Screening for Fabry Disease among Dialysis Patients in Brazil: Findings from the First 18 months of a Nationwide Study

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Aims: To estimate the frequency of Fabry disease (FD) among kidney failure patients on dialysis in Brazil using an algorithm designed to track FD-suspected patients.
Study Design: Cross-sectional study.
Place and Duration of Study: Dialysis Centers in Brazil, from July, 2013 to December, 2014.
Methodology: A total of 25,223 dialysis patients from 188 dialysis centers spread all over the country were analyzed. All collected data were entered in a database created and maintained by DataGenno Interactive Research®. An algorithm was created to sort dialysis patients into three main groups: FD-suspected patients, FD-non suspected patients, and patients for medical analysis. Further up, FD-suspected patients were submitted to *GLA* gene sequencing.
Results: Out of 25,223 patients, 2,956 (11.72%) were considered FD-suspected. From FD-suspected patients, 89 (3.0%; 2.0% female, 1.0% male) were diagnosed with FD. FD-positive patients represented 0.3% (0.2% female, 0.1% male) of all analyzed patients. Average age of FD-positive patients: 37.7 years (±16.6) and of FD-negative patients: 45.1 years (±11.5). Seventeen different mutations were found in FD-positive patients. Missense mutations c.352C>T(R118C), c.1102G>A(A368T) and c.870G>C(M290I) were the most frequent (60.7% of the patients). A368T and R118C were more frequent among 30 patients with depression. Six female patients had cerebrovascular disease and A368T mutation was more frequent. A368T, R118C and M290I were more frequent in patients with heart disease. Angiokeratoma frequency (14.6%) was higher than in previous findings in the Brazilian population.
Conclusion: The natural history and frequency of FD among Brazilian dialysis patients were found, in general, according to literature. Three missense mutations were highly frequent among FD-positive patients; none of them were directly related to end-stage renal disease caused by FD. The algorithm used could be a helpful tool to identify FD.

Keywords: Fabry disease, lysosomal storage disorders, end-stage renal disease, dialysis, screening, mutation.

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1. INTRODUCTION

Fabry disease (MIM: 301500), an X-linked lysosomal storage disorder, with an estimated incidence of 1:40,000–170,000, is caused by a deficiency of the alpha-galactosidase A (α-Gal A) enzyme, resulting in storage of globotriaosylceramide (Gb3/GL3) and related glycosphingolipids in the plasma and cellular lysosomes [1-3]. Currently, 845 variants in the galactosidase, alpha gene (*GLA,* MIM: 300644) have been described [4], most in single families [1]. Fabry disease (FD) is a chronic progressive condition, clinically heterogeneous with symptoms such as chronic neuropathic pain, acute pain crises, abdominal pain, heat and cold intolerance, and fatigue often beginning in childhood [2,3,5,6]. The average presentation age is: 6–8 years (y) (males) and 9y (females), although it may vary from individual to individual even within the same family [5,7,8]. The storage of Gb3/GL3 can result in: angiokeratoma, tinnitus, hearing loss, corneal whorls, vertigo, transient ischemic attacks, stroke, cardiomyopathy, left ventricular hypertrophy, cardiac arrhythmias and valve insufficiency, chronic alternating diarrhea and constipation, obstructive pulmonary disease, proteinuria, progressive renal disease, panic attacks, depression, and adaptive function disorders [2,3,5,7]. Heterozygous women may also be affected, with more variable phenotype [9-11]. Life expectancy is diminished, more apparent in men [12].

FD manifestations tend to be non-specific and often unrecognized. Patients are therefore frequently misdiagnosed or delayed diagnosed [2,3,5,7]. Screening of FD patients in high-risk populations allows the diagnostic investigation and confirmation, the identification of asymptomatic/oligosymptomatic affected relatives and genetic counseling for couples at risk. This corroborates the importance of an early diagnosis of FD in these populations [1,13]. An increasing number of screening studies in high-risk populations and newborn screening studies have been performed since enzyme replacement therapy became available [1,13,14]. The interest of nephrologists in FD increased after the description of the "renal variant" phenotype – patients without classic symptoms of FD who develop end-stage renal disease (ESRD) [15]. Large-scale screening efforts of ESRD populations in dialysis

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treatment have been carried out [1,13,16], as ESRD is an important outcome in FD. Currently, Brazil has around 90,000 patients with ESRD being treated in 692 dialysis centers (Ministry of Health; Figure 1) [17]. Considering a FD prevalence from 0.12-0.94% in dialysis centers [16], the estimative of FD patients in Brazil would be from 108–846. This number can increase as FD may be characterized as a family trait disorder [2,18].

The main objective of this study was to estimate the frequency of FD among Brazilian dialysis patients using a screening tool designed for screening FD-suspects.

2. METHODOLOGY

2.1 Study population

A cross-sectional study was undertaken from July 2013 to December 2014, with kidney failure patients from dialysis centers in Brazil. It's part of a large ongoing study that aims to assess around 90,000 kidney failure patients in order to track FD-suspected patients and may be considered a pilot study. Inclusion criteria: kidney failure patients on hemodialysis or peritoneal dialysis from dialysis centers throughout Brazil (Figure 1), both sexes, having or not underlying causes of chronic kidney disease – CKD: high blood pressure (HBP), diabetes mellitus (DM), obesity, rheumatoid arthritis, polycystic kidney disease, and Berger's disease [16,17]. Exclusion criteria: patients with confirmed laboratory and/or clinical diagnosis of underlying causes of CKD ruling out the possibility of FD. Underlying causes of CKD which might be present in patients enrolled in the study were different from those considered as exclusion criteria.

2.2 Ethics, consent and permissions

The study protocol was approved by the Research Ethics Committee of Campos Medical School (legal opinion #305·988; 06/28/2013). Patients invited to participate were informed about the study purposes and each subject freely signed an individual consent form agreeing to participate. Patients and the health care team involved had their anonymity fully preserved according to Resolution no. 196/96 of the National Board of Health, 1988 Medical

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Ethics Code and 1964 Declaration of Helsinki.

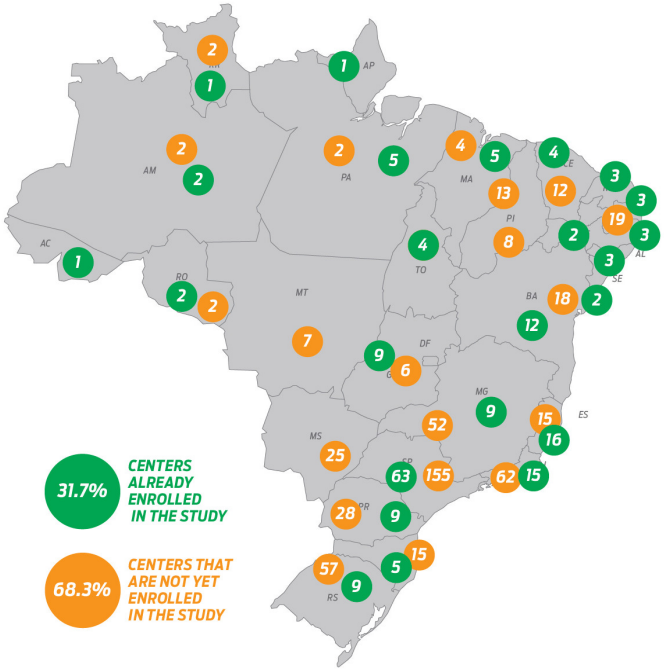

Figure 1. Map of the dialysis centers in Brazil.

Centers already enrolled in the study = 188; Centers that are not yet enrolled or refused to participate in the study = 504.

Total of 692 centers in Brazil [17].

2.3 Screening strategy

The screening strategy started with an invitation letter sent to all Brazilian dialysis centers. After the Ethics Committee's approval and formal acceptance by the head of the dialysis center, clinical questionnaires were sent to be answered by dialysis patients. Dialysis centers' healthcare staffs, mainly nurses, were trained to apply the questionnaire. Questionnaires with filling inconsistencies were sent back to be reapplied. The results were analyzed by a team of medical specialists (MPC - Marcelo Paula Coutinho and MGR – Márcia Gonçalves Ribeiro). It should be clear that this study did not actively request the participant dialysis centers to run FD tests or send FD test results they have decided to run by

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themselves. However, the dialysis centers kindly sent us FD test results of the FD-suspected patients to the study investigators. The flowchart is in Figure 2.

2.3.1 Clinical questionnaire

The clinical questionnaire (Figure 3) has a list of questions about FD signs and symptoms [2,3,5,7] that were divided into seven groups: 1, nefrological; 2, cardiological; 3, rheumatological; 4, neurological; 5, gastrointestinal/otorhinolaryngological; 6, dermatological; 7, ophthalmological. The questionnaire also has questions about underlying causes of CKD [19,20]. Content validity was done by clinical geneticists and nephrologists. The questionnaire was previously applied to 88 dialysis patients: five with FD (positive molecular test) and 83 without FD (negative molecular test); all five FD patients were considered suspected for FD, and the remaining were considered non-suspected by the algorithm (unpublished data).

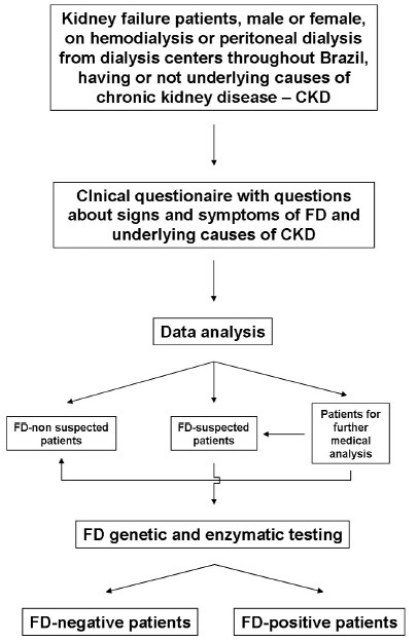

Figure 2. Flowchart depicting a method of screening for FD detection in Brazilian dialysis patients.

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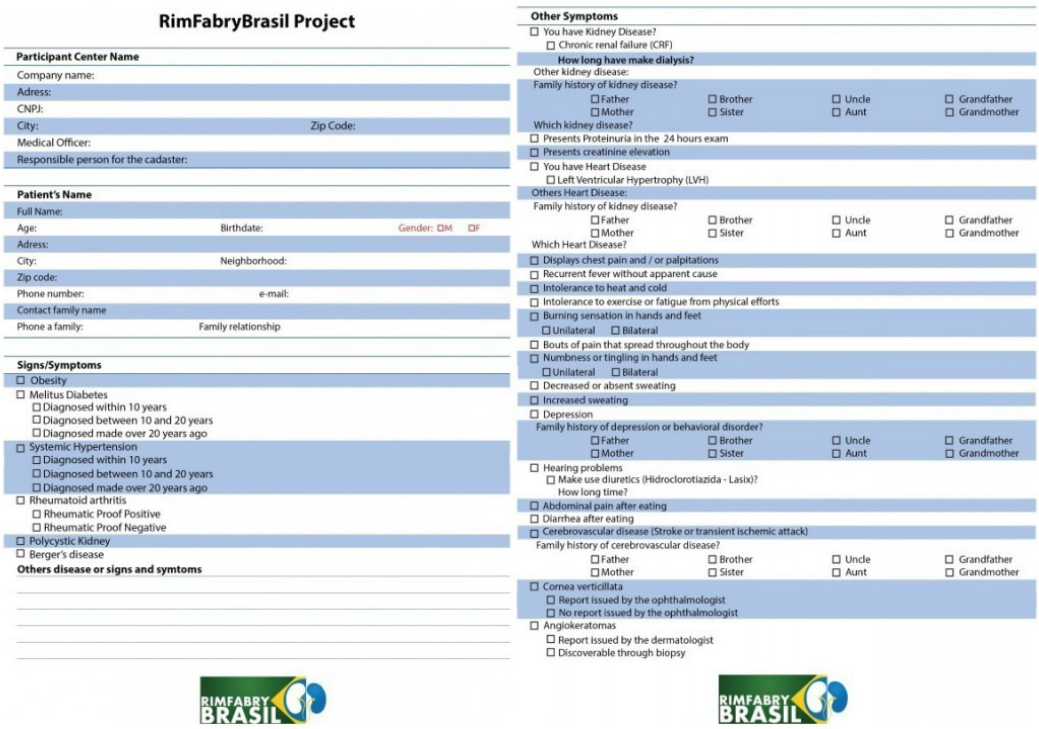

Figure 3. The clinical questionnaire used for interviewing the patients.

2.3.2 Data analysis

All collected data were entered in a database created and maintained by DataGenno Interactive Research® [21]. The algorithm proposed (Figure 4) was created by DataGenno to sort dialysis patients into three main groups: FD-suspected patients, FD-non suspected patients, and patients for further medical analysis.

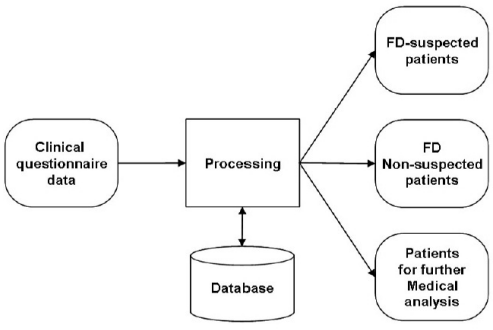

Figure 4. Algorithm for detection of FD-suspected in Brazilian dialysis patients.

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The choice of combinations created to distinguish patients in FD-suspected, FD-non suspected and for further medical analysis was based on combinations of the frequency of signs and symptoms of FD described in the literature, patient's gender and age and the natural history of FD (Tables 1, 2). Patients for further medical analysis were clinically evaluated by a team of medical specialists (MPC, MGR) in order to decide if they could be included either in FD-suspected or FD-non suspected patient groups (Figure 2). Statistical analysis was done by frequency distribution, measures of central tendency, dispersion, and the chi-square test. The results were sent back to the dialysis centers which were entirely responsible for moving forward and order or not FD diagnostic tests for FD-suspected patients.

Table 1. Groups of FD signs and symptoms

Group	FD signs and symptoms	
1 Nefrological	1.1 Family history of kidney disease	1.2 Kidney disease
	1.3 Proteinuria in the 24 hours exam	1.4 Creatinine elevation
2 Cardiological	2.1 Family history of heart disease	2.2 Heart Disease
	2.3 Chest pain and/or palpitations	
3 Rheumatological	3.1 Recurrent fever without apparent cause	
	3.2 Heat and cold intolerance	
	3.3 Exercise intolerance or fatigue from physical efforts	
	3.4 Burning sensation in hands and feet	
	3.5 Bouts of pain that spread throughout the body	
	3.6 Numbness or tingling in hands and feet	
	3.7 Sweating decrease or absence	3.8 Sweating increase
4 Neurological	4.1 Family history of cerebrovascular disease	

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Group	FD signs and symptoms	
	4.2 Cerebrovascular disease (stroke/transient ischemic attack)	
	4.3 Family history of depression/behavioral disorder	
	4.4 Depression	
Gastrointestinal / Otorhinolaryngological	5.1 Hearing problems	5.2 Abdominal pain (after eating)
	5.3 Diarrhea (after eating)	
6 Dermatological	6.1 Angiokeratomas	
7 Ophthalmological	7.1 Cornea verticillata	

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133 **Table 2.** Combinations of FD signs and symptoms and patient's gender and age used to sort
134 dialysis patients into three groups.

	Combinations of FD signs and symptoms, gender or age	Group of patients
A	1+ 2+3 (with four or more FD signs and symptoms of Group 3, without sweating increase)	FD-suspected
B	Male patient >60y with 1+2+3 (with four or more FD signs and symptoms of Group 3, without sweating increase)	Analysis
C	1+1.1+3 (with three or more FD signs and symptoms of Group 3, without sweating increase)	FD-suspected
D	Male patient >60y with 1+1.1+3 (with three or more FD signs and symptoms of Group 3, without sweating increase)	Analysis
E	1+2+ 2.1+3 (with three or more FD signs and symptoms of Group 3, without sweating increase)	FD-suspected
F	Male patient >60y with 1+2+2.1+3 (with three or more FD signs	Analysis

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	Combinations of FD signs and symptoms, gender or age	Group of patients
	and symptoms of Group 3, without sweating increase)	
G	Combinations A, B or C where Group 3 of FD signs and symptoms includes sweating increase	Analysis
H	Male patient >60y with combinations of signs and symptoms A,C or E where Group 3 of FD signs and symptoms includes sweating increase	FD-non suspected
I	Male patient with 1.1 and/or 2.1 whose father has 1.1 and/or 2.1 (healthy mother)	Analysis
J	1+2+7 (one or more FD signs and symptoms of Group 7)	FD-suspected
K	Patient with polycystic kidney disease, excluding FD signs and symptoms of Groups 5 and/or 6	Analysis
L	1+5	FD-suspected
M	1+6	FD-suspected
N	1	Analysis
O	1+5	FD-suspected
P	1+6	FD-suspected
Q	1+2+3 (four or more FD signs and symptoms of Group 3)	Analysis
R	1+1.1+3 (three or more FD signs and symptoms of Group 3)	Analysis
S	1+2+2.1+3 (three or more FD signs and symptoms of Group 3)	Analysis
T	1+3 (three or more FD signs and symptoms of Group 3) +4 or 5	Analysis
U	Male patient >60y with combinations of signs and symptoms R,S or T where the FD signs and symptoms of Group 3 includes sweating increase	FD-non suspected
V	Patients with confirmed laboratory and/or clinical diagnosis of	FD-non suspected

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Combinations of FD signs and symptoms, gender or age	Group of patients
underlying causes of CKD ruling out the possibility of FD	

A to V – combinations of FD signs/symptoms, gender or age used to define three main groups of patients: FD-suspected, FD-non suspected, and analysis.

1 to 7 – groups of FD signs/symptoms: 1, nefrological; 2, cardiological; 3, rheumatological; 4, neurological; 5, gastrointestinal/otorhinolaryngological; 6, dermatological; 7, ophthalmological.

2.3.3 FD laboratory testing

Male FD-positive patients: positive enzymatic test (low/undetectable α-Gal A activity) and/or presence of pathogenic mutation in the *GLA* gene. Female FD-positive patients: pathogenic mutation in the *GLA* gene. FD genetic and enzymatic tests were run independently by the participant dialysis centers.

2.3.4 Searches in human mutation databases

Searches for *GLA* gene mutations were performed at the Human Gene Mutation Database – HGMD [4], Leiden Open-source Variation Database – LOVD platform [22], available at Zhejiang University Center for Genetic and Genomic Medicine website [23], and at NCBI's ClinVar [24].

3. RESULTS

3.1 Patient demographics

A total of 25,223 dialysis patients from 188 dialysis centers were analyzed. Nine of the invited dialysis centers did not join the study. Male patients were 59.3% (14,957) and female, 40.7% (10,266) (Figure 5A). FD-suspected patients were 2,956 (11.7%). The total of FD-negative patients was 2,867, among FD-suspected patients and the total of FD-positive patients was 89, most female (58 [65.2%] female; 31 [34.8%] male) (Figure 5C). Female predominance in FD-positives was significant ($\chi^2 = 7.39$; $P = 0.0065$). FD-positive patients

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represented 3.0% (2.0% female, 1.0% male) of all FD-suspected patients and 0.3% (0.2% female, 0.1% male) of all participant dialysis patients. FD-negatives were 2,867 (Figure 5B), both sexes in similar proportions (50.5% female; 49.5% male).

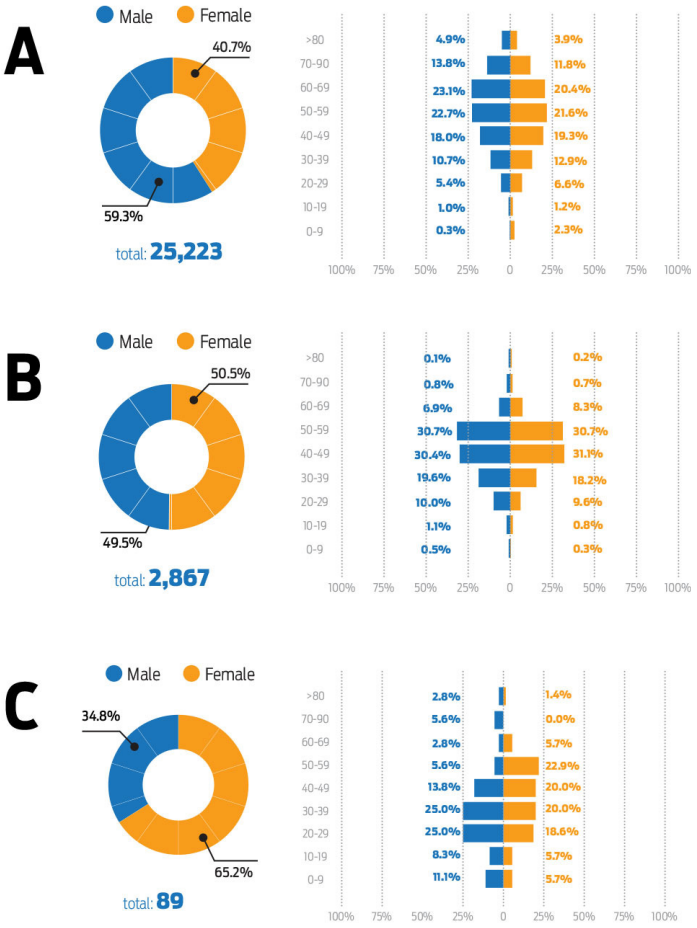

Figure 5. Distribution of the Brazilian dialysis patients by gender, age group, and FD diagnosis. **A)** Total; **B)** FD-negatives; **C)** FD-positives.

The average age of FD-positive patients was 37.7y (±16.6); for FD-negative patients it was 45.1y (±11.5). FD-positive patients showed lower average age (male: 33.8±16.0; female: 37.8±17.6) when compared to FD-negatives (male: 44.9±11.6; female: 45.2±11.5). It is possible to see a sharp decline in the number of FD-positive male patients from 40y (Figure

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5C) while the decline in the number of FD-positive female patients becomes more significant from 60y. The distribution of FD-negative patients by gender and age was also different from the total of analyzed patients (Figure 5B,5A). In FD-negative patients, both genders, a sharp decline in the number of patients is only seen from 60y (Figure 5B) and in the total of analyzed patients that happens from 70y (Figure 5A).

3.2 *GLA* gene mutation analysis

A total of 17 different mutations were found in FD-positive patients' *GLA* gene (Table 3). Three mutations were highly frequent: c.1102G>A (A368T), c.352C>T (R118C) and c.870G>C (M290I). Mutations 194+1G>A, 370-1G>T and 801+36G>A were located in intronic regions of the *GLA* gene.

Table 3. Mutations found in FD-positive patients' *GLA* gene

Mutation No.	*GLA* gene nucleotide change	Location	Mutation	Number of patients with the mutation
M1	c.194+1G>A	intron	unknown	3
M2	c.370-1G>T	intron	splice acceptor variant	1
M3	c.801+36G>A	intron	unknown	1
M4	c.337T>C	exon 2	p.Phe113Leu (F113L)	3
M5	c.352C>T	exon 2	p.Arg118Cys (R118C)	18
M6	c.413delG	exon 3	p.Gly138Glufs (G138E)	1
M7	c.427G>A	exon 3	p.Ala143Thr (A143T)	1
M8	c.679C>T	exon 5	p.Arg227X (R227X)	4
M9	c.803T>C	exon 6	p.Leu268Ser (L268S)	1
M10	c.870G>A	exon 6	p. Met290Ile (M290I)	5
M11	c.870G>C	exon 6	p.Met290Ile (M290I)	17

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Mutation No.	*GLA* gene nucleotide change	Location	Mutation	Number of patients with the mutation
M12	c.877C>T	exon 6	p.Pro293Ser (P293S)	3
M13	c.937G>T	exon 6	p.Asp313Tyr (D313Y)	4
M14	c.1025G>A	exon 7	p.Arg342Gln (R342Q)	4
M15	c.1067G>A	exon 7	p. Arg356Gln (R356Q)	1
M16	c.1102G>A	exon 7	p.Ala368Thr (A368T)	19
M17	c.1117G>A	exon 7	p.Gly373Ser (G373S)	1
**	c.870G>C/ c.376A>G	exon 6 exon 3	p.Met290Ile (M290I)/ p.Ser126Gly (S126G)	2

** compound heterozygous (M11/variant of uncertain significance)

After renal involvement, heart disease was the most prevalent symptom; it was present in 56 patients (62.9%). Three mutations were more frequent: c.1102G>A (A368T), c.325C>T (R118C) and c.870G>C (M290I); 30.3%, 19.6% and 14.3% respectively. Thirty patients had depression and the most prevalent mutations were c.1102G>A (A368T; 40.0%) and c.352C>T (R118C; 13.4%). Six female had cerebrovascular disease and the mutation c.1102G>A (A368T) was frequent (66.7%). Thirteen patients had angiokeratoma and only three were male (23.1%); the mutations c.870G>A (M290I), c.870G>C (M290I) and c.870G>C/c.376A>G (M290I/S126G) were equally frequent (15.4% each). A total of 10 FD-positive had cornea verticillata; the mutations c.352C>T (R118C), c.870G>C (M290I) and c.937G>T (D313Y) were equally frequent (20.0% each).

3.3 Frequency of FD symptoms

Heart and rheumatologic symptoms were more frequent below 59y and neurological and gastrointestinal/otorhinolaryngologic symptoms were more frequent below 39y in FD-positive patients (Figure 6B), unlike what was observed in FD-negative patients among FD suspected

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patients; it reminds the gaussian distribution (Figure 6A). Symptoms in general occurred earlier in male then female FD-positive patients (Figure 6C,6D).

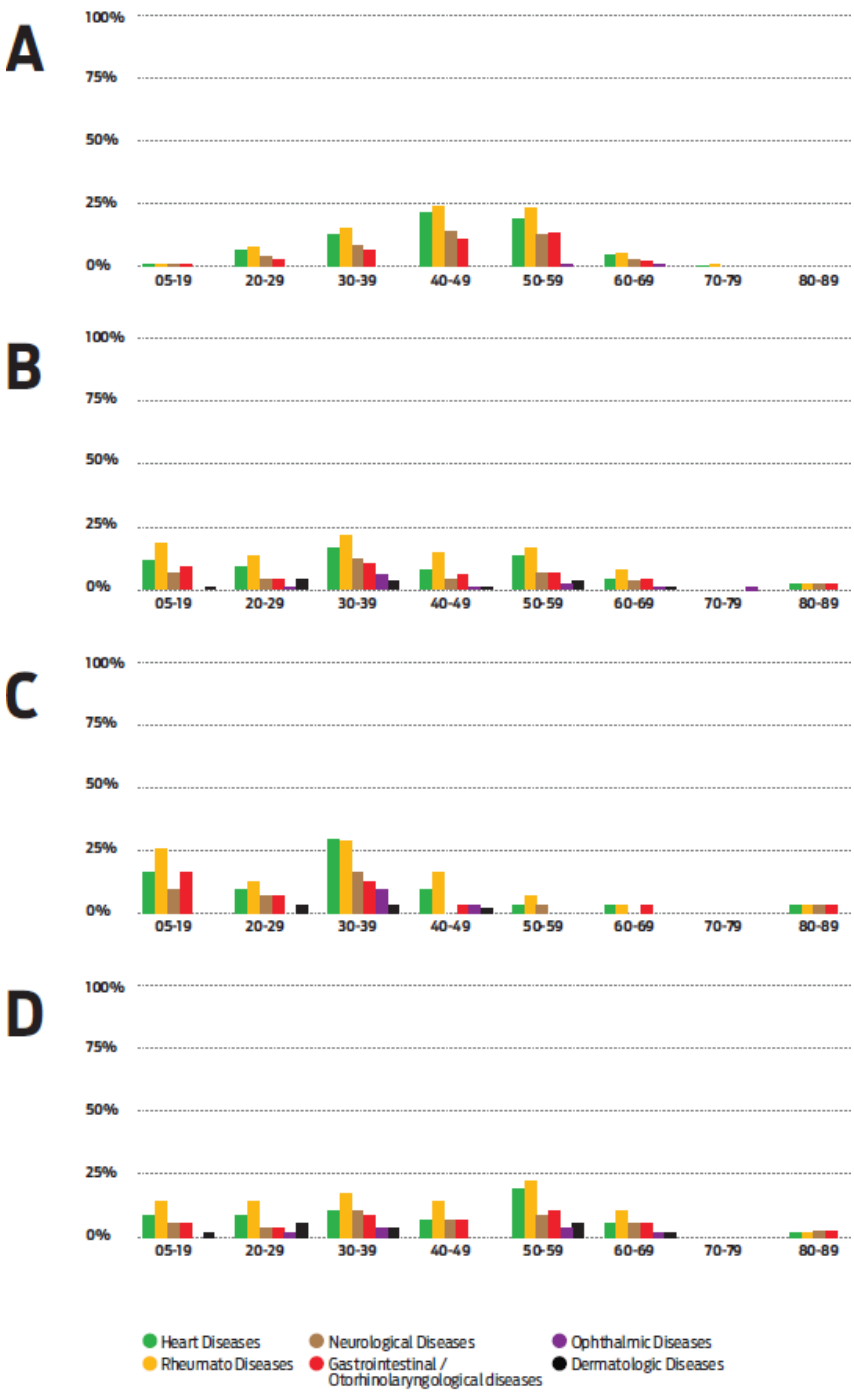

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Figure 6. Frequency of FD symptoms by gender, age group, and FD diagnosis. **A)** FD-negative; **B)** FD-positive; **C)** FD-positive male; **D)** FD-positive female.

3.4 Frequency of underlying causes of CKD

HBP (80.1%) and DM (33.3%) were highly frequent underlying causes of CKD in FD-negative patients (Figure 7), followed by obesity (9.6%), rheumatoid arthritis (9.2%), and polycystic kidney disease (6.7%). However, underlying causes of CKD were way less frequent (13.4%) in FD-positive patients (Figure 7). HBP was the most frequent (10.1%) followed by DM, obesity and rheumatoid arthritis (1.1% each). There were no cases of Polycystic Kidney or Berger's Disease in FD-positive patients.

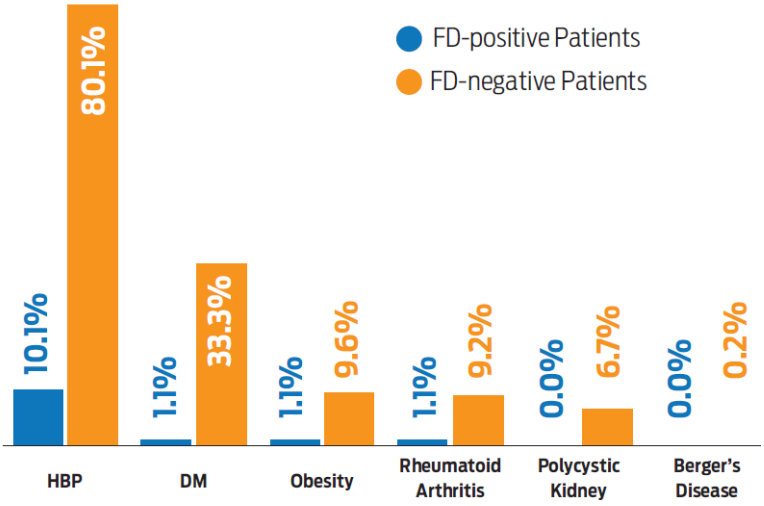

Figure 7. Frequency of underlying causes of CKD in FD-positive and negative patients.

Most of the FD-negative patients with HBP were males (59.9%; Figure 8B). In FD-positive individuals only a small part (9; 10.1%; Figure 7) of the patients had HBP, most of them were female (52.1%) (Figure 8A).

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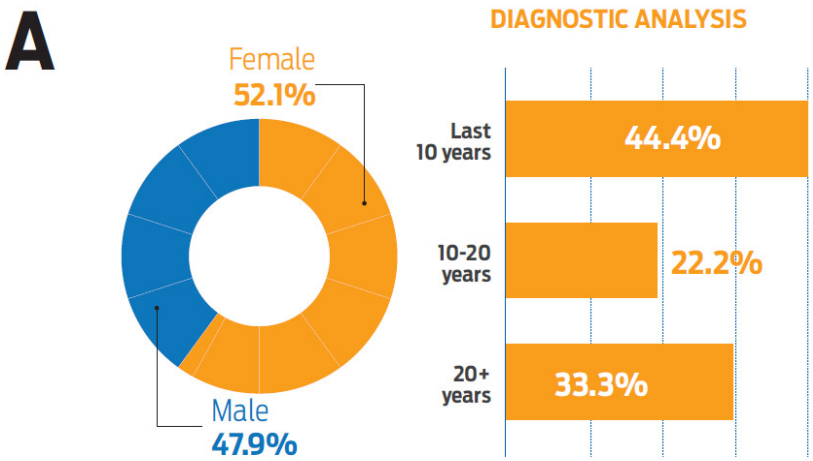

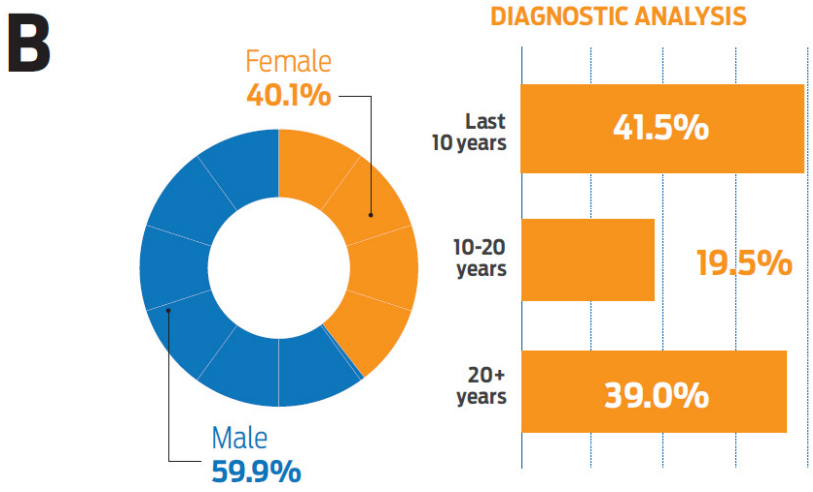

Figure 8. Diagnosis time and distribution of HBP by gender in FD patients. **A)** FD-positive; **B)** FD-negative.

HBP in FD-negative patients was observed in all age groups, in about the same proportion in both sexes (Figure 9A), and starts to become more common from 40y. In FD-positive female patients, the number of individuals with HBP increased significantly from 30y (Figure 9B) while in FD-positive men the number of individuals with HBP was quite high in the age groups of 20-29y and above 69y.

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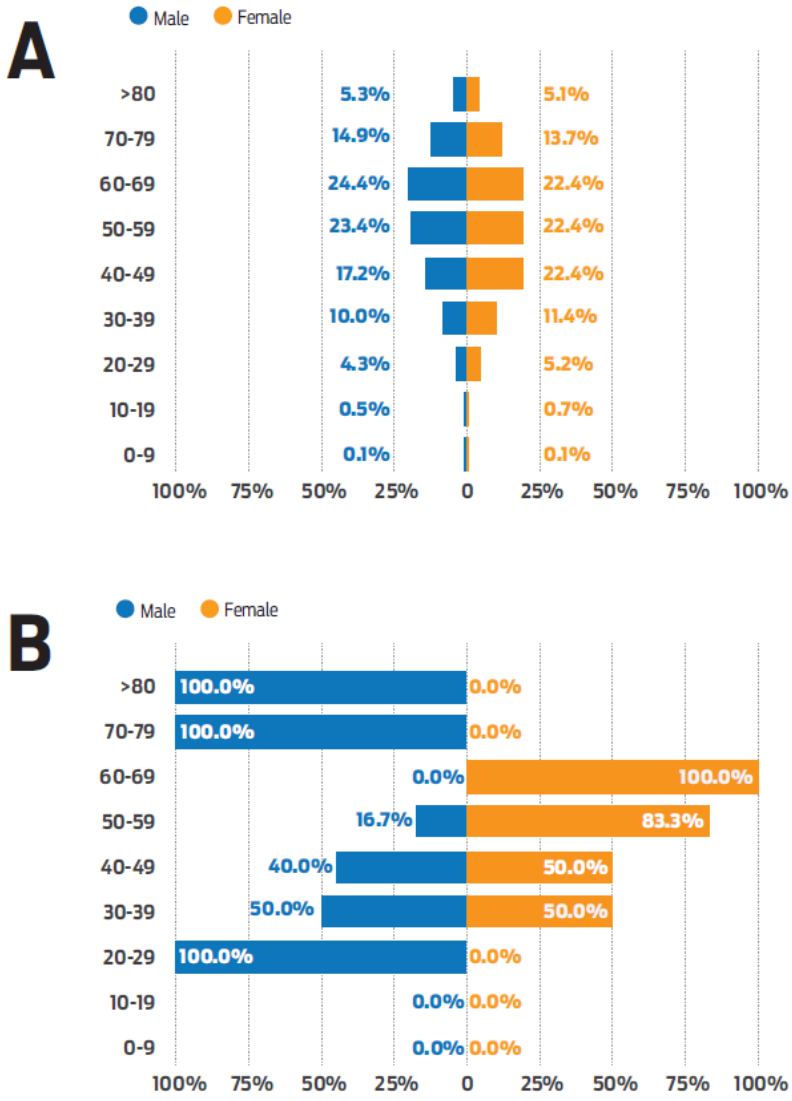

Figure 9. Frequency of HBP by gender and age group in FD patients. **A)** FD-negative; **B)** FD-positive.

In FD-negative patients, DM is observed in both sexes and in all age groups, but it starts to become more common above 40-49y. However, the number of FD-positive patients with DM was very small (one female).

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The distribution of underlying causes of CKD according to age group revealed that the highest prevalence occurs between 40-49y for FD-positive patients and between 50-69y for negative patients; FD-positive patients showed higher frequency before 50y (81.8% *versus* 48.4% in FD-negative patients), (*P* (Fisher) = 0.03). Similar findings occurred with HBP (88.9% versus 33.8%; *P* (Fisher) = 0.001). Comparing FD-negative and positive patients regarding to HBP, FD-negative patients showed higher frequencies (χ^2 = 218.15; *P* < 0.0001). The small number of FD-positive patients with DM, Obesity and Rheumatoid Arthritis prevented this kind of analysis.

4. DISCUSSION

As far as we know, this is the first study with such a large sample carried out in a continental multiethnic country like Brazil, in order to identify FD patients among dialysis patients enrolled in dialysis centers from all over the country. FD has a vast phenotypic spectrum, lacking a clear genotype-phenotype correlation [4,7,13]. Thus, an algorithm based on combinations of signs and symptoms, age and gender (Figure 4, Table 2) was proposed and used to screen for FD-suspected patients in a population of 25,223 dialysis patients. In this study, this strategy allowed to reduce to 88.3% the number of dialysis patients for FD enzymatic and genetic testing. The prevalence of 0.3% (0.2% female, 0.1% male) is in line with previous studies carried out in hemodialysis centers. Porsch *et al.* (2008) [25] evaluated 558 southern Brazilian male patients with ESRD and only two had low α-Gal A activity and were diagnosed with FD (0.36%). Other FD screening studies performed in Brazilian patients with CKD undergoing hemodialysis in Paraná and in patients on dialysis treatment in Rio de Janeiro found a prevalence of 0.24% [26,27]. In Latin American countries, FD prevalence in Peru was 0.3% and in Colombia, 0.4% [28,29]. A review encompassing all screening studies [16] showed 55 patients (44 males/11 females) detected in a total of 18,837 hemodialysis patients; mean prevalence of 0.29% (0.23% female/0.06% male).

The decline in the life expectancy of FD patients [12] not associated to underlying causes of

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CKD (Figures 7,9), reinforces previous findings that FD is a devastating disorder [2,3,12]. Life expectancy of male FD patients, according to The United States Fabry Registry (58.2y male) [12] was diminished to about a quarter of the average in the Brazilian general population (74.9y) [30]. Lack of diagnosis may have contributed to the early deaths due to FD in Brazilian dialysis patients, since FD progresses quickly and kidney failure may occur by the third or fourth decade of life [2,12].

Rheumatologic symptoms were the most frequent in general (Figure 6A,6B). However, the frequency was much higher in FD-positives as these symptoms are common in FD since childhood [2,3,31]. Three mutations (c.1102G>A [A368T], c.325C>T [R118C] and c.870G>C [M290I]) were highly frequent in FD-positive patients (Table 3). However, no mutation previously confirmed with manifestation confined to the kidney or heart [15,32] was found in this study.

Heart diseases were the second most prevalent amongst FD-positive patients. Mutation c.352C>T (R118C) was found in 11 of these patients with heart disease. This same mutation was previously found in Italian male neonates [33]. It was frequent in unrelated hemodialysis patients in Spain [34] and in young Portuguese patients with stroke [35]. Historically, Brazil has received large numbers of Italian, Spanish, and Portuguese immigrants. The country itself was a colony of Portugal which may explain the high frequency of this mutation among Brazilian FD patients. This mutation has been described in Brazilian families suspected of FD [36]. R118C is considered by HMGD a disease causing mutation since it has been found in young adults with stroke, in a patient with apical left ventricular hypertrophy, and may be a cardiomyopathy phenotype modifier thought not to cause classic FD phenotype in a Medelian fashion [35,37-39].

Depression was the third most frequent symptom amongst FD-positive patients and mutations c.1102G>A (A368T) and c.352C>T (R118C) were frequent. Dementia, cognitive impairment, and depression occur in patients with FD [40,41]. However, additional studies are needed to establish a direct link of these morbidities to FD.

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289 An analysis of a large cohort of 2,446 patients in the Fabry Registry (Fabryregistry.com)
290 reported that stroke occurs in 6.9% of men and 4.3% of women [42]. Six female patients had
291 cerebrovascular disease, four had c.1102G>A (A368T) mutation, making it highly prevalent;
292 mutation that was previously reported in Brazilian hemodialysis patients [43,44].
293 Nevertheless, it is not considered a disease causing mutation by HMGD [4]. Despite of being
294 considered a mutation that is associated with cerebrovascular disease, slight decrease of
295 Alpha-galactosidase A activity, normal lyso-Gb3 and less severe typical signs and symptoms
296 of FD, c.427G>A (A143T) mutation was not found in our patients. It seems to be most likely
297 a neutral variant or a possible modifier instead of a disease-causing mutation [45,46].
298 Angiokeratoma, a classic sign of FD was frequent among FD-positive patients (14.6%). It's not
299 directly related to kidney failure [2]. A previous study carried out in Brazil found 6.7% of FD
300 patients after reviewing angiokeratomas' biopsies [47]. Another Brazilian study about FD
301 patients' registry identified angiokeratomas in 8.7% of FD patients [48]. Despite of different
302 methodologies, the percentage of FD patients with angiokeratomas in this study was higher.
303 Six of the patients with angiokeratoma had M290I mutation that was originally identified as
304 causing FD classic phenotype in 66 unrelated families [49]. On the other hand, cornea
305 verticillata, the main ocular finding in FD [50], was present in only 11.2% of the patients. Its
306 prevalence in FD ranges from 44% to 94.5% in men and 88.0% in women [51]. The striking
307 low incidence found in this study may be explained due to the need of a more specific
308 evaluation by an ophthalmologist for a more precise diagnosis.
309 Although the intronic mutations found in this study (Table 3) have been found in FD patients
310 before [11,24], their effects on the *GLA* gene expression or in alpha-galactosidase protein
311 remain unknown.
312 HBP and DM were highly prevalent in FD-negative patients while FD-positive patients
313 presented much less underlying causes of CKD (Figure 7). These results reinforce previous
314 findings [52] that FD is the main cause of kidney failure in FD patients.
315 The algorithm (Figure 4, Table 2) used in the present study to track FD-suspected allowed to

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316 reduce significantly (by 88.3%) the number of dialysis patients for genetic and enzymatic
317 testing. The natural history and frequency of FD among dialysis patients in this study were in
318 line with literature. This indicates the algorithm could be a helpful tool in screening studies
319 set to identify FD patients among large numbers of dialysis patients.

320 **5. CONCLUSION**

321 The initial findings of this large long-term study ongoing in Brazil emphasize the importance
322 of early diagnosis in order to detect and treat FD before it may causes irreversible renal,
323 cardiac, and/or neurologic damages. Although the algorithm used for screening of FD-
324 suspected patients would have been a useful tool, it still needs to be statistically validated
325 (sensitivity, specificity, predictive value). The encouraging results obtained from this first 18
326 months abet us to move forward with this country-wide study since it will allow us to have a
327 better understanding of FD natural history in Brazil. More importantly, it will contribute to the
328 development of an optimized diagnosis strategy which can save resources from public health
329 system and provide early disease identification for an appropriate timely treatment.

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335 data and in the writing of the manuscript.

336 **COMPETING INTERESTS**

337 The authors declare that they have no competing interests.

338 **AUTHORS' CONTRIBUTIONS**

339 MPC: Literature search, study design, data collection, data analysis, data interpretation,
340 figures, tables, writing, critical review, final version approval; OMVN: Literature search, data
341 collection, data interpretation, critical review, final version approval; JCBA: data analysis,
342 data interpretation, figures, final version approval; TMS: Writing, literature search, data

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analysis, data interpretation, figures and tables preparation, critical review, references, final version approval; JEPL: Literature search, data collection, data interpretation, critical review, final version approval; LRB: Literature search, data collection, final version approval; MGR: Literature search, study design, data collection, data analysis, data interpretation, writing, critical review, final version approval.

CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this study. The patients invited to participate were informed about the study purposes and each subject freely signed an individual consent form agreeing to participate in the study. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

The study protocol was approved by the Research Ethics Committee of Campos Medical School (legal opinion #305·988; 06/28/2013). Patients and the health care team involved had their anonymity fully preserved according to Resolution no. 196/96 of the National Board of Health, 1988 Medical Ethics Code and 1964 Declaration of Helsinki.

REFERENCES

1. van der Tol L, Smid BE, Poorthuis BJHM, Biegstraaten M, Lekanne Deprez RH, Linthorst GE, et al. A systematic review on screening for Fabry disease: prevalence of individuals with genetic variants of unknown significance. J. Med. Genet. [Internet]. 2013;51:1–9.

2. Germain DP. Fabry disease. Orphanet J. Rare Dis. [Internet]. BioMed Central Ltd; 2010;5:1–49.

3. Zarate YA, Hopkin RJ. Fabry's disease. Lancet[Internet]. Elsevier Ltd; 2008;372:1427–35.

E-mail address: mcoutinho@datagenno.com

4. Stenson PD, Mort M, Ball E V, Shaw K, Phillips AD, Cooper DN. The Human Gene Mutation Database: Building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. Hum.Genet. 2014;133:1–9.

5. Desnick RJ, Brady R, Barranger J, Collins AJ, Germain DP, Goldman M, et al. Fabry disease, an under-recognized multisystemic disorder: Expert recommendations for diagnosis, management, and enzyme replacement therapy. Ann. Intern. Med. [Internet]. 2003;138:338–46. Available from: http://annals.org/article.aspx?doi=10.7326/0003-4819-138-4-200302180-00014.

6. Sestito S, Ceravolo F, Concolino D. Anderson-Fabry disease in children. Curr Pharm Des. 2013;19(33):6037-45.

7. Laney DA, Bennett RL, Clarke V, Fox A, Hopkin RJ, Johnson J, et al. Fabry disease practice guidelines: Recommendations of the national society of genetic counselors. J. Genet. Couns. 2013;22:555–64.

8. Rigoldi M, Concolino D, Morrone A, Pieruzzi F, Ravaglia R, Furlan F, Santus F, Strisciuglio P, Torti G, Parini R. Intrafamilial phenotypic variability in four families with Anderson-Fabry disease. Clin Genet. 2014 Sep;86(3):258-63.

9. Deegan PB, Baehner AF, Barba Romero M, Hughes DA, Kampmann C, Beck M. Natural history of Fabry disease in females in the Fabry Outcome Survey. J. Med. Genet. 2006;43:347–52.

10. Wilcox WR, Oliveira JP, Hopkin RJ, Ortiz A, Banikazemi M, Feldt-Rasmussen U, et al. Females with Fabry disease frequently have major organ involvement: Lessons from the Fabry Registry. Mol. Genet. Metab. 2008;93:112–28.

11. Echevarria L, Benistan K, Toussaint A, Dubourg O, Hagege AA, Eladari D, et al. X-chromosome inactivation in female patients with Fabry disease. Clin. Genet. 2016;89:44–54.

12. Waldek S, Patel MR, Banikazemi M, Lemay R, Lee P. Life expectancy and cause of death in males and females with Fabry disease: Findings from the Fabry Registry. Genet. Med. [Internet]. 2009;11:790–6.

E-mail address: mcoutinho@datagenno.com

13. Linthorst GE, Bouwman MG, Wijburg FA, Aerts JMFG, Poorthuis BJHM, Hollak CEM. Screening for Fabry disease in high-risk populations: a systematic review. J. Med. Genet. 2010;47:217–22.

14. Rombach SM, Smid BE, Linthorst GE, Dijkgraaf MGW, Hollak CEM. Natural course of Fabry disease and the effectiveness of enzyme replacement therapy: A systematic review and meta-analysis: Effectiveness of ERT in different disease stages. J. Inherit. Metab. Dis. 2014;37:341–52.

15. Nakao S, Kodama C, Takenaka T, Tanaka A, Yasumoto Y, Yoshida A, et al. Fabry disease: Detection of undiagnosed hemodialysis patients and identification of a “renal variant” phenotype. Kidney Int. 2003;64:801–7.

16. Okur I, Ezgu F, Biberoglu G, Tumer L, Erten Y, Isitman M, et al. Screening for Fabry disease in patients undergoing dialysis for chronic renal failure in Turkey: Identification of new case with novel mutation. Gene [Internet]. Elsevier B.V.; 2013;527:42–7.

17. Brasil - Ministério da Saúde. 2014. Saúde amplia tratamento para doentes renais crônicos. http://www.brasil.gov.br/saude/2014/03/saude-amplia-tratamento-para-doentes-renais-cronicos. Last access: March/2017.

18. Gutiérrez-Amavizca BE, Orozco-Castellanos R, R. Padilla-Gutiérrez J, Valle Y, Figuera LE. Pedigree analysis of Mexican families with Fabry disease as a powerful tool for identification of heterozygous females. Genet. Mol. Res. [Internet]. 2014;13:6752–8.

19. Snyder S, Pendergraph B. Detection and evaluation of chronic kidney disease. Am. Fam. Physician. 2005;72:1723–34.

20. Levey AS, Coresh J. Chronic kidney disease. Lancet [Internet]. Elsevier Ltd; 2012;379:165–80.

21. Costa F, Costa F, Foly L, Coutinho M. DataGenno: building a new tool to bridge molecular and clinical genetics. Appl. Clin. Genet. 2011;4:45–54.

22. Fokkema IFAC, Taschner PEM, Schaafsma GCP, Celli J, Laros JFJ, den Dunnen JT. LOVD v.2.0: The next generation in gene variant databases. Hum. Mutat. 2011;32:557–63.

E-mail address: mcoutinho@datagenno.com

422 23. http://www.genomed.org/lovd2/home.php?select_db=GLA. Last access: March/2017.
423 24. Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, et al. ClinVar:
424 Public archive of relationships among sequence variation and human phenotype. Nucleic
425 Acids Res. 2014;42:980–5.
426 25. Porsch DB, Nunes ACF, Milani V, Rossato LB, Mattos CB, Tsao M, et al. Fabry disease
427 in hemodialysis patients in southern Brazil: prevalence study and clinical report. Ren. Fail.
428 2008;30:825–30.
429 26. Biagini G *et al*. Prevalence of Fabry disease in patients with chronic renal insufficiency
430 undergoing hemodialysis in the State of Paraná—Brazil. World Congress of Nephrology,
431 abstract, 2007.
432 27. Delgado V *et al*. Fabry disease epidemiology in Rio de Janeiro State: Partial outcome of
433 screening in patients on dialysis treatment. World Congress of Nephrology, abstract, 2007.
434 28. Prevalence in Peru of Fabry disease among males with chronic renal insufficiency. X
435 Latin American Symposium in Lysosomal Storage Disease, abstract, 2005.
436 29. Martínez JA, Ardila ME, Gamarra G, *et al*. Prevalence of Fabry disease in Colombia—
437 preliminary report. Book of Abstracts. San Jose, Costa Rica: X Latin American Symposium
438 in Lysosomal Storage Disease 2005; 237.
439 30.http://www.ibge.gov.br/home/estatistica/populacao/tabuadevida/2013/defaulttab_pdf.shtm
440 Last access: July/2016.
441 31. Ellaway C. Paediatric Fabry disease. Transl Pediatr. 2016;5:37–42.
442 32. Nakao S, Takenaka T, Maeda M, Kodama C, Tanaka A, Tahara M, et al. An atypical
443 variant of Fabry's disease in men with left ventricular hypertrophy. N. Engl. J. Med.
444 1995;333:288–93.
445 33. Spada M, Pagliardini S, Yasuda M, Tukel T, Thiagarajan G, Sakuraba H, et al. High
446 incidence of later-onset fabry disease revealed by newborn screening. Am. J. Hum. Genet.
447 [Internet]. 2006;79:31–40.

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448 34. Gaspar P, Herrera J, Rodrigues D, Cerezo S, Delgado R, Andrade CF, et al. Frequency
449 of Fabry disease in male and female haemodialysis patients in Spain. BMC Med. Genet.
450 [Internet]. 2010;11:19.
451 35. Baptista MV, Ferreira S, Pinho-E-Melo T, Carvalho M, Cruz VT, Carmona C, et al.
452 Mutations of the GLA Gene in Young Patients with Stroke: The PORTYSTROKE Study-
453 Screening Genetic Conditions in PORTuguese Young STROKE Patients. Stroke.
454 2010;41:431–6.
455 36. Turaça LT, Pessoa JG, Motta FL, Muñoz Rojas MV, Müller KB, Lourenço CM, et al. New
456 mutations in the GLA gene in Brazilian families with Fabry disease. J. Hum. Genet.
457 [Internet]. 2012;57:347–51.
458 37. Caetano F, Botelho A, Mota P, Silva J, Marques AL. Fabry disease presenting as apical
459 left ventricular hypertrophy in a patient carrying the missense mutation R118C. Rev. Port.
460 Cardiol. [Internet]. Sociedade Portuguesa de Cardiologia; 2014;33:183.e1–183.e5.
461 38. Golbus JR, Puckelwartz MJ, Dellefave-Castillo L, Fahrenbach JP, Nelakuditi V, Pesce
462 LL, et al. Targeted analysis of whole genome sequence data to diagnose genetic
463 cardiomyopathy. Circ. Cardiovasc. Genet. 2014;7:751–9.
464 39. Ferreira S, Ortiz A, Germain DP, Viana-Baptista M, Caldeira-Gomes A, Camprecios M,
465 et al. The alpha-galactosidase A p.Arg118Cys variant does not cause a Fabry disease
466 phenotype: Data from individual patients and family studies. Mol. Genet. Metab. [Internet].
467 Elsevier Inc.; 2015;114:248–58.
468 40. Schermuly I, Müller MJ, Müller KM, Albrecht J, Keller I, Yakushev I, et al.
469 Neuropsychiatric symptoms and brain structural alterations in Fabry disease. Eur. J. Neurol.
470 2011;18:347–53.
471 41. Cole AL, Lee PJ, Hughes DA, Deegan PB, Waldek S, Lachmann RH. Depression in
472 adults with Fabry disease: A common and under-diagnosed problem. J. Inherit. Metab. Dis.
473 2007;30:943–51.

E-mail address: mcoutinho@datagenno.com

42. Sims K, Politei J, Banikazemi M, Lee P. Stroke in Fabry disease frequently occurs before diagnosis and in the absence of other clinical events: Natural history data from the Fabry Registry. Stroke. 2009;40:788–94.

43. Pereira EM, do Monte SJH, do Nascimento FF, de Castro JAF, Sousa JLM, Filho HCSALC, et al. Lysosome-associated protein 1 (LAMP-1) and Lysosome-associated protein 2 (LAMP-2) in a larger family carrier of Fabry disease. Gene [Internet]. Elsevier B.V.; 2014;536:118–22.

44. Silva, C. A. B., Barreto, F. C., dos Reis, M. A., Junior, J. A. M., & Cruz, C. M. S. (2016, May). Family Screening for Fabry Disease based on Male Index Cases with End-Stage Renal Disease. In *Nephrology Dialysis Transplantation* (Vol. 31, pp. 357-357). GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND: OXFORD UNIV PRESS.

45. Brouns R, Thijs V, Eyskens F, Van Den Broeck M, Belachew S, Van Broeckhoven C, et al. Belgian Fabry study: Prevalence of Fabry disease in a cohort of 1000 young patients with cerebrovascular disease. Stroke. 2010;41:863–8.

46. Rolfs A, Fazekas F, Grittner U, Dichgans M, Martus P, Holzhausen M, et al. Acute cerebrovascular disease in the young: The stroke in young Fabry patients study. Stroke. 2013;44:340–9.

47. Kelmann SV, Quaio CRDC, Honjo RS, Bertola DR, Rosa Neto NS, Lourenço CM, et al. Multicentric study on the diagnosis of Fabry's disease using angiokeratoma biopsy registries. Int. J. Dermatol. [Internet]. 2014;54:1–3.

48. Martins AM, Kyosen SO, Garrote J, Marques FM V, Guilhem JG, Macedo E, et al. Demographic characterization of Brazilian patients enrolled in the Fabry Registry. Genet. Mol. Res. 2013;12:136–42.

49. Shabbeer J, Yasuda M, Benson SD, Desnick RJ. Fabry disease: identification of 50 novel alpha-galactosidase A mutations causing the classic phenotype and three-dimensional structural analysis of 29 missense mutations. Hum. Genomics. 2006;2:297–309.

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50. Pitz S, Kalkum G, Arash L, Karabul N, Sodi A, Larroque S, et al. Ocular Signs Correlate Well with Disease Severity and Genotype in Fabry Disease. PLoS One [Internet]. 2015;10:1–13.

51. Nguyen TT, Gin T, Nicholls K, Low M, Galanos J, Crawford A. Ophthalmological manifestations of Fabry disease: a survey of patients at the Royal Melbourne Fabry Disease Treatment Centre. Clin. Experiment. Ophthalmol. [Internet]. 2005;33:164–8.

52. Branton M, Schiffmann R, Kopp JB. Natural history and treatment of renal involvement in Fabry disease. J. Am. Soc. Nephrol. 2002;13 Suppl 2:S139–43.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

α-Gal A: Alpha-galactosidase A

CKD: chronic kidney disease

DM: diabetes mellitus

ESRD: end-stage renal disease

FD: Fabry disease

Gb3/GL3: globotriaosylceramide

GLA: galactosidase, alpha gene

HBP: high blood pressure

HGMD: Human Gene Mutation Database

LOVD: Leiden Open-source Variation Database

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