Frequency of Fabry disease in a Juvenile Idiopathic Arthritis Cohort

Luciana Paim-Marques
University of Florida
Amanda Virginia Cavalcante
Escola de Saude Publica do Ceara
Islane Verçosa
Hospital Geral de Fortaleza
Paula Carneiro
Hospital Geral de Fortaleza
Marcia Souto-Maior
Universidade de Fortaleza
Erlane Marques
Hospital Infantil Albert Sabin
Simone Appenzeller (appenzellersimone@gmail.com)
Universidade Estadual de Campinas Hospital de Clinicas https://orcid.org/0000-0001-5075-4474

Research article

Keywords: Fabry disease, Juvenile arthritis, chronic arthritis, alpha-galactosidase A, GLA variants

Posted Date: October 14th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-60936/v2

Abstract

Background: Fabry disease (FD) is a rare, X-linked, multisystemic lysosomal storage disorder (LSD) that results from a deficiency in the hydrolase alpha-galactosidase A (-GalA). During childhood, classic FD symptomatology is rare. The majority of children may show non-specific symptoms, including in the musculoskeletal system. The prevalence of FD among juvenile idiopathic arthritis (JIA) patients is unknown.

Objective: The aim of this study was to identify the frequency of FD in a JIA cohort, characterizing early clinical symptoms, enzyme titers and *GLA* genotyping.

Methods: Children with JIA followed in a tertiary Children Hospital cohort were selected. Clinical, laboratorial, and familiar information were recorded. Molecular genetic testing to detect *GLA* gene mutations was performed in girls and enzymatic analysis in boys.

Results: In 89 patients (56.2% female, age at disease onset: 8.93 ± 4.35 years), one male (1.12%) patient presented pathogenic mutation in *GLA* gene, *c.1244T>C p.L415P*, one female patient had a variant of uncertain significance *c.38C>T (p.Ala13Val).* The enzymatic activity of alpha galactosidase was slightly decreased in 3 additional (3.4%) patients. We observed the presence of intronic variants in 44.44% patients in our cohort: *c.1000-22C> T*; *c.370-81_-77del*; *c.640-16A> G*; *c.10C>T*; *c.548-125C> G* and *c.-12G> A.* These variants and their combination were associated with clinical symptoms in our cohort.

Conclusions: The incidence of FD in our cohort was 1.12%. Intronic variants were associated with symptoms previously described in literature. Screening for FD in JIA may be a reasonable strategy for those with atypical pattern of pain.

Background

Fabry disease (FD) is a rare, X-linked, multisystemic lysosomal storage disorder (LSD) that results from a deficiency in the hydrolase alpha-galactosidase A (-GalA) caused by a *GLA* gene mutation. Its birth prevalence is estimated at 1:40.000-170.000 (1). Recently, a neonatal screening has found a higher incidence of FD: 1:3.100 in Italy and 1:1.500 among males in Taiwan (2,3).

FD, a recessive X-linked disease, affects predominately male patients. Female carrier may present milder symptoms if X inactivation is present. These differences influence the diagnostic methods, clinical signs and life expectancies. Male patients can be screened with enzyme dosage, while female patients should have genetic test done directly.

The -GalA deficiency in patient lysosomes with FD causes a progressive accumulation of the glycosphingolipid globotriaosylceramide (Gb3) in cells of many organ systems resulting in a chronic inflammatory process. (4). FD should be suspected in individuals presenting acroparesthesias or other classic manifestations such as angiokeratomas, gastrointestinal symptoms, exercise intolerance, ocular abnormalities (*cornea verticillata*), decreased sweating, renal and cardiac involvement. Central nervous system presentation may include transient ischemic attacks and strokes predominantly in the vertebrobasilar system. However, in early childhood, FD may present with mild non-specific symptoms affecting frequently the musculoskeletal system. Peripheral neuropathic pain, fever, arthritis, and elevated erythrocyte sedimentation rate (ESR) can be observed (5-8).

High disease suspicion is necessary at early stages of the disease and screening in high-risk patients is a cost-effective strategy for identifying FD patients (10). Since musculoskeletal features are frequently observed in FD, and juvenile idiopathic arthritis (JIA) is the most frequent chronic, inflammatory arthritis disorder observed in childhood, this study aimed to identify the frequency of FD in a JIA cohort by characterizing early clinical symptoms, enzyme titers and *GLA* genotyping.

Materials And Methods

Consecutive JIA patients, classified according to ILAR criteria (9) followed in the pediatric rheumatology outpatient clinic at Albert Sabin Children's Hospital were invited to participate in this cross-sectional study from December 2014 to December 2017.

The local ethics committee approved this study (Albert Sabin Childhood Hospital, Fortaleza, Ceará, Brazil, **CAAE:** 37270414.0.0000.5042), and all patients, and their legal representatives if children under 18, have signed the informed consent and assent form.

JIA history

The following demographic and disease characteristics were obtained through a careful chart review for each patient such as age, sex, age at disease-onset, JIA subtype, articular, and extra-articular manifestations. Immunological features [rheumatoid factor (RF) by latex agglutination test, antinuclear antibodies (ANA) by indirect immunofluorescence assay (IIFA) on Human epithelial type 2 (HEp-2 cells), anti- ds DNA, anti-Smith, anti-RNP, anti-SSa, anti-SSb, anti-cardiolipin antibodies by enzyme-linked immunosorbent assay (ELISA), and human leukocyte antigen (HLA) B27 by polymerase chain reaction (PCR)] were retrieved from medical charts.

Study questionnaire

JIA patients were inquired about FD features through a structured questionnaire based on early signs and symptoms applied by the treating physician (11). This questionnaire contained queries about clinical symptoms (heat intolerance, hypo/hyperhidrosis, chronic fatigue, abdominal distension, dyspepsia, diarrhea, gastric fullness sensation, weight gain difficulty, tinnitus, dysacusis, acroparesthesia), physical exam findings (telangiectasia, angiokeratoma), past medical history (stroke and transient ischemic attack), and family history (stroke, transient stroke, sudden death, end-stage renal or FD).

Clinical evaluation

All patients were evaluated by a board-certified pediatric rheumatologist who performed a complete clinical, osteoarticular and neurological exam performed at study entry.

JIA patients underwent a complete ophthalmological examination by a board-certified pediatric ophthalmologist and the positive findings were reviewed by a second board-certified ophthalmologist. Refractive errors were measured by a hand-held auto-refractor keratometer retinomax K plus 2. The best-corrected visual acuity of each eye was determined by Snellen letters and numbers chart, measured at a distance of 6 meters for children above 6 years old. Non-literate children's visual acuity was evaluated by the Snellen E and Snellen number chart at an appropriate distance. Anterior segment (cornea, iris, and lens crystalline) was evaluated by slit-lamp examination. The optic nerve, macula, and posterior pole vessels were analyzed with direct ophthalmoscopy. Intraocular

pressure (IOP) was measured by applanation with the Perkins tonometer method and was considered normal if ranged from 10-20 mmHg. A tear breakup time (TBUT) test was performed after placing a drop of fluorescein in the cul-de-sac in order to determine keratoconjunctivitis. The presence of *cornea verticillata* was evaluated by a board-certified pediatric ophthalmologist during complete ophthalmological evaluation.

The patients were also evaluated through a transthoracic 2D echocardiogram (Echo), and conduction disturbance was evaluated through 12 channels electrocardiogram (ECG) analyzed by a board-certified pediatric cardiologist.

Kidney involvement was evaluated through serum creatinine, 24-hour urinary microalbuminuria, and urinary sodium.

Genetic testing

The genetic test was sponsored by Shire and carried out in the outpatient clinic by a trained nurse. Blood was drawn after patients and legal representatives re-authorization and placed at 5 blood spots on filter paper duly identified with the patient's, doctor's and nurse's data.

For males, an initial screening of the α-GalA enzyme and the acidic sphingomyelinase (control) enzyme activity and measurement of globotriaosylsphingosine (lyso-Gb3) by high-performance liquid chromatography (HPLC) was performed by tandem mass spectrometry at Centogene (Germany).

For women or male patients with abnormal enzyme activity, the analysis of the *GLA* gene (ref: NM_000169.2) was conducted by PCR and sequencing of the entire coding region and the highly conserved exon-intron splice junctions. This test has been developed and validated by Centogene AG for clinical purposes. Patients with genetic abnormalities or α-GalA enzyme below normal values had their first and second-degree relatives (parents, grandparents, and siblings), when possible, screened for FD with appropriate genetic investigation (enzyme levels or gene identification), and referred to genetic monitoring.

Statistical Analyses

All statistical analysis were performed using SPSS 20.0 software package. Results are shown in absolute number and percentage or mean and standard deviation (SD). Chi-square or Fischer exact test was used to compare categorical variables. The continuous variables were compared by analysis of variance (ANOVA). A p-value ≤ 0.05 was considered clinically significant.

Results

A total of 89 JIA patients (mean age of 15.80 ± 3.95) were included and a predominance of female (56.17%) and pauciarticular JIA subgroup (47.2%) was observed. The mean age of disease onset was 8.93 ± 4.35 years. The subtype classification, clinic, laboratory abnormalities of JIA patients were summarized in Tables 1 and 2. We did not observe significant differences in patient demographics and clinical characteristics among those who were enrolled in the study and those who did not agree to participate (data not shown).

Table 1: Frequency of subtypes, clinical, laboratorial and drugs used in JIA patients:

Features	N (%)
JIA subtypes:	
Pauciarticular	42 (47.20)
Poliarticular RF Negative	17 (19.10)
Enthesitis related	15 (16.90)
Systemic	10 (11.20)
Poliarticular RF Positive	3 (3.36)
Psoriatic	1 (1.12)
Indeterminate	1 (1.12)
Symptoms:	
Acroparesthesia	47 (52.80)
Difficulty gaining weight	30 (33.70)
Heat Intolerance	26 (29.50)
Hyperhidrosis	22 (24.70)
Dyspepsia	19 (21.30)
Tinnitus	18 (20.20)
Peripheric Neuropathy	17 (19.10)
Abdominal distention	14 (15.70)
Chronic fatigue	14 (15.70)
Diarrhea	11 (12.40)
Dysacusis	8 (9.00)
Gastric fullness sensation	6 (6.74)
Angiokeratoma	2 (2.24)
Telangiectasia	00
Family History:	
Stroke	38 (42.70)
Sudden death	22 (24.71)
kidney failure	11 (12.40)
Transient attack	2 (2.24)
Fabry	1 (1.12)
Laboratory:	
ANA	13 (14.60)

Microalbuminuria (66 patients)	9 (12.32)
HLA-B27	6 (6.74)
Rheumatoid Factor	4 (4.49)
Anti-RNP antibody	1 (1.12)

Table 2: Cardiac Abnormalities in JIA Cohort and subtype descriptions - 74 patients – 20 cardiac abnormalities occurrence in 17 (22.90%) patients.

Features	**Total N (%)**	**Presentation x JIA Subtype**
Right bundle branch block	10 (52.63)	4 polyarticular 1 systemic 3 oligoarticular 2 Entesitis-related
Mitral Valvar Prolapse	4 (20)	1 polyarticular 2 oligoarticular
Ventricular Hypertrophy	3 (15.78)	1 polyarticular -VE (conc.) 1 systemic - VD 1 Oligoarticular + FD - VE (conc.)
Valvar regurgitation:	3 (15.78)	Mitral: 1 Oligoarticular + FD Pulmonary: 1 Systemic Tricuspid: 1 Systemic

Legends: Conc: concentric

The genetic tests were performed in all (56.17%) female patients, while enzyme activity was performed in 39 (43.82%) males of our cohort. The results identified 4 (4.49%) males with decreased enzyme activity. For that reason, we had a total of 54 (60.67%) patients with genetic tests done.

One of 89 (1.12%) patients (male, admixture of Caucasian and Native South American (Indian), pauciarticular JIA with positive ANA and RNP antibodies with no previous treatment) presented diminished α-GalA values and abnormal lyso-Gb3 levels. His genetic test showed the *GLA* variant *c.1244T>C p.L415P* (Ref: Serebrinsky, 2006) confirming FD (12). That patient presented hands and feet pain at the age of 5 years old, associated with sporadic low-grade fever, especially after exercising, and significant anhidrosis. At the age of eleven, he reported bilateral ankle pain and swelling. He was referred to Pediatric Rheumatology for evaluation. The chronic ankles arthritis was clinically confirmed, however, the complaint of absence of sweat, fever after activities, burning pain, abdominal pain, some of them uncommon in JIA set of symptoms, raised the hypothesis of FD. His physical exam and work up showed presence of angiokeratomas around the belly bottom, cardiac abnormalities, and *cornea*

verticillata. His pedigree was rich for strokes, heart attack and, transient ischemic attack in family members under the age of 50. This patient also had intronic *GLA* variants, c.370-81_-77del (rs5903184) on intron 2, c.640-16A> G (rs2071397) on intron 4, c.1000-22C> T (rs2071228) on intron 6 e, c.-10C> T (rs2071225) in region 5'UTR exon 1. Once he started the enzyme replacement, his symptoms improved: hypohidrosis, abdominal pain, dyspepsia, heat intolerance, acroparesthesias, and angiokeratomas. However, no changes in the *cornea verticillata* and arthritis was observed. Magnetic resonance imaging of the right ankle showed increased synovial fluid and thickening. Methotrexate (15mg/m^2/week) was added to his therapeutic plan with subsequent resolution of his synovitis.

Another female pauciarticular JIA patient presented a previously unreported heterozygous variant in exon 1 of the *GLA* gene *c.38C>T p.Ala13Val* (No reference). This variant is located in a non-conserved nucleotide, and a frankly conserved amino acid position, with small physical-chemical difference between the amino acid alanine and valine (Alamut v.2.4). Polyphen-2, SIFT and MutationTaster analysis predict this variant as probably benign. The patient also presented the following intronic *GLA* variants, *c.370-81_-77del* (rs5903184) on intron 2, *c.640-16A> G* (rs2071397) on intron 4, *c.1000-22C> T* (rs2071228) at intron 6 e, *c.-10C> T* (rs2071225) in region 5'UTR exon 1. Clinically, *cornea verticillata* was observed on her ophthalmologic exam. Although she denied any other symptoms related to FD, her mother presented an early stroke with a negative genetic test.

A third pauciarticular JIA female presented a variant on *GLA gene* exon 1 heterozygous *c.48T>G* p.Leu16Leu (rs201449986) considered benign due to the change of the same codons, and because it is not located in a splicing sequence. This variant was recently checked in 3 genetic banks: Online Mendelian Inheritance in Man (OMIM), Clinvar and HGNC (HUGO Gene Nomenclature Committee) and is still described as neutral. The statistical association of this variant with FD clinical symptoms, laboratory and *GLA* variants are described in Table 3.

Table 3: Statistic correlation (p value) between genetic variants and CIHs and clinical signs of Fabry disease, significant $p<0.05$.

Symptoms	c.1000-22C> T	c.370-81_-77del	c.640-16 A> G	c.-10 C> T	*c1244 T>C*	*c.38 C>T*	Hap1	Hap2	Hap3
AlfaGal Abn.	0.624	1.000	1.000	0.429	0.073	0.927	1.000	1.000	1.000
GB3 Abn	1.000	0.501	0.501	0.240	0.036	0.964	0.448	0.448	1.000
Heat Intolerance	0.012	0.022	0.022	0.116	0.091	0.091	0.098	0.098	0.325
fatigue	0.756	1.000	1.000	0.531	0.236	0.764	0.719	0.719	1.000
Tinnitus	0.202	1.000	1.000	0.531	0.236	0.764	0.719	0.719	0.234
Dysacusis	0.643	1.000	1.000	0.492	0.909	1.000	1.000	1.000	0.325
Acroparesthesias	0.278	0.775	0.775	0.437	1.000	0.473	0.764	0.764	0.613
Hyperhidrosis	0.222	0.102	0.102	0.624	0.273	0.273	0.493	0.493	1.000
Abdominal Dist	0.745	0.730	0.730	0.519	0.218	0.782	1.000	1.000	1.000
Dyspepsia	1.000	0.478	0.478	0.571	0.200	0.800	0.709	0.709	1.000
Diarrhea	0.718	0.212	0.212	0.733	0.145	0.145	0.405	0.405	1.000
Weight Gain Diff	0.775	0.213	0.213	*0.024*	0.309	0.691	0.322	0.322	1.000
Angiokeratoma	0.436	0.291	0.291	0.127	*0.018*	0.982	0.255	0.255	1.000
Valvar Abn	0.132	0.419	0.419	0.308	0.186	0.814	0.217	0.217	*0.031*
Arrhythmias	1.000	0.602	0.602	0.465	1.000	0.907	0.572	0.572	1.000
Visual Changes	0.073	*0.046*	0.046	*0.005*	0.213	0.787	*0.01*	*0.01*	0.521
Corneal Changes	0.002	*0.003*	*0.003*	*0.012*	0.255	0.401	*0.05*	*0.05*	1.000
Cornea Vertic	0.070	0.208	0.208	0,292	0.064	0.064	1.000	1.000	0.183
Cataract	0.426	1.000	1.000	0.894	1.000	0.979	1.000	1.000	0.064
Ant. Chamb Ch	*0.004*	*0.006*	*0.006*	*0.011*	1.000	0.128	0.164	0.164	0.343
Stroke FH	0.184	0.565	0.565	0.645	0.436	0.436	0.756	0.756	0.307
Trans. attack FH	0.186	0.081	0.081	0.240	*0.036*	*0.001*	0.448	0.448	1.000
Sudden death FH	1.000	0.346	0.346	0.586	0.327	0.673	0.510	0.510	1.000
kidney failure FH	0.686	0.660	0.660	0.423	1.000	0.891	1.000	1.000	0.379
Fabry FH	0.436	0.291	0.291	0.127	*0.018*	0.982	0.255	0.255	1.000

10C>T, c.370-77_-81del, c.640-16A>G, c.1000-22C>T, **Hap 2**- c.370-77_-81del, c.640-16A>G, c.1000

ap 3= c.548-125C>G, c.1000-22C>T, c.-12G>A. Abn: abnormal, Dist: Distention, Diff: difficulty, cornea *rnea verticillata*, Ant. Chamb Ch: Anterior chamber changes, , FH: Family history.

The enzymatic activity of -GalA was slightly decreased in 3 (3.4%) additional patients with a total of four (4,49%) abnormal enzymatic essay. Two patients had history and clinical symptoms suggestive of FD (hypohidrosis, acroparesthesia and dyspepsia, weight gain difficulty, familial history of sudden death and end-stage renal disease). The third one had no symptoms or family history suggestive of FD. All three patients had negative genetic test for FD.

In addition, we identified a total of 18 (22.90%) patients with cardiac abnormalities, 10 (52.63%) patients had right bundle branch block, 4 (20%) presented mitral valve prolapse, 3 (15.78%) had valvar regurgitation. We also observed 3 (15.78%) patients with ventricular hypertrophy; one systemic JIA patient with right ventricular hypertrophy secondary to pulmonary hypertension, one polyarticular JIA patient with left ventricular hypertrophy, and our index patient with a left concentric ventricular hypertrophy.

A total of 54 (60.67%) patients had genetic testing. Twenty-six of 54 (48.14%) had *GLA* variants (intronic and exonic). The overwhelming majority of these patients (92.30%) presented multiple intronic *GLA* variants. 24 (92.30%) of the 26 showed *c.1000-22C> T* (rs2071228) on intron 6, 16 (61.53%) with variant *c.370-81_-77del* (rs5903184) on intron 2, 15 (57.69%) patients with variant *c.640-16A> G* (rs2071397 on intron 4; 8 (30.76%) of them with *c.10C>T* (rs2071225) on 5'UTR exon 1; 7 (26.92%) patients with variant **c.548-125C> G** (rs2071396) on intron 3, and 4 with c.-12G> A (rs3027585) on 5'UTR exon 1.

We also observed presence of complex intronic haplotypes (CIH) in 44.44% of the total tests performed. The intronic variants as well as the CIH had positive correlations with FD symptoms (Table 3). They were grouped as **Haplotype 1**- *c.-10C>T, c.370-77_-81del, c.640-16A>G, c.1000-22C>T* in 8 (14.81%), **Haplotype 2**- *c.370-77_-81del, c.640-16A>G, c.1000-22C>T* in 7 (12.97%), **Haplotype 3**- *c.548-125C>G, c.1000-22C>T, c.-12G>A* in 4 (7.40%), ; **Haplotype 4**- *c.548-125C>G, c.1000-22C>T* in 2 (3.70%), **Haplotype 5**- *c.370-81_-77del, c.548-125C>G, c.640-16A>G, c.1000-22C>T in 1(1,85%)* patient.

Discussion

We observed a frequency of 1.12% of FD in our cohort. There are no epidemiological studies of FD in Brazil, however, the frequency expected for live male births in the general population is 0.0025% (p= 0.0088) (13). In the pediatric population, a Portuguese group studied a cohort of 292 patients with JIA and its association with FD, however, they did not find a classic pathogenic mutation (10).

The ethnicity of the affected child [admixture of Caucasian and Native South American (Indian)] is not included in the populations of higher incidence for FD (2,3).

In this cohort three exonic *GLA* variants were found. The first variant, *c.1244T>C,* was in our index case described as pathogenic and referenced by Serebrinsky et al in 2006 as disease-causing according to ACMG variant classification recommendations (17).

The second variant (*c.38C>T p.Ala13Val*) has not been described before, and it is located in a non-conserved nucleotide and weakly conserved amino acid position, with small physicochemical differences between the amino

acids alanine and valine (Alamut v.2.4). Software analyses by Polyphen-2, SIFT, MutationTaster and Align-GVGD predict this variant as probably benign according to ACMG recommendations of interpretation of sequence variations (17).

The third variant in *GLA* exon 1 *c.48T>G* (rs201449986) was considered likely benign (17). This abnormal sequence is not expected to have clinical significance because it does not alter an amino acid residue and is not located within the splice consensus sequence, according to Sequence Project (http://evs.washington.ed/EVS/;). This allele frequency is 1/6728.

Patients with classic FD have no residual or around 30-35% of α-GalA enzyme activity (14). For diagnosis, an increased levels of Gb3 in lysosomes is required (**15**). The inheritance is X-linked and recessive, which means that female heterozygous genotype presents with incomplete penetrance, due to X inactivation. The mildest disease allows women to have residual enzyme activity; for that reason, the genetic analysis is the gold standard for diagnosis (16). The enzymatic activity can be measured in peripheral blood cells or dry blood spots. In our study, all analysis was done with dry blood spots. The enzymatic activity is very variable among FD patients, and different organs (18). These variations are a challenge to establish thresholds of FD pathogenicity (18).

In this study, we identified 4 (4.5%) patients with decreased levels of α-GalA activity. Three children had a family history and symptoms suggestive of FD. One patient was asymptomatic.

A defective α-GalA leads to accumulation of undegraded substrates [globotriaosylceramide and globotriaosylsphingosine (Gb3 and Lyso Gb3)] inside lysosomes, acting as damage-associated molecular patterns (DAMPs) or stimulating DAMP production. This production activates an inflammatory pathway, inducing apoptosis and a toll-like receptor- 4 (TLR4) mediated innate immune system pro-inflammatory cytokines secretion (IL-1β and TNF-α) (19). These different cellular mechanisms contribute to the different phenotypic expression of FD (20). These cytokines secretion are a characteristic trace of autoinflammatory disorders (21). The recognition of Gb3 or lyso-Gb3 as antigens also influences the invariant natural killer T cell (iNKTs) to induce the release of others inflammatory cytokines such as interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukins (IL): IL-4, IL-5, IL-9, IL10, IL13, and IL-17. This inflammatory cascade produces a continuous stimulus responsible for the induction and maintenance of the autoimmune response. The activation of the above mentioned inflammatory pathway explains the presence of autoimmune and autoinflammatory features in FD. Our index patient had, beyond the classic FD symptoms, a positive ANA and RNP antibodies, and chronic oligoarthritis (bilateral ankles).

The high frequency of cardiac involvement in our cohort (Table 2) is probably related to JIA, which can involve all of the cardiac structures, including pericardium, myocardium, endocardium; coronary vessels; valves and conduction system (22). However, FD also causes cardiac abnormalities including conduction abnormalities, valvular dysfunction, arrhythmias in childhood, progressing to ventricle concentric hypertrophy in non-treated patients (23). Our index patient had mitral valve prolapse with reflux and left ventricle concentric hypertrophy, cardiac manifestations frequently observed in FD.

The main musculoskeletal symptoms described in early FD is acroparesthesia. However, chronic inflammatory joint and bone diseases (polyarticular, oligo and monoarticular, gout, osteoporosis), degenerative joint conditions, neurologic arthropathy (Charcot's foot) (7), Heberden-like nodules (24) and also myositis have been described (8). Nowadays, the coexistence of FD and autoimmune disease has gained increased visibility in

the medical literature, and patients with FD and systemic lupus erythematosus (25,26) rheumatoid arthritis (27), autoimmune hypothyroidism (28), Ig A nephropathy (29) and granulomatosis with polyangiitis (30) have also been described. Patients with FD and rheumatic manifestations have a significant delay in FD diagnosis that can last up to 16 years or more (31). The most common associated mutations observed in FD patients presenting with rheumatic manifestations were R118C and A143T (31).

Other interesting finding in our study was the presence of *GLA* intronic mutations in patients with JIA. Six intronic variants were identified, *c.370-81_-77del*, *c.548-125C>G* and *c.548-162A> T*, *c.640-16A> G*, *c.1000-22C> T*, *c.-10C > T* and *c.-12G> A* located in introns 2, 3, 4, 6 and in the 5'UTR region of *GLA* exon 1, respectively. Three single nucleotide polymorphisms (SNP) [*c.370-81_-77del* (rs5903184), *c.-12G>A* (rs3027585) and *c.-10C> T* (rs2071225)] are relatively common to different ethnic groups, with a frequency of the minor allele about 10% in the British population (32), and 12% in Latin populations (OMIM). These SNP are common to the Portuguese population, the greatest ancestor of the Brazilian citizens (33). Those variants were found in 12 (22.22%) *c.370-81_-77del*, 8 (14.81%) *c.-10C> T* and 4 (7.40%) *c.-12G> A* in the 54 tested patients.

As the previous variants, other SNP and its combination, denominated CIH, have been described as associated with Fabry-similar symptoms.

The two SNP of the α-GalA gene *c.1000-22C> T* [rs2071228] and *c.640-16A> G* (rs2071397) were associated with the presence of angiokeratomas and acroparesthesias in patients with hypertrophic cardiomyopathies without FD (34). In vitro and in vivo analyzes have shown that polymorphisms in the 5'UTR region can alter the expression of the α-GalA gene, with possible clinical relevance, particularly in male patients with *GLA* variants associated with a high reduction in enzyme activity (35). The *c.-10T* allele, found in 15% of the positive results of this study, was previously associated with a decrease in α-GalA activity in leukocytes (33). It has a possible correlation with neurological injuries such as stroke, transient ischemic attack, white matter injury and fine fiber neuropathy, in patients with peripheral neuropathy (36), as well as in patients with FD (35). In our study, this variant was associated with the difficulty of weight gain and ocular changes. Classic ocular manifestations in FD are observed by the age of 4, while heterozygotes present it later, around the age of 10 (37).

The *c.-10T* allele, located in the 5' non-coding region, has been associated with a decrease in the expression of α-GalA (34), altering in the promoter gene the nuclear protein binding site (38). Studies are still needed to determine the real role of this variant in *GLA*. Recent data suggested that reduced enzyme activity, with normal levels, may be a risk factor in Parkinson's disease (39). There are numerous descriptions of the *c.-10T* allele and Fabry-simile manifestations (34).

The most frequent intronic variant in our cohort was *c.1000-22C> T* (rs2071228), observed in 24 (44.44%) of 54 patients. This variant, located in intron 6, is phenotypically associated with FD, and idiopathic hypertrophic cardiomyopathy by the bank Vep Ensembl. This allele is also associated with some CIH that seem to translate enzymatic alteration with the accumulation of glycosphingolipids (38). Haplotypes are a combination of inherited alleles at adjacent *loci*. There are numerous reports of groups of alleles causing Fabry simile changes and FD per se. Gervas-Arruga et al, studied a ICH (*c.-10C> T , c.369 + 990C> A, c.370-81_370-77delCAGCC, c.640-16A> G, c.1000-22C> T*) in the *GLA* gene. They evaluated the enzymatic levels in cells (fibroblasts and leukocytes) in the plasma and the enzyme's quantitative expression. The results suggested an altered expression pattern of the studied gene, without sufficient abnormality of enzyme levels in plasma, leukocytes, and skin fibroblasts to cause

FD. However, glycosphingolipids accumulation in fibroblasts, renal and glomerular tubular cells has been described (38).

Another study described a similar CIH on a *GLA* in a patient with the early systemic onset of FD. The patient carried only the haplotype (*-10C> T, c.370-77_-81del, c.640-16A> G*, *c.1000-22C> T)*, suggesting that those variants located in a promoter and intronic regulatory region, could cause disease even without the presence of exonic abnormalities (40). In our cohort, we had 8 (14.8%) of the 54 tests, that presented this same haplotype (*-10C> T, c.370-77_-81del, c.640-16A> G, c.1000-22C> T*), including the patient with FD and the patient with *c.38C>T variant* who presented *cornea verticillata*. Haplotypes 1 and 2 were associated with visual changes and corneal abnormalities, and haplotype 3 had a positive association with valve changes (table 1).

There was no association of acroparesthesias/peripheral neuropathies with ICHs, despite their incidence in half of our sample. A limited number of genetic tests in our cohort may have influenced the possible positive associations between the variants found and reported clinical signs.

In our JIA cohort we observed a variety of clinical symptoms related to FD. Almost 50% described acroparesthesias, and a third of the patients had difficulty of weight gain, while 42% had family history of stroke. All these features could be associated with chronic arthritis and its treatment. Our index patient was initially treated with enzyme replacement, considering FD was misdiagnosed as JIA. Despite a significant improvement of anhidrosis, muscular, abdominal pain and GB3 levels, he had persistent chronic arthritis course with synovial thickening, suggesting the co-existence of JIA. Methotrexate significantly improved his symptoms. We showed another patient with a VOUS for FD, unfortunately, young age is an obstacle for identification of FD or FD-like symptoms. These patients need extensive follow-up to determine if these mutations are pathogenic or not, especially in females.

We observed FD as a comorbidity in 1.12% of our JIA cohort but the small number of JIA patients in this cohort was a limitation for this study. We only included 50% of our cohort, mostly due to logistic issue (missing appointments, incomplete clinical evaluation).

Other limitation is the genetic test, which should have been done for all patients. Although we can assume that patients with normal enzyme levels do not present exonic pathogenic changes, the same cannot be concluded for intronic variants and *GLA* haplotypes. We also had no access to patient's family medical records. Therefore, we could not explain the high incidence of familiar history of vascular events and confirm other possible confounders such as diabetes, obesity or antiphospholipid syndrome.

Conclusions

In our cohort, FD was present in 1.12% of JIA patients. FD may have autoimmune features, and a high index of suspicion is necessary for the diagnosis. Pediatric rheumatologists should be aware that FD could present with similar classic autoimmune disease features, but they can also co-exist. We need to be careful with those patients with JIA and "dysautonomia" symptoms (persistent extremities or diffuse pain, gastroparesis or abdominal pain, absence of sweat, and arrhythmias), and be more meticulous with the family history, looking for strokes, sudden death and heart attacks at a young age (<50), and kidney disease leading to transplant. Those questions are crucial for FD diagnosis, and early disease identification is an effective strategy to avoid kidney transplants and

early death with the enzyme replacement (10). In the future, we hope to perform enzyme activity and genetic tests in all suspected patients.

List Of Abbreviations

-GalA - Alpha-galactosidase A

ANA - antinuclear antibodies

ANOVA - Analysis of variance

CIH - Complex intronic haplotypes

DAMPs - Damage-associated molecular patterns

ds DNA – Double stranded deoxyribonucleic acid

ECG - Electrocardiogram

Echo - Echocardiogram

ELISA - Enzyme-Linked Immunosorbent Assay

ESR - Erythrocyte sedimentation rate

FD - Fabry disease

Gb3 - Globotriaosylceramide

HEp-2- Human epithelial type 2

HGNC - HUGO Gene Nomenclature Committee

HLA - Human leukocyte antigen

HPLC - High-performance liquid chromatography

IFN-γ - Interferon-gamma

IIFA - immunofluorescence assay

IL – Interleukins

ILAR – International League against Rheumatism

IOP - Intraocular pressure

JIA - Juvenile idiopathic arthritis

LSD - Lysosomal storage disorder

Lyso-Gb3- globotriaosylsphingosine

OMIM - Online Mendelian Inheritance in Man

PCR - Polymerase Chain Reaction

RF - rheumatoid factor

RNP – Ribonucleoprotein

SNP - Single nucleotide polymorphism

TBUT - Tear breakup time

TLR4 - Toll-like receptor- 4

TNF-α - Tumor necrosis factor alpha

Declarations

Ethics approval and consent to participate

The local ethics committee approved this study (Albert Sabin Childhood Hospital, Fortaleza, Ceará, Brazil, **CAAE:** 0.0000.5042). All patients (and legal representatives if children under 18 years) included signed the informed consent and assent form.

Consent for publication – Not applicable

Availability of data and materials:

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Conflict of Interest disclosure:

All authors declare that they have no conflict of interest related to the study.

Funding

Conselho Nacional Pesquisa Desenvolvimento-Brasil CNPq 306723/2019-0),

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) Finance Code 001

Shire Brasil for genetic tests.

Authors' contributions – Please use the author's initials (AI, ZE, SA):

LPM, EM, SA– Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work.

LPM, AVC, IV, PC, MSM, SA- Drafted the work or revising it critically for important intellectual contente.

All authors gave final approval of the version to be published.

All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgements:

Aureliana Euclides and Fábia Sardinha.

Correspondence: Simone Appenzeller-Department of Medicine, Faculty of Medical Science, State University of Campinas, Cidade Universitária, Campinas SP, Brazil, CEP 13083-970; FAX: +55 19 3289-1818

Email: appenzel@unicamp.br

References

1. Biegstraaten M, Arngrímsson R, Barbey F, Boks L, Cecchi F, Deegan PB, et al. Recommendations for initiation and cessation of enzyme replacement therapy in patients with Fabry disease: The European Fabry Working Group consensus document. *Orphanet Journal of Rare Diseases.* 2015;*10* (1):1-10. org/10.1186/s13023-015-0253-6.
2. Spada M, Pagliardini S, Yasuda M, Tukel T, Thiagarajan G, Sakuraba H, Ponzone A, Desnick RJ. High incidence of later-onset Fabry disease revealed by newborn screening. Am J Hum Genet. 2006;79:31–40. doi.org/1086/504601.
3. Chien YH, Lee NC, Chiang SC, Dobrovolny R, Huang AC, Yeh HY, Chao MC, Lin SJ, Kitagawa T, Desnick RJ, Hsu LW. Newborn screening for Fabry disease in Taiwan reveals a high incidence of the later-onset *GLA* mutation c.936+919G>A (IVS4+919G>A). Hum Mutat. 2009;30(10):1397–1405. doi.org/1086/504601.
4. Brady RO, Gal AE, Bradley RM, Martensson E, Warshaw AL, Laster L. Enzymatic defect in Fabry's disease. Ceramidetrihexosidase deficiency. N. Engl. J. Med. 1967;276:1163–1167. org/10.1056/NEJM196705252762101.
5. Cimaz R, Guillaume S, Hilz MJ, Horneff G, Manger B, Thorne JC, Torvin Moller A, Wulffraat NM, Roth J. Awareness of Fabry disease among rheumatologists-current status and perspectives. Clinical Rheumatology. 2011;30(4):467–475. org/10.1007/s10067-010-1445-z.
6. Ivleva A, Weith E, Mehta A & Hughes DA. The Influence of Patient-Reported Joint Manifestations on Quality of Life in Fabry Patients. *JIMD Reports.* 2018;*41*:37–45. doi.org/10.1007/8904_2017_84.
7. Thévenot C, Crrouzet J, Villiaumey J, Avouac B, Le Charpentier Y, Voisin MC. Les manifestations articulaires de la maladie de Fabry. A propos de deux observations. *HÔP PARIS*. 1992;486–493.
8. Chimenti C, Padua L, Pazzaglia C, Morgante E, Centurion C, Antuzzi D et al. Cardiac and skeletal myopathy in Fabry disease: A clinicopathologic correlative study. *Human Pathology.* 2012;*43*(9): 1444–1452. doi.org/10.1016/j.humpath.2011.09.020.
9. Petty RE, Southwoood TR, Baum J, Bhettay E, Glass DN, Manners P, Maldonado-Coco J, Suarez-Almazor M, Orozco-Alcala JPA. JIA criteria Article ILAR 1997. *The Journal of Rheumatology.* 1998;*25*:1991–1994.
10. Gonçalves MJ, Mourão AF, Martinho A, Simões O, Melo-Gomes J, Salgado M et al. Genetic Screening of Mutations Associated with Fabry Disease in a Nationwide Cohort of Juvenile Idiopathic Arthritis Patients. *Frontiers in Medicine.* 2017;*4*(12):1–5. org/10.3389/fmed.2017.00012.

11. Sestito S, Ceravolo F & Concolino D. Anderson- Fabry Disease in Children. *Current Pharmaceutical Design*.2013;*19*:6037–6045. doi.org/10.2174/ 13816128113199990345.
12. Serebrinsky GP, Pascucelli V, Politei JM. Gene symbol: *GLA*. Disease: Fabry disease. Hum Genet. 2006;119:361. PMID: 17230649.
13. Matern D, Gavrilov D, Oglesbee D, Raymond K, Rinaldo P & Tortorelli S. Newborn screening for lysosomal storage disorders. *Seminars in Perinatology.* 2015;*39*: 206–216. doi.org/10.1053/j.semperi.2015.03.005
14. Chien YH, Lee NC, Chiang SC, Desnick RJ & Hwu WL. Fabry Disease: Incidence of the Common Later-Onset α-Galactosidase A IVS4+919G→ A Mutation in Taiwanese Newborns- Superiority of DNA-Based to Enzyme-Based Newborn Screening for Common Mutations. *Molecular Medicine*. 2012;*18*(5):780–784. org/10.2119/molmed.2012.00002.
15. Desnick RJ, Allen KY, Desnick SJ, Raman MK, Bernlohr RW, Krivit W. Fabry's disease: enzymatic diagnosis of hemizygotes and heterozygotes. Alpha- galactosidase activities in plasma, serum, urine, and leukocytes. J Lab Clin Med. 1973;81:157–171. PMID: 4683418.
16. Pagnini I, Borsini W, Cecchi F, Sgalambro A, Olivotto I, Frullini A, et al. Distal extremity pain as a presenting feature of Fabry's disease. Arthritis Care Res (Hoboken). 2011;63:390–395. org/10.1002/acr.20385.
17. Richards CS, Bale S, Bellissimo DB, et al. ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007. *Genet Med*. 2008;10(4):294-300. doi:10.1097/GIM.0b013e31816b5cae.
18. Havndrup O, Christiansen M, Stoevring B, Jensen M, Hoffman-Bang J, Andersen PS, et al. Fabry disease mimicking hypertrophic cardiomyopathy: Genetic screening needed for establishing the diagnosis in women. *European Journal of Heart Failure*. 2010;*12*(6): 535–540. doi.org/10.1093/eurjhf/hfq073.
19. De Francesco PN, Mucci JM, Ceci R, Fossati CA & Rozenfeld PA. Fabry disease peripheral blood immune cells release inflammatory cytokines: Role of globotriaosylceramide. *Molecular Genetics and Metabolism.* 2013;*109*(1):93–99. doi.org/10.1016/j.ymgme.2013.02.003.
20. Aerts JM, Groener JE, Kuiper S, Donker-Koopman WE, Strijland A, Ottenhoff R et al. Elevated globotriaosylsphingosine is a hallmark of Fabry disease. *Proceedings of the National Academy of Sciences of the United States of America.* 2008;*105*(8): 2812–2817. org/10.1073/pnas.0712309105.
21. Jesus AA, Canna SW, Liu Y & Goldbach-Mansky R. Molecular Mechanisms in Genetically Defined Autoinflammatory Diseases: Disorders of Amplified Danger Signaling. Annu Rev Immunol. 2015;33:823–874. org/10.1146/annurev-immunol-032414-112227.
22. Koca B, Sahin S, Adrovic A, Barut K, Kasapcopur O. Cardiac involvement in juvenile idiopathic arthritis. Rheumatol Int. 2017;37(1):137–142. doi:10.1007/s00296-016-3534-z)
23. Germain DP, Fouilhoux A, Decramer S, Tardieu M, Pillet P, Fila M, Rivera S, et al. Consensus recommendations for diagnosis, management and treatment of Fabry disease in paediatric patients. Clin Genet. 2019;96(2):107–117. doi:10.1111/cge.13546.
24. Lidove O, Zeller V, Chicheportiche V, Meyssonnier V, Sené T, Godot S & Ziza J M. Musculoskeletal manifestations of Fabry disease: A retrospective study. *Joint Bone Spine*. 2016;*83*(4): 421–426. org/10.1016/j.jbspin.2015.11.001.
25. Rahman P, Gladman DD, Wither JSM. Coexistence de Fabry's disease and Systemic Lupus Erythematous. *Clinical and Experimental Rheumatology.* 1998;*16*:475–478. PMID: 9706432.

26. Chatre C, Filippi N, Roubille F & Pers Y-M. Heart Involvement in a Woman Treated with Hydroxychloroquine for Systemic Lupus Erythematosus Revealing Fabry Disease. *The Journal of Rheumatology.* 2016;*43*(5):997–998. doi.org/10.3899/jrheum.150726.
27. Martinez P, Aggio M & Rozenfeld P. High incidence of autoantibodies in Fabry disease patients. *Journal of Inherited Metabolic Disease.* 2007;*30*(3):365–369. doi.org/10.1007/s10545-007-0513-2.
28. Katsumata N, Ishiguro A & Watanabe H. Fabry disease superimposed on overt autoimmune hypothyroidism. *Clinical Pediatric Endocrinology.* 2011;*20*(4):95–98. doi.org/10.1297/cpe.20.95.
29. Yin G, Wu Y, Zeng CH, Chen HP & Liu ZH. Coexistence of Fabry disease and IgA nephropathy: a report of two cases. *Irish Journal of Medical Science.* 2014;*183*(4): 671–675. doi.org/10.1007/s11845-014-1161-9
30. Hanaoka H, Hashiguchi A, Konishi K, Ishii T & Kuwana M. A rare association between Fabry's disease and granulomatosis with polyangiitis: A potential pathogenic link. *BMC Nephrology.* 2014;*15*(1): 1–5. https://doi.org/10.1186/1471-2369-15-157.
31. Rosa Neto NS, Bento J & Pereira R. Higher rate of rheumatic manifestations and delay in diagnosis in Brazilian Fabry disease patients. *Advances in rheumatology*. 2020;*60*(1), 7. doi:10.1186/s42358-019-0111-7.
32. Davies JP, Winchester BG & Malcolm S. Sequence variations in the first exon of alpha-galactosidase A. *Journal of medical genetics*. 1993;*30*(8),658–663. https://doi.org/10.1136/jmg.30.8.658.
33. Oliveira JP, Ferreira S, Reguenga C, Carvalho F, Mansson JE. The g.1170C>T polymorphism of the 5' untranslated region of the human alphagalactosidase gene is associated with decreased enzyme expression– evidence from a family study. J Inherit Metab Dis. 2008;31 Suppl 2:S405-13.
34. Poliakova AA, Gudkova AYA. P989. Association of acroparesthesias and angiokeratomas with the alpha-galactosidase A gene polymorphisms in females with hypertrophic cardiomyopathy. *European Heart Journal*. 2019; 40 (1) ehz747.0494, https://doi.org/10.1093/eurheartj/ehz747.0494.
35. Ferreira S, Reguenga C & Oliveira JP. The Modulatory Effects of the Polymorphisms in *GLA* 5'-Untranslated Region Upon Gene Expression Are Cell-Type Specific. *JIMD reports*. 2015; *23*, 27–34. https://doi.org/10.1007/8904_2015_424.
36. Schelleckes M, Lenders M, Guske K, et al. Cryptogenic stroke and small fiber neuropathy of unknown etiology in patients with alpha-galactosidase A -10T genotype. *Orphanet J Rare Dis*. 2014; 9:178. doi:10.1186/s13023-014-0178-5.
37. Michaud L. Longitudinal study on ocular manifestations in a cohort of patients with Fabry disease. *PloS one*, 2019;*14*(6), e0213329. https://doi.org/10.1371/journal.pone.0213329.
38. Gervas-Arruga, J., Cebolla, J. J., Irun, P., Perez-Lopez, J., Plaza, L., Roche, J. C., Capablo, J. L., Rodriguez-Rey, J. C., Pocovi, M., & Giraldo, P. Increased glycolipid storage produced by the inheritance of a complex intronic haplotype in the α-galactosidase A (*GLA*) gene. *BMC genetics*. 2015; *16*,109. https://doi.org/10.1186/s12863-015-0267-z.
39. Alcalay RN, Wolf P, Levy OA, et al. Alpha galactosidase A activity in Parkinson's disease. *Neurobiol Dis*. 2018; 112:85-90. doi:10.1016/j.nbd.2018.01.012.
40. Pisani A, Imbriaco M, Zizzo C, Albeggiani G, Colomba P, Alessandro R, Iemolo F & Duro G. A classical phenotype of Anderson-Fabry disease in a female patient with intronic mutations of the *GLA* gene: a case report. *BMC cardiovascular disorders*. 2012; *12*, 39. https://doi.org/10.1186/1471-2261-12-39