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Original article

FABRY DISEASE IN ARGENTINA: CLINICAL, BIOCHEMICAL AND MOLECULAR CORRELATION IN ALL REPORTED *GLA* VARIANTS

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Abstract

Introduction: Fabry disease is an X-linked trait due to pathogenic variants in the *GLA* gene, causing deficient GLA enzyme activity, and producing a chronic accumulation of globotriaosylceramide and its derivative globotriaosylsphingosine (LysoGb3) in tissues and fluids. Conflicting or discordant results of interpretation of multiple variants in *GLA* were reported in literature. The aim of this article is to report the spectrum of *GLA* variants in Argentine population, as well as the revised interpretation of variants classification. Moreover, we intend to find a possible correlation between biochemical parameters, clinical manifestations and genetic variants of adult male Fabry patients that could be of help for interpretation of variants.

Materials and methods: Blood samples from patients with clinical suspicion of Fabry disease were evaluated for specific laboratory tests: alfa-galactosidase A enzyme activity, LysoGb3 and *GLA* genetic test.

Results: There are 44 males with pathogenic *GLA* variants which showed deficient enzyme activity. Among them, thirty-two presented the classic phenotype (72%) and twelve the late onset clinical features (28%). Mean percentage of enzyme activity was 0.9% for classical patients and 3.2% for later onset ones. LysoGb3 values were increased in all males, with classic patients showing considerable higher values than that of late onset.

Discussion: Our results showed that the combined analysis of the clinical picture, leukocyte enzyme activity, globotriaosylsphingosine concentration and a detailed exhaustive study of the genetic variant lead to a definite diagnosis in those cases previously interpreted as of unknown significance, together with a revised interpretation of the phenotype.

Key words: Fabry disease, *GLA*, genetic test, diagnosis, LysoGb3

Resumen

Enfermedad de Fabry en Argentina: Correlacion Clínica, Bioquímica y Molecular en todas las variantes en GLA informadas

Introducción: La enfermedad de Fabry es una enfermedad genética ligada al cromosoma X, causada por variantes patogénicas en el gen *GLA*, que produce deficiencia en la enzima alfa-galactosidasa A y acumulación de globotriaosilesfingosina (LysoGb3). Hay resultados conflictivos en la literatura acerca de la interpretación de los estudios de diagnóstico. El objetivo de este trabajo es reportar el espectro de variantes en *GLA* con interpretación revisada y la correlación entre parámetros bioquímicos, clínicos y genéticos en pacientes adultos de Argentina.

Materiales y métodos: Se recibieron muestras de sangre de pacientes con sospecha clínica de enfermedad de Fabry. Se realizaron los test específicos de laboratorio, que incluyeron medida de actividad de la enzima alfa-galactosidasa A, concentración de LysoGb3 y estudio genético del gen *GLA*.

Resultados: Se detectaron 44 varones con variantes patogénicas en *GLA*. La medida de actividad enzimática fue deficiente, con valores de porcentaje medios respecto de los controles de 0.9 % para pacientes con las formas clásicas y 3.2% para los de las formas tardías. La concentración de LysoGb3 fue elevada en todos los varones, con valores significativamente mayores en pacientes clásicos que los tardíos.

Discusión: Nuestros resultados mostraron que el análisis combinado de la clínica, actividad enzimática en leucocitos, concentración de globotriaosilesfingosina y variante genética llevó a un diagnóstico definitivo en todos los pacientes, aun en aquellos interpretados previamente como de significado incierto.

Palabras clave: enfermedad de Fabry, *GLA*, estudio genético, diagnostico, LysoGb3

KEY POINTS
Current knowledge

- Fabry disease caused by pathogenic variants in the *GLA* gene, resulting in deficient *GLA* enzyme activity, and accumulation of globotriaosylceramide and globotriaosylsphingosine (LysoGb3).
- Conflicting or discordant results of interpretation of multiple variants in *GLA* were reported in literature.

Contribution of the article to current knowledge

- We report the spectrum of *GLA* variants in Argentine population, as well as the revised interpretation of variants classification.
- We show a that the combined analysis of the clinical picture, leukocyte enzyme activity, LysoGb3 concentration and a detailed exhaustive study of the genetic variant lead to a definite and accurate diagnosis.

Fabry disease (FD, OMIM 301500) is an X-linked trait due to pathogenic variants in the *GLA* gene, causing deficient alfa galactosidase A (GLA) enzyme activity (EC:3.2.1.22), and producing a chronic accumulation of globotriaosylceramide (Gb3) and its derivative globotriaosylsphingosine (LysoGb3) in tissues and fluids[1].

FD patients may present two phenotypes, the most severe one is the "classic" (C) form, while the other, a milder form, is called the "late onset" (LO) variant. Classic males initiate their clinical manifestations at childhood with acroparesthesias, angiokeratomas, hypohidrosis, abdominal pain, heat/exercise intolerance and *cornea verticillata*. With age, the disease progresses altering the function of kidney, heart and central nervous system, with microalbuminuria/proteinuria, renal insufficiency, cardiomyopathy and/or stroke. The late onset form lacks the symptoms in infancy, and manifestations are generally restricted mainly to heart, with a wide range of renal affection[1]. Heterozygous females with same variants may exhibit clinical heterogeneity, from an asymptomatic to a severe disease course, explained by the random X-inactivation phenomena and lack of cross-correction[2].

FD biochemical confirmation in males is based on demonstration of deficient enzyme activity in leukocytes from peripheral blood, along with elevated LysoGb3 concentration and the finding of a pathogenic variant in *GLA* gene[3]. Measurement of enzyme and/or LysoGb3 in females is inconclusive for diagnosis[4]. In the last years, conflicting or discordant results of interpretation of multiple variants in *GLA* were reported in literature. Although there is consensus regarding the non-pathogenicity of many benign variants, some reports intend to show a pathogenic effect[5].

The aim of this study was to report the spectrum of *GLA* variants in Argentine population, as well as the revised interpretation of variants classification. Moreover, we intend to find a possible correlation between biochemical parameters, clinical manifestations and genetic variants of adult male patients that could be of help for interpretation of variants. We also included *GLA* variants found in females in which there were no affected males in their families

and the genetic variant was previously classified in scientific reports.

Materials and methods

Between 2003 and 2023, patients with clinical suspicion of FD were evaluated for specific laboratory tests in specialised centres for lysosomal diseases diagnosis in Argentina. Samples from patients consisted of blood samples collected on filter paper or dried blood spots (DBS), 9 ml heparin blood and 1 ml EDTA blood.

The test in male samples for enzyme activity in dried blood spots (DBS) (reference value > 2.10 µmol/L.h) was carried out according to the method developed by Chamoles et al[6]. Those samples which showed deficient values in DBS were analysed for confirmation in leukocytes (reference value > 4.66 nmol/mg.h). Percentage of enzyme activity in leukocytes was calculated as the percentage of the value in the patient sample divided by the mean value of control individuals' samples.

LysoGb3 analysis in DBS was carried out when available (reference value < 0.9 nmol/L), according to the method described in Politei et al[7].

Genetic test by Sanger sequencing of the 7 exons along with exon-intron boundaries was performed in males with deficient GLA activity and in females as the 1st test. The reference sequence of *GLA* gene used is NM_000169.3.

Phenotype assignment between C or LO was based on the consensus by Arends et al[8], which establishes the requirement of the presence of at least one of these 4 manifestations: acroparesthesia, gastrointestinal pain/diarrhoea, angiokeratomas/hypohidrosis and/or *cornea verticillata* during childhood, for the C phenotype.

The study conformed to the principles outlined in the Declaration of Helsinki, and the Ethics Committee of the National University of La Plata. Informed consent was obtained from all patients or their guardians.

Statistical analysis was performed using GraphPad Prism 8.0 software (GraphPad Software, La Jolla, CA, USA) applying unpaired t-test. Data are reported as mean ± SD

Results

We have compiled *GLA* variants found in patients from Argentina. After that, we have revised and re-interpreted them based on the actual knowledge on variant classification[9]. We have re-classified the variants found in male patients in 2 groups: pathogenic or benign (Table 1). There are 44 males with pathogenic *GLA* variants. Among them, thirty-two presented the C phenotype (72%) and twelve the LO clinical features (28%).

We could assess the enzyme activity in 43 DBS samples and 34 leukocytes from peripheral blood, and, in all the cases resulted deficient. Enzyme values assayed in DBS samples were not significantly different among both groups (C: 0.39 ± 0.28 micromol/l.h vs LO: 0.49 ± 0.28 micromol/L.h). A tendency towards higher values of enzyme was observed in leukocytes from late onset patients as compared to classic ones although overlap is present and no statistical difference was observed (C: 0.30 ± 0.29 nmol/mg.h vs LO: 1.04 ± 0.55 nmol/mg.h). Mean percentage of enzyme activity for both groups was 0.9% for classical patients with a range of 0 – 2.9% and 3.2% for later onset ones, with a range of 0.9-6.4%.

LysoGb3 was evaluated in 32 samples from Fabry patients, 21 with C and 11 with LO Fabry disease. LysoGb3 concentrations showed elevated values in all Fabry patients, with an overall mean value of 62.4± 41.3 nmol/L. When analysing values from both male Fabry phenotypes, there is a significant ($p<0.001$) difference between them, classic patients showing considerable higher values (89.7 ± 21.1 nmol/L) than that of late onset (13.6 ± 35.4 nmol/L) (Fig. 1).

Among males with benign *GLA* variants (n=6), they all showed reduced enzyme activities when tested in DBS. However, we only had access to leukocytes of five of these cases, all of which had activities within the normal value. Scientific consensus states that FD is ruled out with normal leukocyte GLA activity. For this reason, detection of low enzyme activity in DBS in these individuals was considered a false positive result, reinforcing the need for enzyme test in leukocytes for definitive diagnosis in males. LysoGb3 values were obtained from 5 individuals of this group. Three of these individuals showed normal values, however two of them presented values slightly increased.

This study also included for evaluation 6 females (Table 2), who displayed *GLA* variants. One of them presented the benign variant p.Ala143Thr excluding FD diagnosis, while the others presented known pathogenic variants related to classic phenotype. Only one Fabry female showed a high LysoGb3 value.

Table 1 | *GLA* variants in Argentine male patients. The table shows the information regarding *GLA* variant, the phenotype, enzyme activity in dried blood spots (DBS, in µmol/l.h) and leukocytes (expressed as nmol/mg.h and as percentage from mean controls values), and LysoGb3 concentration at baseline

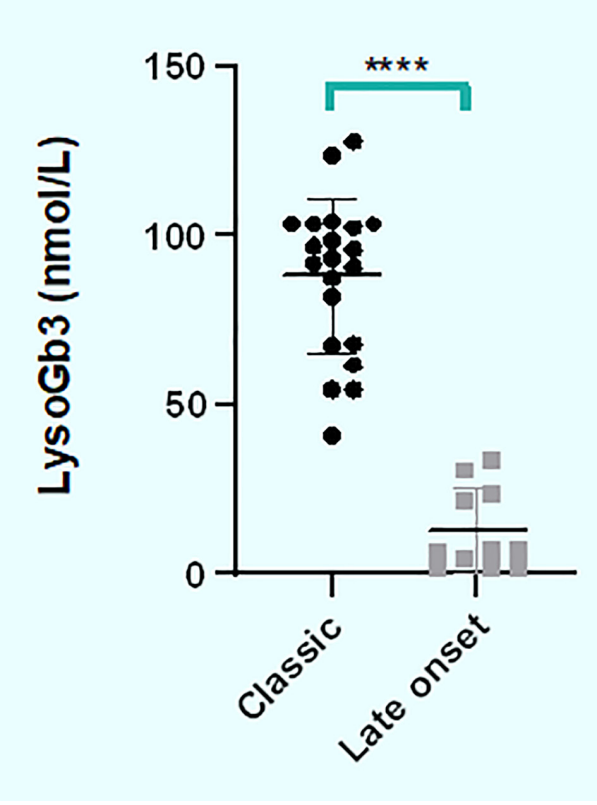
n	cDNA	Exon	Protein	Inter-preta-tion of	Classi-fication variant	Pheno-type	Enzyme activity (DBS)[a]	Enzy-me activity (leuko-cytes)[b]	% (leuko-cytes)[c]	Lyso Gb3 (nmol/L)[c]
1	c.1122_1125delAGGA	7	p.Gly375Trpfs*	Pathogenic	Frameshift	Classic	ND	ND	ND	ND
2	c.448delG	3	p.Gly150Aspfs*	Pathogenic	Frameshift	Classic	0.34	ND	ND	ND
3	c.718_719delAA	5	p.Lys240Glufs*	Pathogenic	Frameshift	Classic	0.00	0.95	2.94	ND
4	c.441_444delGAGT	3	p.Ser148Leufs*	Pathogenic	Frameshift	Classic	0.48	0.09	0.28	ND
5	c.1033_1034delTC	7	p.Ser345Argfs*	Pathogenic	Frameshift	Classic	0.28	0.10	0.31	124.0
6	c.886_887delAT	2	p.Met296Valfs*	Pathogenic	Frameshift	Classic	1.09	ND	ND	ND
7	c.1045_1049delTGGGC	7	p.Trp349Cysfs*	Pathogenic	Frameshift	Classic	0.00	0.30	0.93	92.0
8	c.902_905insTGTC	6	p.Arg301Leufs*	Pathogenic	Frameshift	Classic	0.43	0.73	2.26	68.1
9	c.728T>G	5	p.Leu243Trp	Pathogenic	Missense	Classic	0.18	ND	ND	ND
10	c.463G>C	3	p.Asp155His	Pathogenic	Missense	Classic	0.47	0.10	0.31	102.5
11	c.1244T>C	7	p.Leu415Pro	Pathogenic	Missense	Classic	0.07	0.11	0.34	103.7
12	c.281G>A	2	p.Cys94Tyr	Pathogenic	Missense	Classic	0.45	ND	ND	ND
13	c.572T>C	4	p.Leu191Pro	Pathogenic	Missense	Classic	0.60	ND	ND	ND
14	c.581C>T	4	p.Thr194Ile	Pathogenic	Missense	Classic	0.79	0.59	1.83	96.0
15	c.874G>A	6	p.Ala292Thr	Pathogenic	Missense	Classic	0.41	0.02	0.06	127.9
16	c.1145G>A	7	p.Cys382Tyr	Pathogenic	Missense	Classic	0.95	ND	ND	ND
17	c.680G>A	5	p.Arg227Gln	Pathogenic	Missense	Classic	0.10	0.20	0.62	104.3
18	c.790G>T	5	p.Asp264Tyr	Pathogenic	Missense	Classic	0.38	0.44	1.36	61.8
19	c.100A>G	1	p.Asn34Asp	Pathogenic	Missense	Classic	0.76	0.14	0.43	103.5
20	c.712A>G	5	p.Ser238Gly	Pathogenic	Missense	Classic	0.44	0.37	1.15	41.0
21	c.145C>G	1	p.Arg49Gly	Pathogenic	Missense	Classic	0.24	0.92	2.85	90.9
22	c.504A>T	3	p.Lys168Asn	Pathogenic	Missense	Classic	0.23	0.10	0.31	67.5
23	c.467C>T	3	p.Ala156Val	Pathogenic	Missense	Classic	0.85	0.00	0.00	82.3
24	c.1118G>A	7	p.Gly373Asp	Pathogenic	Missense	Classic	0.00	0.10	0.31	97.0
25	c.679C>T	5	p.Arg227X	Pathogenic	Nonsense	Classic	0.15	0.43	1.33	103.4
26	c.772G>T	5	p.Gly258X	Pathogenic	Nonsense	Classic	0.68	0.35	1.08	ND
27	c.658C>T	5	p.Arg220X	Pathogenic	Nonsense	Classic	0.44	0.12	0.37	ND
28	c.1024C>T	7	p.Arg342X	Pathogenic	Nonsense	Classic	0.37	ND	ND	54.7
29	c.243G>A	2	p.Trp81X	Pathogenic	Nonsense	Classic	0.33	0.63	1.95	54.7
30	c.1192G>T	7	p.Glu398X	Pathogenic	Nonsense	Classic	0.10	ND	ND	87.4
31	c.639G>T	4	p.Lys213Asn	Pathogenic	Splicing	Classic	0.42	0.01	0.03	93.6
32	c.801+4A>G	IVS5	p.Met267_Leu268ins4*	Pathogenic	Splicing - Intron 5 partial inclusion	Classic	0.10	0.00	0.00	98.6
33	c.1284_1287delACTT	7	p.Leu428Phefs*	Pathogenic	Frameshift	Late onset	0.43	1.64	5.08	22.3
34	c.888G>A	6	p.Met296Ile	Pathogenic	Missense	Late onset	0.71	0.59	1.83	ND
35	c.1088G>A	7	p.Arg363His	Pathogenic	Missense	Late onset	0.77	1.12	3.47	1.8
36	c.520 T>G	3	p.Cys174Gly	Pathogenic	Missense	Late onset	0.59	0.88	2.73	7.2
37	c.644A>G	5	p.Asn215Ser	Pathogenic	Missense	Late onset	0.32	0.49	1.52	7.2
38	c.647A>G	5	p.Tyr216Cys	Pathogenic	Missense	Late onset	0.24	ND	ND	24.0
39	c.335G>A	2	p.Arg112His	Pathogenic	Missense	Late onset	0.48	0.83	2.57	6.5
40	c.902G>A	6	p.Arg301Gln	Pathogenic	Missense	Late onset	0.35	0.66	2.05	34.3
41	c.613C>T	4	p.Pro205Ser	Pathogenic	Missense	Late onset	0.38	0.28	0.87	6.7
42	c.695T>C	5	p.Iso232Thr	Pathogenic	Missense	Late onset	0.70	1.32	4.09	1.5
43	c.1095T>A	7	p.Tyr365X	Pathogenic	Nonsense	Late onset	0.30	1.50	4.65	31.0
44	c.547+404T>G	IVS3	p.Gly183Alafs*	Pathogenic	Splicing - Intron 3 partial inclusion	Late onset	0.58	2.08	6.45	5.1
1	c.870G>A	6	p.Met290Ile	Benign	Missense	Benign	0.94	6.93	21.48	0.5
2	c.239G>A	2	p.Gly80Asp	Benign	Missense	Benign	1.14	5.59	17.33	0.1
3	c.1147T>C	7	p.Phe383Leu	Benign	Missense	Benign	2.85	ND	ND	1
4	c.352C>T	2	p.Arg118Cys	Benign	Missense	Benign	1.15	9.47	29.36	1.7
5	c.376A>G	3	p.Ser126Gly	Benign	Missense	Benign	6.13	11.77	36.48	ND
6	c.937G>T	6	p.Asp313Tyr	Benign	Missense	Benign	ND	7.11	22.04	0.8

Reference values for enzyme activity in DBS > 2.10 µmol/l.h[a], and in leukocytes > 4.66 nmol/mg.h[b]. Reference values for LysoGb3 concentration < 0.9nmol/L[c]

Discussion

Clinical manifestations in FD are nonspecific and clinically similar to other common disorders. These factors make clinical suspicion of FD complicated[10], and sometimes, this situation leads to false positive diagnosis due to common assumptions that believe that every clinical picture is a direct cause of FD.

Figure 1 | LysoGb3 concentration in classic and late onset male patients (reference value < 0.9 nmol/L)
Unpaired t-test **** $p < 0.0001$. Data are reported as mean ± SD



Currently, there are more than a thousand genetic variants in the *GLA* gene described (The Human Gene Mutation Database)[11]. However, the analysis of the results is far away from being an easy task, and a lot of genetic variants may remain of unknown significance. For genetic variants of unknown significance, a thorough multidisciplinary analysis is mandatory to establish the phenotypic significance of the genetic variant, which should include functional analysis of the effect of a variant in the protein, biochemical testing, and clinical studies. The clinical picture and natural course of the disease in each patient is diverse and associated not only to specific genetic variants but also to other factors such as environmental, cardiovascular risk factors or epigenetics. However, there does exist a genotype–phenotype correlation in FD. So, in those cases in which the GLA variant has been previously reported, patient´s phenotype can be predicted.

The GLA DBS test was introduced by Dr. Chamoles in 2001[6]. His development makes these screening test accessible to patients worldwide. However, the possibility to offer a first tier DBS test, does not exclude the need for confirmatory tests. The enzyme test in DBS is a screening assay, and although weighting for the highest diagnostic sensitivity, there is a compromise in the diagnostic specificity which leads to a percentage of false positives. In our study, 3 DBS samples displayed deficient enzyme activities, implying a 6% rate of false positives. The DBS test provides a way to detect all possible

Table 2 | Variants in *GLA* gene in Argentine female patients. The table shows the information regarding *GLA* variant, the phenotype, and LysoGb3

n	cDNA	Exon	Protein	Interpretation of variant	Classification of variant	Phenotype according to bibliography	LysoGb3 at baseline (nmol/l)*
1	c.427G>A	3	p.Ala143Thr	Benign	Missense	Benign	1.1
2	c.802-1G>C	6	Splicing	Pathogenic	Exon 6 exclusion	Classic	ND
3	c.394G>A	3	p.Gly132Arg	Pathogenic	Missense	Classic	ND
4	c.605G>A	4	p.Cys202Tyr	Pathogenic	Missense	Classic	4.6
5	c.254G>A	2	p.Gly85Asp	Pathogenic	Missense	Classic	0.5
6	c.1069C>T	7	p.Gln357X	Pathogenic	Nonsense	Classic	ND

**Reference values for LysoGb3 concentration < 0.9 nmol/L*

patients, with the lowest false negative rate, and where all positive screening tests should be confirmed in leukocyte samples. The reason for the false positives in DBS may be due to neutral pH of blood that makes the GLA enzyme unstable as observed for the p.Asp313Tyr variant[12]. Misinterpretation of a pathogenic value in DBS without the confirmatory assay would lead to misdiagnosis of FD.

Leukocyte residual enzyme activity expressed as percentage from the wild-type value has been considered as a useful tool to classify the phenotype of the patient[13]. The results from our study showed values comparable to bibliographic reports, showing higher mean values for classical patients than that found in late-onset ones.

Many variants previously reported in bibliography as causing FD were re-analyzed in terms of new available biochemical, pathological and/or genetic data. These studies led to a change in the classification of the genetic variant. Although these studies are worldwide available there are still misreporting of these benign *GLA* variants as disease causing by some labs or professionals, and ultimately in scientific papers[13-18].

Analysis of our data showed two variants p.Gly80Asp and p.Met290Ile initially considered as pathogenic, based on a deficient GLA activity in DBS, and are now re-classified as benign. The bibliographic revision for information regarding the p.Gly80Asp variant revealed that has been found in high frequency in Ecuatorian Hispanic newborns, and the 1000 genomes report for Latin-American population[18], expressing enzyme activity above the affected range. The GLA activity expressed *in vitro* was 28% of mean normal range[19]. Patient carrier of p.Gly80Asp variant had been included in a hemodialysis national screening of FD. Since the clinical manifestations were no characteristic, the young age at which hemodialysis was initiated, together with normal LysoGb3 values and leukocyte enzyme activities, this variant was finally re-classified as benign.

The variant p.Met290Ile was found in a 65-year-old female, after family screening based on an index case from Spain with a possible FD diagnosis. She presented no typical signs of the disease. Her evaluation showed normal kidney function, mitral insufficiency in cardiac ultrasound and white matter lesions in cerebral MRI. Her son, a 47-year-old male hemizygous for the variant, presented proteinuria and type I diabetes. His enzyme activity in leukocytes was normal. Both individuals showed normal LysoGb3 level. Recently, a Spanish patient with proteinuria and renal insufficiency bearing the same variant was reported. In this case the renal biopsy showed absence of lipid deposits, proposing the reinterpretation of this variant as benign (Unpublished results). Other recognized benign *GLA* variants found in Argentina were p.Phe383Leu, p.Arg118Cys, p.Ser126Gly and p.Asp313Tyr. Leukocyte enzyme testing results in males bearing these variants were normal, confirming the benign nature of these mutations. LysoGb3 was normal in 3 of the patients with benign variants, and slightly above normal range in 2 of them.

One male patient with suspicion of FD based on renal insufficiency of unknown aetiology had deficient enzyme activity both in DBS and leukocytes. Genetic variant p.Asp55Gly was previously assigned as benign due to a normal LysoGb3 value[20]. Re-analysis of this patient confirmed the enzyme deficiency in leukocytes, and a mild LysoGb3 increase. Assessment of relatives from the family tree showed various patients with deficient leukocyte enzyme values and LysoGb3 increases, suggesting a LO clinical picture. Although kidney biopsy was requested to assess specific glycolipid deposits, it has not been performed to date. Tissue biopsy, generally a renal specimen, has been suggested as a final diagnosis confirmation tool based on the presence or absence of glycolipid intralysosomal deposits, mainly in podocytes and vascular smooth muscle cells[21-23]. However, typical associated deposits are not pathognomonic of FD. Other conditions such as iatrogenic phospholipidosis, genetic nephropathies and other lysosomal disorders may present similar kidney biopsy findings. Therefore, the definitive significance of kidney biopsy should be carefully interpreted and should be revised[24-26]. However, based on the clinical picture, leukocyte enzyme deficiency and LysoGb3 analysis, and X-linked segregation of several affected direct relatives, the p.Asp55Gly variant was re-classified as pathogenic.

Deacylated sphingolipids have been thought to be closely related to the pathogenesis of many sphingolipidoses[27-29], due to its toxicity. In 2008, Aerts et al. reported that LysoGb3 was increased in the plasma of classically affected male Fabry patients and in the plasma and tissues of Fabry mice, and they suggested that circulating LysoGb3 could be a candidate biomarker for monitoring Fabry disease[30]. In our study, male FD patients display elevated LysoGb3, and values from classic patients are higher than those from late onset ones. These results are in accordance with those reported by several authors[31-34], reflecting LysoGb3 values between 40 to 127 nmol/L for classical males and in the range of 1.5 to 34.3 for men with LO phenotype.

Levels of LysoGb3 are reported to be strongly associated with both disease type (C or LO) and sex of the patient[35,36]. LysoGb3 was found to be associated with several disease severity parameters in small studies, including left ventricular mass, white matter lesions and overall disease severity[37,38]. In 2023, Van der Veen et al. confirmed that plasma LysoGb3 reaches a stable level in childhood and predicts which expected disease burden later in life[36], and they also showed that plasma levels can differentiate C and LO phenotypes. Recently, Ouyang et al., reported that LysoGb3 significantly improves the predictive capacity for prognosis in the C phenotype, but C-statistics only slightly increase from 0.82 to 0.84 in the LO phenotype. This might be related to the fact that in the LO phenotype, plasma concentrations are usually only slightly increased or even normal in some females, making it difficult to establish a cut-off point[39]. A new tandem mass spectrometry method for quantification proved plasma LysoGb3 to be the best biomarker for all FD types, that discriminated FD male patients with both phenotypes as well as classic FD females from controls[40].

When revising LysoGb3 values belonging to patients with variants previously considered as benign, we found two male patients with values slightly above the cut off in this group. This reinforces to cautiously interpret LysoGb3 during the diagnosis process. LysoGb3 levels should never be used for Fabry diagnosis as a unique second tier confirmation test.

In conclusion, our results showed that the combined analysis of the clinical picture, leukocyte enzyme activity, LysoGb3 concentration and a detailed exhaustive study of the genetic variant lead to a definite diagnosis in those cases previously interpreted as of unknown significance, together with a revised interpretation of the phenotype. As a whole, it will be possible to avoid misdiagnosis and exposure of a patient to unnecessary, burdensome and costly treatment.

Conflict of interest: None to declare

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