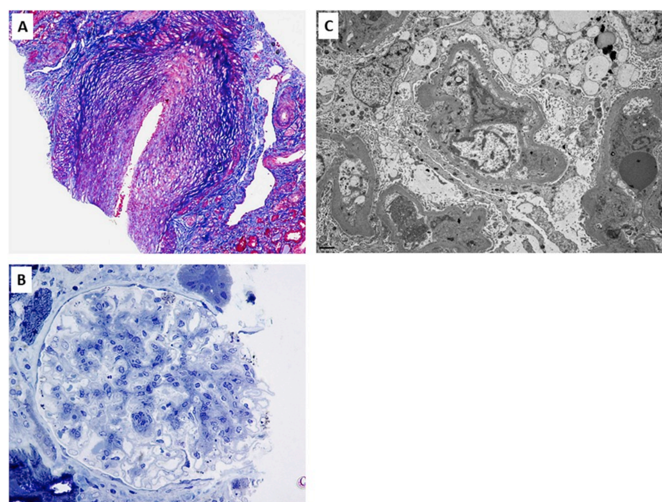

Fig. 3. (*continued*)

3.6. Microsatellite haplotypes

Remarkably, the Leu113 allele segregated with the same *GLA* microsatellite haplotype in all 10 families (Fig. 4), while their mothers and daughters who were genetically studied carried the Leu113 haplotype in heterozygosity with one of several other distinct *GLA* microsatellite haplotypes. Conversely, the haplotype associated with the Leu113 allele was not identified in any of the patient's second- and third-degree healthy female relatives, who tested homozygous for the wildtype *GLA* allele, nor in Portuguese individuals carrying other *GLA* gene variants, including the Cys118 allele (data not shown). Furthermore, the *GLA* Leu113 microsatellite haplotype identified in the two boys diagnosed in the Italian newborn screening was identical to the one found in the Portuguese families.

4. Discussion and conclusions

The *GLA* Leu113 variant was first recognized as causative of FD in a patient with a mild cardiac phenotype, whose ethnic origin was not reported (Eng et al., 1997). It was subsequently identified in two out of 37,104 consecutive Italian male neonates screened for FD using a dried blood spot (DBS) enzyme assay (Spada et al., 2006); in a Korean patient diagnosed with an atypical variant of FD (Park et al., 2009); and in two apparently unrelated Portuguese immigrants in France, enrolled in a FD screening study of patients with idiopathic LVH/HCM (Hagège et al., 2011).

The *in vitro* α-galactosidase activity in the Italian children hemizygous for the *GLA* Leu113 variant was undetectable in the DBS assay but was ≈1–2% of the control average when measured in the plasma (Spada et al., 2006). Notably, their family histories were relevant for cardiac complications of FD including LVH, HCM and atrioventricular block needing permanent pacemaker implantation, in affected male and female relatives, respectively aged 34 and 62 years.

Our findings that hemizygous males for the *GLA* Leu113 variant develop a later-onset, predominantly cardiac FD phenotype, manifesting invariably with LVH/HCM and frequently with conduction defects, arrhythmias and myocardial ischemia in the absence of overt obstructive coronary disease, are also consistent with the formerly described genotype-phenotype correlation. However, the etiologic interpretation of these patients' cardiac disorders, as well as of the cerebrovascular and renal complications additionally observed in some of them, and their imputation to FD, is confounded by the coexistence of major atherosclerotic cardiovascular risk factors (Perk et al., 2012), including long-standing severe and/or inadequately controlled HT, T2DM, obesity, hyperlipidemia, and tobacco smoking. Furthermore, both HCV-related cryoglobulinemia, and repeated exposure to radiocontrast agents, are well-known risk factors for CKD initiation and/or progression (Goldenberg and Matetzky, 2005; Levey et al., 2003). In the patient with T2DM, albuminuria was kept at relatively low levels following optimization of glycemic control and institution of treatment with an angiotensin-converting enzyme inhibitor, which is the expected response in patients with diabetic nephropathy (Gross et al., 2005). In the patient diagnosed with FSGS, the urinary protein excretion reached nephrotic level, which is a typical manifestation of FSGS but an uncommon finding in males with in FD nephropathy (Branton et al., 2002).

Kidney biopsies were obtained in three of the probands, allowing the diagnosis of FD only in one case, and of glomerular disorders unrelated to FD in the others; two patients, including the one with histological evidence of FD, additionally showed advanced lesions of HTN, which may have been a major contributor to CKD development (Klag et al., 1996; Levey et al., 2003; Luke, 1999). Ultrastructural examination of the biopsy specimen – which is the most sensitive test for the diagnosis of kidney involvement in FD (Warnock et al., 2010) –, was performed only in one patient, and did not reveal the presence the lamellar inclusions typical of lysosomal GL-3 deposits (i.e., "zebra bodies" or "myelin figures"), particularly in podocytes. Unfortunately, the other two kidney biopsies were not evaluated by EM, precluding definite exclusion of the diagnosis of FD nephropathy in the patient diagnosed with FSGS, or its confirmation in the patient diagnosed with FD nephropathy on LM examination (van der Tol et al., 2015). However, in the former case, the lack of podocyte or distal tubular cell vacuolation in the LM study, as well as the history of microscopic hematuria, were not strikingly suggestive of FD nephropathy (Warnock et al., 2010). Anyhow, podocyte GL-3 storage disease may be observed in kidney biopsies obtained from totally asymptomatic hemizygous carriers of the *GLA* Leu113 allele, but elucidation of the long-term prognostic significance of such findings requires further investigation. Our findings of predominant GL-3 storage involvement of cardiomyocytes in the heart and of podocytes in the kidneys, sparing the microvascular endothelium in both organs, is consistent with the histopathology described in the later-onset forms of FD (Desnick et al., 2003).

Owing to the natural history of its clinical phenotype, the *GLA* p.Phe113Leu variant is not expected to reduce the reproductive fitness of the affected individuals: therefore, it is not subject to purifying selection and most probably behaves as a neutral polymorphism in the population (Hartl, 2000). It should also be emphasized that the clinical relevance of the FD phenotype caused by the p.Phe113Leu variant is only recognizable in populations with relatively long life expectancies: for example, 75 years ago, when life expectancy in Portugal was 49 years for males and 53 years for females (Tiago-de-Oliveira and Mendes, 2010), such affected families would hardly be ascertained as carrying a Mendelian risk factor for severe heart disease and premature death.

Like in the northwestern Italian population, where the minor allele frequency of the *GLA* p.Phe113Leu variant mutation directly determined from newborn screening (Spada et al., 2006) is at least 2½-fold higher than the prevalence of all pathogenic *GLA* variants associated with classic FD, the p.Phe113Leu is the most frequent disease-causing *GLA* variant so far identified in Portuguese individuals (Soares et al., 2016). It seems to be particularly frequent in the district of Braga, in Northwest Portugal, where 7 apparently unrelated families with cardiac FD were identified by systematic screening of patients with HCM, an observation which was interpreted as a relatively recent "local founder effect" (Azevedo et al., 2013), with subsequent spread to other Western European countries by Portuguese migration, during the 20th century. Overall, our study expands on those observations, showing that families with FD caused by the p.Phe113Leu variant originating from geographic regions other than Braga in mainland Portugal, as well as from Madeira Island, all share the same genetic haplotype. Notably, p.Phe113Leu variant was not identified in the large Spanish FD case-

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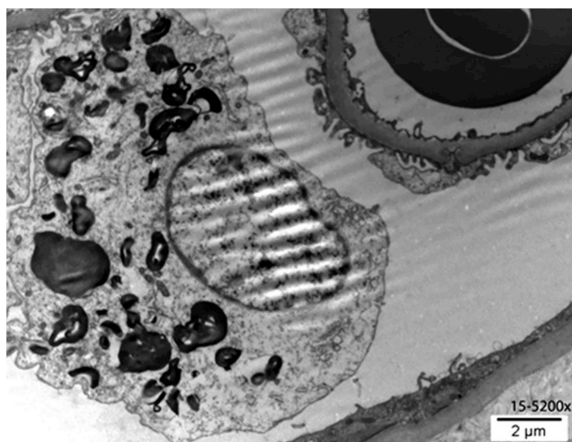

Fig. 3. (*continued*)

finding studies among patients with HCM (Monserrat et al., 2007) or undergoing chronic hemodialysis (Gaspar et al., 2010; Herrera and Miranda, 2014), suggesting that its prevalence in Spain is much lower than in Portugal; this contrasts with the available population genetics data for the benign *GLA* variant p.Arg118Cys, which seems to be particularly frequent in the two Iberian populations (Ferreira et al., 2015). However, in contrast with the common ancestor origin of the Leu113 allele in the Portuguese population, the Cys118 alleles identified in the two Iberian populations arose from multiple independent mutational events, occurring in a CpG mutational hotspot (Ferreira et al., 2015).

Molecular modelling studies (Guce and Garman, 2010; Matsuzawa et al., 2005; Spada et al., 2006) have shown that a leucine residue at amino acid position 113 can be entirely accommodated within the three-dimensional structure of the α-galactosidase polypeptide, influencing only one atom in the main chain of the enzyme, without affecting the active site. These structural features are comparable to other *GLA* variants associated with attenuated FD phenotypes (Spada et al., 2006).

COS-7 cells transfected with the mutant *GLA* c.337T > C cDNA construct express an immature form of α-galactosidase (Park et al., 2009) and the REA of the α-galactosidase Leu113 variant overexpressed in such cells ranged from ≅12% (Ishii et al., 2007) to 38% (Spada et al., 2006) of the wild-type. *In vitro* assays of the catalytic performance of α-galactosidase purified from transiently transfected COS-7 cells, after pre-incubation at different pH conditions, showed that the Leu113 enzyme completely loses its function within 15 min, at pH 7.5 (Ishii et al., 2007), indicating that it is physically unstable at neutral pH. Since the luminal microenvironment of the ER has a neutral pH, the Leu113 polypeptide probably misfolds within the ER, being subject to excessive ERAD (Fan and Ishii, 2007). These hypotheses are supported by the following experimental observations: (*i*) treatment with pharmacological ERAD inhibitors increased the amount of the Leu113 enzyme expressed in the transiently transfected COS-7 cells (Ishii et al., 2007); (*ii*) when the transfected COS-7 cells were cultured in presence of DGJ, the Leu113

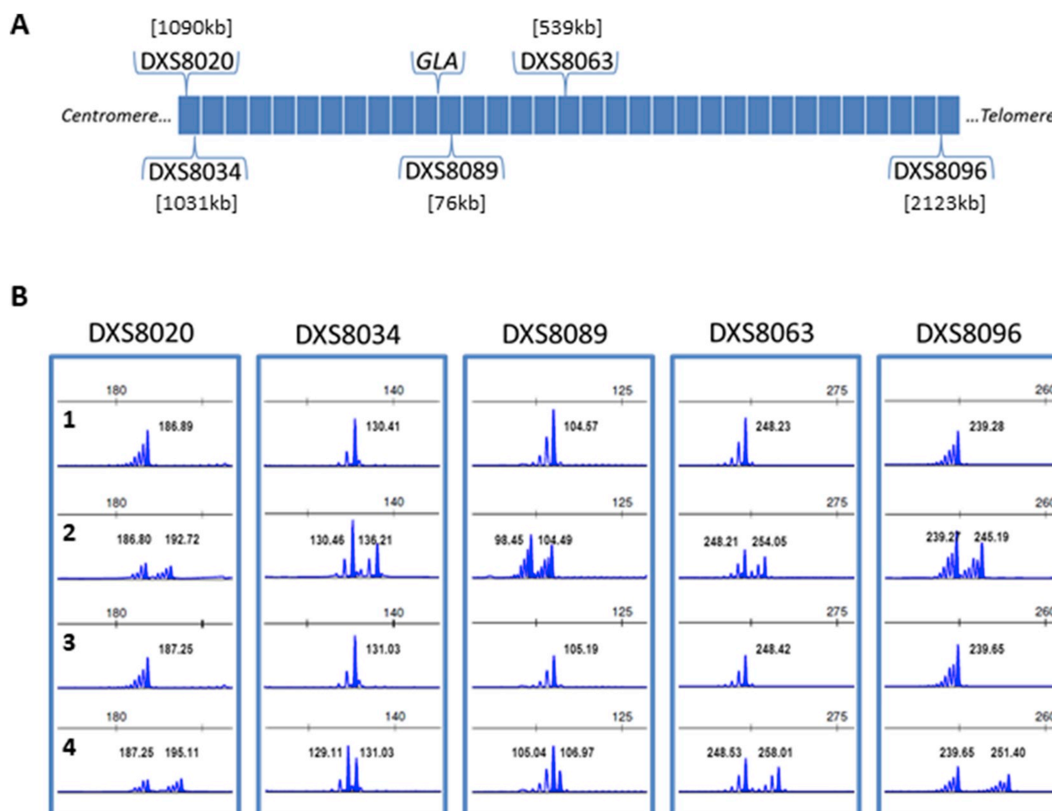

Fig. 4. Schematic representation of the genomic region encompassing the *GLA* gene and the DXS8020, DXS8034, DXS8089, DXS8063 and DXS8096 microsatellite markers, and illustrative results of the haplotyping studies.

A. Relative genomic positions of the *GLA* gene and of the microsatellite markers used for haplotyping. The diagram is drawn at scale, with each rectangle representing 100 kb. Shown between brackets are the approximate distances in kb between the p.Phe113Leu *GLA* gene variant and each microsatellite marker, according to Genome Reference Consortium Human Build 38 patch release 12 (GRCh38.p12-Primary Assembly).

B. Illustrative examples of GeneMapper® Software (Version 4.1; Applied Biosystems) microsatellite analysis. The haplotype associated with the GLA p.Phe113Leu variant was the same in all carriers, irrespective of their population ancestry or region of origin in Portugal. 1 – Portuguese male proband; 2 – heterozygous daughter of the Portuguese proband; 3 – Italian boy; 4 – heterozygous mother of the Italian boy.

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enzyme activity increased significantly, with enhancement ratios of 1.5 (Spada et al., 2006), 2.6 (Park et al., 2009), and ≅4.5 (Ishii et al., 2007) in different studies. Overall, these data explain the (pH-related) discrepancy between the levels of REA of the α-galactosidase Leu113 variant measured in plasma – as well as in dried blood spots (Spada et al., 2006) – and in the transfected COS-7 cells, and suggest that patients with attenuated FD variants associated with the Leu113 allele are candidates for treatment with pharmacological chaperones. Availability of DGJ for oral pharmacological chaperone therapy might represent a therapeutic alternative for these patients (Germain et al., 2016).

In summary, the *GLA* p.Phe113Leu variant, which apparently originated from a common ancestor in Portuguese and Italian families, segregates with a later-onset cardiac form of FD mainly characterized by LVH/HCM and consequences thereof, associated with a relatively small but definite risk of multi-organ involvement, including progressive CKD. In such patients, the α-galactosidase deficiency may add to other major cardiovascular risk factors, enhancing the overall risk of vascular complications, including cerebrovascular, coronary and renal disease; nevertheless, the imputation of such complications to FD should be made with caution, and a kidney biopsy should be considered for differential diagnosis in all cases exhibiting albuminuria/proteinuria with deteriorating renal function, particularly in patients with concurrent risk factors for CKD. Finally, given the lack of controlled clinical trial evidence that ERT is effective for the treatment of the later-onset cardiac forms of FD, and the *in vitro* biochemical features of the α-galactosidase Leu113 variant, patients with the *GLA* Leu113 allele may be candidates for enrollment in long-term clinical trials addressing the efficacy of ERT and/or pharmacological chaperone therapy for FD cardiomyopathy.

Conflicts of interest

None of the authors declare any conflict of interest in relation to the content of this manuscript.

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References

Azevedo, O., Gal, A., Rodrigues, D., Sá-Miranda, C., Sousa, F., Medeiros, R., Lourenço, A., Sousa, N., Cunha, D., 2013. Miocardiopatia hipertrófica secundária a Doença de Fabry: evidência de um efeito de fundador na região de Guimarães. Rev. Port. Cardiol. 32, 28 (Espec Congr)).

Benjamin, E.R., Della Valle, M.C., Wu, X., Katz, E., Pruthi, F., Bond, S., Bronfin, B., Williams, H., Yu, J., Bichet, D.G., Germain, D.P., Giugliani, R., Hughes, D., Schiffmann, R., Wilcox, W.R., Desnick, R.J., Kirk, J., Barth, J., Barlow, C., Valenzano, K.J., Castelli, J., Lockhart, D.J., 2017. The validation of pharmacogenetics for the identification of Fabry patients to be treated with migalastat. Genet. Med. 19 (4), 430–438.

Branton, M.H., Schiffmann, R., Sabnis, S.G., Murray, G.J., Quirk, J.M., Altarescu, G., Goldfarb, L., Brady, R.O., Balow, J.E., Austin Iii, H.A., Kopp, J.B., 2002. Natural history of Fabry renal disease: influence of alpha-galactosidase A activity and genetic mutations on clinical course. Medicine (Baltim.) 81 (2), 122–138.

den Dunnen, J.T., Dalgleish, R., Maglott, D.R., Hart, R.K., Greenblatt, M.S., McGowan-Jordan, J., Roux, A.F., Smith, T., Antonarakis, S.E., Taschner, P.E., 2016. HGVS recommendations for the description of sequence variants: 2016 update. Hum. Mutat. 37 (6), 564–569.

Desnick, R.J., Brady, R., Barranger, J., Collins, A.J., Germain, D.P., Goldman, M., Grabowski, G., Packman, S., Wilcox, W.R., 2003. Fabry disease, an under-recognized multisystemic disorder: expert recommendations for diagnosis, management, and enzyme replacement therapy. Ann. Intern. Med. 138 (4), 338–346.

Echevarria, L., Benistan, K., Toussaint, A., Dubourg, O., Hagège, A.A., Eladari, D., Jabbour, F., Beldjord, C., De Mazancourt, P., Germain, D.P., 2016. X-chromosome inactivation in female patients with Fabry disease. Clin. Genet. 89 (1), 44–54.

Authors/Task Force members,Elliott, P.M., Anastasakis, A., Borger, M.A., Borggrefe, M., Cecchi, F., Charron, P., Hagège, A.A., Lafont, A., Limongelli, G., Mahrholdt, H., McKenna, W.J., Mogensen, J., Nihoyannopoulos, P., Nistri, S., Pieper, P.G., Pieske, B., Rapezzi, C., Rutten, F.H., Tillmanns, C., Watkins, H., 2014. 2014 ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy: the task Force for the diagnosis and management of hypertrophic cardiomyopathy of the european society of cardiology (ESC). Eur. Heart J. 35 (39), 2733–2779.

Eng, C.M., Ashley, G.A., Burgert, T.S., Enriquez, A.L., D'Souza, M., Desnick, R.J., 1997. Fabry disease: thirty-five mutations in the alpha-galactosidase A gene in patients with classic and variant phenotypes. Mol. Med(Cam. Mass) 3 (3), 174–182.

Fan, J.Q., Ishii, S., 2007. Active-site-specific chaperone therapy for Fabry disease. Yin and Yang of enzyme inhibitors. FEBS J. 274 (19), 4962–4971.

Ferreira, S., Ortiz, A., Germain, D.P., Viana-Baptista, M., Caldeira-Gomes, A., Camprecios, M., Fenollar-Cortes, M., Gallegos-Villalobos, A., Garcia, D., Garcia-Robles, J.A., Egido, J., Gutierrez-Rivas, E., Herrero, J.A., Mas, S., Oancea, R., Peres, P., Salazar-Martin, L.M., Solera-Garcia, J., Alves, H., Garman, S.C., Oliveira, J.P., 2015. The alpha-galactosidase A p.Arg118Cys variant does not cause a Fabry disease phenotype: data from individual patients and family studies. Mol. Genet. Metab. 114 (2), 248–258.

Frustaci, A., Chimenti, C., Ricci, R., Natale, L., Russo, M.A., Pieroni, M., Eng, C.M., Desnick, R.J., 2001. Improvement in cardiac function in the cardiac variant of Fabry's disease with galactose-infusion therapy. N. Engl. J. Med. 345 (1), 25–32.

Gal, A., 2010. Molecular genetics of fabry disease and genotype–phenotype correlation. In: Elstein, D., Altarescu, G., Beck, M. (Eds.), Fabry Disease. Springer, Dordrecht, pp. 3–19.

Gaspar, P., Herrera, J., Rodrigues, D., Cerezo, S., Delgado, R., Andrade, C.F., Forascepi, R., Macias, J., del Pino, M.D., Prados, M.D., de Alegria, P.R., Torres, G., Vidau, P., Sa-Miranda, M.C., 2010. Frequency of Fabry disease in male and female haemodialysis patients in Spain. BMC Med. Genet. 11, 19.

Germain, D.P., 2010. Fabry disease. Orphanet J. Rare Dis. 5, 30.

Germain, D.P., Fan, J.Q., 2009. Pharmacological chaperone therapy by active-site-specific chaperones in Fabry disease: in vitro and preclinical studies. Int. J. Clin. Pharmacol. Ther. 47 (Suppl. 1), S111–S117.

Germain, D.P., Hughes, D.A., Nicholls, K., Bichet, D.G., Giugliani, R., Wilcox, W.R., Feliciani, C., Shankar, S.P., Ezgu, F., Amartino, H., Bratkovic, D., Feldt-Rasmussen, U., Nedd, K., Sharaf El Din, U., Lourenco, C.M., Banikazemi, M., Charrow, J., Dasouki, M., Finegold, D., Giraldo, P., Goker-Alpan, O., Longo, N., Scott, C.R., Torra, R., Tuffaha, A., Jovanovic, A., Waldek, S., Packman, S., Ludington, E., Viereck, C., Kirk, J., Yu, J., Benjamin, E.R., Johnson, F., Lockhart, D.J., Skuban, N., Castelli, J., Barth, J., Barlow, C., Schiffmann, R., 2016. Treatment of Fabry's disease with the pharmacologic chaperone migalastat. N. Engl. J. Med. 375 (6), 545–555.

Germain, D.P., Brand, E., Burlina, A., Cecchi, F., Garman, S.C., Kempf, J., Laney, D.A., Linhart, A., Marodi, L., Nicholls, K., Ortiz, A., Pieruzzi, F., Shankar, S.P., Waldek, S., Wanner, C., Jovanovic, A., 2018. Phenotypic characteristics of the p.Asn215Ser (p.N215S) GLA mutation in male and female patients with Fabry disease: a multicenter Fabry Registry study. Mol.Genet. Genomic. Med. 6 (4), 492–503.

Goldenberg, I., Matetzky, S., 2005. Nephropathy induced by contrast media: pathogenesis, risk factors and preventive strategies. CMAJ (Can. Med. Assoc. J.) 172 (11), 1461–1471.

Gross, J.L., de Azevedo, M.J., Silveiro, S.P., Canani, L.H., Caramori, M.L., Zelmanovitz, T., 2005. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care 28 (1), 164–176.

Guce, A.I., Garman, S.C., 2010. The structure of human α-galactosidase a and implications for fabry disease. In: Elstein, D., Altarescu, G., Beck, M. (Eds.), Fabry Disease. Springer, Dordrecht, pp. 21–38.

Hagège, A.A., Caudron, E., Damy, T., Roudaut, R., Millaire, A., Etchecopar-Chevreuil, C., Tran, T.C., Jabbour, F., Boucly, C., Prognon, P., Charron, P., Germain, D.P., 2011. Screening patients with hypertrophic cardiomyopathy for Fabry disease using a filter-paper test: the FOCUS study. Heart 97 (2), 131–136.

Hagège, A.A., Réant, P., Habib, G., Damy, T., Barone-Rochette, G., Soulat, G., Donal, E., Germain, D.P., 2019. Epub ahead of print]. Fabry disease in cardiology practice: literature review and expert point of view. pii: S1875-2136. Arch Cardiovasc Dis Feb 27 (19). https://doi.org/10.1016/j.acvd.2019.01.002. 30039-7.

Hartl, D.L., 2000. A Primer of Population Genetics, third ed. Sinauer Associates, Sunderland, MA, U.S.A.

Herrera, J., Miranda, C.S., 2014. Prevalence of Fabry's disease within hemodialysis patients in Spain. Clin. Nephrol. 81 (2), 112–120.

HGMD®, 2019. Human Gene Mutation Database. accessed on the March 1, 2019. http://www.hgmd.cf.ac.uk/ac/gene.php?gene=GLA.

Hindieh, W., Weissler-Snir, A., Hammer, H., Adler, A., Rakowski, H., Chan, R.H., 2017. Discrepant measurements of maximal left ventricular wall thickness between cardiac magnetic resonance imaging and echocardiography in patients with hypertrophic cardiomyopathy. Circ. Cardiovasc. Imaging. 10 (8).

Ishii, S., Chang, H.H., Kawasaki, K., Yasuda, K., Wu, H.L., Garman, S.C., Fan, J.Q., 2007. Mutant alpha-galactosidase A enzymes identified in Fabry disease patients with residual enzyme activity: biochemical characterization and restoration of normal intracellular processing by 1-deoxygalactonojirimycin. Biochem. J. 406 (2), 285–295.

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Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group, 2013. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int. Suppl. 3, 1–150.
Klag, M.J., Whelton, P.K., Randall, B.L., Neaton, J.D., Brancati, F.L., Ford, C.E., Shulman, N.B., Stamler, J., 1996. Blood pressure and end-stage renal disease in men. N. Engl. J. Med. 334 (1), 13–18.
Lang, R.M., Bierig, M., Devereux, R.B., Flachskampf, F.A., Foster, E., Pellikka, P.A., Picard, M.H., Roman, M.J., Seward, J., Shanewise, J.S., Solomon, S.D., Spencer, K.T., Sutton, M.S., Stewart, W.J., Chamber Quantification Writing, G., American Society of Echocardiography's, G., Standards, C., European Association of, E., 2005. Recommendations for chamber quantification: a report from the American society of echocardiography's guidelines and standards committee and the chamber quantification writing Group, developed in conjunction with the european association of echocardiography, a branch of the european society of cardiology. J. Am. Soc. Echocardiogr. 18 (12), 1440–1463.
Lavalle, L., Thomas, A.S., Beaton, B., Ebrahim, H., Reed, M., Ramaswami, U., Elliott, P., Mehta, A.B., Hughes, D.A., 2018. Phenotype and biochemical heterogeneity in late onset Fabry disease defined by N215S mutation. PLoS One 13 (4) e0193550.
Levey, A.S., Coresh, J., Balk, E., Kausz, A.T., Levin, A., Steffes, M.W., Hogg, R.J., Perrone, R.D., Lau, J., Eknoyan, G., National Kidney, F., 2003. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann. Intern. Med. 139 (2), 137–147.
Luke, R.G., 1999. Hypertensive nephrosclerosis: pathogenesis and prevalence. Essential hypertension is an important cause of end-stage renal disease. Nephrol. Dial. Transplant. 14 (10), 2271–2278.
Matsuzawa, F., Aikawa, S., Doi, H., Okumiya, T., Sakuraba, H., 2005. Fabry disease: correlation between structural changes in alpha-galactosidase, and clinical and biochemical phenotypes. Hum. Genet. 117 (4), 317–328.
Mignani, R., Feriozzi, S., Schaefer, R.M., Breunig, F., Oliveira, J.P., Ruggenenti, P., Sunder-Plassmann, G., 2010. Dialysis and transplantation in Fabry disease: indications for enzyme replacement therapy. Clin. J. Am. Soc. Nephrol. 5 (2), 379–385.
Monserrat, L., Gimeno-Blanes, J.R., Marin, F., Hermida-Prieto, M., Garcia-Honrubia, A., Perez, I., Fernandez, X., de Nicolas, R., de la Morena, G., Paya, E., Yague, J., Egido, J., 2007. Prevalence of fabry disease in a cohort of 508 unrelated patients with hypertrophic cardiomyopathy. J. Am. Coll. Cardiol. 50 (25), 2399–2403.
Nakanishi, R., Berman, D.S., Budoff, M.J., Gransar, H., Achenbach, S., Al-Mallah, M., Andreini, D., Cademartiri, F., Callister, T.Q., Chang, H.J., Cheng, V.Y., Chinnaiyan, K., Chow, B.J., Cury, R., Delago, A., Hadamitzky, M., Hausleiter, J., Feuchtner, G., Kim, Y.J., Kaufmann, P.A., Leipsic, J., Lin, F.Y., Maffei, E., Pontone, G., Raff, G., Shaw, L.J., Villines, T.C., Dunning, A., Min, J.K., 2015. Current but not past smoking increases the risk of cardiac events: insights from coronary computed tomographic angiography. Eur. Heart J. 36 (17), 1031–1040.
Nakao, S., Takenaka, T., Maeda, M., Kodama, C., Tanaka, A., Tahara, M., Yoshida, A., Kuriyama, M., Hayashibe, H., Sakuraba, H., et al., 1995. An atypical variant of Fabry's disease in men with left ventricular hypertrophy. N. Engl. J. Med. 333 (5), 288–293.
Oliveira, J.P., Ferreira, S., 2019. Multiple phenotypic domains of Fabry disease and their relevance for establishing genotype–phenotype correlations. Appl. Clin. Genet. 12, 1–16.
Oliveira, J.P., Ferreira, S., Reguenga, C., Carvalho, F., Mansson, J.E., 2008. The g.1170C > T polymorphism of the 5' untranslated region of the human alpha-galactosidase gene is associated with decreased enzyme expression–evidence from a family study. J. Inherit. Metab. Dis. 31 (Suppl. 2), S405–S413.
Park, J.Y., Kim, G.H., Kim, S.S., Ko, J.M., Lee, J.J., Yoo, H.W., 2009. Effects of a chemical chaperone on genetic mutations in alpha-galactosidase A in Korean patients with Fabry disease. Exp. Mol. Med. 41 (1), 1–7.
Perk, J., De Backer, G., Gohlke, H., Graham, I., Reiner, Z., Verschuren, M., Albus, C., Benlian, P., Boysen, G., Cifkova, R., Deaton, C., Ebrahim, S., Fisher, M., Germano, G., Hobbs, R., Hoes, A., Karadeniz, S., Mezzani, A., Prescott, E., Ryden, L., Scherer, M., Syvanne, M., Scholte op Reimer, W.J., Vrints, C., Wood, D., Zamorano, J.L., Zannad, F., European Association for Cardiovascular, P., Rehabilitation Guidelines, E.S.C.C.f.P., 2012. European guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth joint task Force of the european society of cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). Eur. Heart J. 33 (13), 1635–1701.
Ponikowski, P., Voors, A.A., Anker, S.D., Bueno, H., Cleland, J.G.F., Coats, A.J.S., Falk, V., Gonzalez-Juanatey, J.R., Harjola, V.P., Jankowska, E.A., Jessup, M., Linde, C., Nihoyannopoulos, P., Parissis, J.T., Pieske, B., Riley, J.P., Rosano, G.M.C., Ruilope, L.M., Ruschitzka, F., Rutten, F.H., van der Meer, P., Group, E.S.C.S.D., 2016. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur. Heart J. 37 (27), 2129–2200.
Sachdev, B., Takenaka, T., Teraguchi, H., Tei, C., Lee, P., McKenna, W.J., Elliott, P.M., 2002. Prevalence of Anderson-Fabry disease in male patients with late onset hypertrophic cardiomyopathy. Circulation 105 (12), 1407–1411.
Schiffmann, R., 2009. Fabry disease. Pharmacol. Ther. 122 (1), 65–77.
Senechal, M., Germain, D.P., 2003. Fabry disease: a functional and anatomical study of cardiac manifestations in 20 hemizygous male patients. Clin. Genet. 63 (1), 46–52.
Soares, A.R., Laranjeira, F., Caseiro, C., Ribeiro, I., Silva, E., Pinto, E., Ferreira, C., Rocha, S., Fortuna, A., Quelhas, D., Lacerda, L., 2016. Fabry disease in Portugal: insights from the male patients. Nascer e Crescer 25 13-13.
Spada, M., Pagliardini, S., Yasuda, M., Tukel, T., Thiagarajan, G., Sakuraba, H., Ponzone, A., Desnick, R.J., 2006. High incidence of later-onset fabry disease revealed by newborn screening. Am. J. Hum. Genet. 79 (1), 31–40.
Tiago-de-Oliveira, I., Mendes, M.F., 2010. A diferença de esperança de vida entre homens e mulheres: Portugal de 1940 a 2007. Análise Soc. XLV (194), 115–138.
Tuttolomondo, A., Pecoraro, R., Simonetta, I., Miceli, S., Pinto, A., Licata, G., 2013. Anderson-Fabry disease: a multiorgan disease. Curr. Pharmaceut. Des. 19 (33), 5974–5996.
US Department of Health and Human Services and US Department of Agriculture, 2015. 2015 – 2020 Dietary Guidelines for Americans, eighth ed. U.S. Department of Health and Human Services, Washington, DC.
van der Tol, L., Svarstad, E., Ortiz, A., Tondel, C., Oliveira, J.P., Vogt, L., Waldek, S., Hughes, D.A., Lachmann, R.H., Terryn, W., Hollak, C.E., Florquin, S., van den Bergh Weerman, M.A., Wanner, C., West, M.L., Biegstraaten, M., Linthorst, G.E., 2015. Chronic kidney disease and an uncertain diagnosis of Fabry disease: approach to a correct diagnosis. Mol. Genet. Metab. 114 (2), 242–247.
Warnock, D.G., Valbuena, C., West, M., Oliveira, J.P., 2010. Renal manifestations of fabry disease. In: Elstein, D., Altarescu, G., Beck, M. (Eds.), Fabry Disease. Springer, Dordrecht, pp. 211–243.
Weidemann, F., Sanchez-Nino, M.D., Politei, J., Oliveira, J.P., Wanner, C., Warnock, D.G., Ortiz, A., 2013. Fibrosis: a key feature of Fabry disease with potential therapeutic implications. Orphanet J. Rare Dis. 8, 116.
Wilcox, W.R., Oliveira, J.P., Hopkin, R.J., Ortiz, A., Banikazemi, M., Feldt-Rasmussen, U., Sims, K., Waldek, S., Pastores, G.M., Lee, P., Eng, C.M., Marodi, L., Stanford, K.E., Breunig, F., Wanner, C., Warnock, D.G., Lemay, R.M., Germain, D.P., 2008. Females with Fabry disease frequently have major organ involvement: lessons from the Fabry Registry. Mol. Genet. Metab. 93 (2), 112–128.

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