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The Challenge of Diagnosis and Indication for Treatment in Fabry Disease

Marco A. Curiati, MD[1], Carolina S. Aranda, MD, MSc[1], Sandra O. Kyosen, MD, MSc[1], Patricia Varela, MSc[2], Vanessa G. Pereira, PhD[3], Vania D'Almeida, PhD[3], João B. Pesquero, PhD[2], and Ana M. Martins, MD, PhD[1]

Abstract

Fabry disease, caused by deficient alpha-galactosidase A lysosomal enzyme activity, remains challenging to health-care professionals. Laboratory diagnosis in males is carried out by determination of alpha-galactosidase A activity; for females, enzymatic activity determination fails to detect the disease in about two-thirds of the patients, and only the identification of a pathogenic mutation in the *GLA* gene allows for a definite diagnosis. The hurdle to be overcome in this field is to determine whether a mutation that has never been described determines a "classic" or "nonclassic" phenotype, because this will have an impact on the decision-making for treatment initiation. Besides the enzymatic determination and *GLA* gene mutation determination, researchers are still searching for a good biomarker, and it seems that plasma lyso-Gb3 is a useful tool that correlates to the degree of substrate storage in organs. The ideal time for treatment initiation for children and nonclassic phenotype remains unclear.

Keywords

genotype-phenotype correlation, enzyme replacement therapy, alpha-galactosidase A deficiency, dried blood spot on filter paper, screening

Introduction

Fabry disease (FD) is an X-linked lysosomal storage disorder caused by deficiency of alpha-galactosidase A (α-gal A) activity involved in the degradation of glycosphingolipids. Its deficiency leads to the accumulation of globotriaosylceramide (Gb3) inside the cells, causing several clinical abnormalities; the GLA gene is located on Xq22.1.[1]

The clinical suspicion of FD begins with a selection of characteristic signs and symptoms, such as acroparesthesia, angiokeratomas, recurring headache, abdominal pain, chronic diarrhea, progressive loss of renal function, cardiomyopathy generally associated with myocardial fibrosis, central nervous system microangiopathy,[1–4] and/or positive family history, or also families with high prevalence of kidney disease, cardiomyopathy, or ischemic encephalopathy.[1,2]

After diagnostic hypothesis of FD is raised, the diagnosis is made using laboratory testing. In males, the enzymatic activity of α-gal A in plasma, leukocytes, skin biopsy, or dried blood spot (DBS) samples is measured. In females with clinical suspicion, it is widely described in literature that there is a limitation in performing enzymatic activity assay, since women can range from almost undetectable, as can be found in males, up to a normal range of values, as found in healthy individuals.[1,2,5–7] In those patients, a diagnostic is performed with *GLA* gene molecular analysis.

Molecular analysis of the *GLA* gene is an essential diagnostic tool in women or in patients who have very strong clinical suspicion due to the presented symptoms but the enzyme activity is within the normal range. Furthermore, it can inform the treating physician about the exact kind of mutation, which is generally related to the clinical

[1] Reference Center in Inborn Errors of Metabolism, Universidade Federal de São Paulo, São Paulo, Brazil
[2] Center for Research and Molecular Diagnosis of Genetic Diseases, Universidade Federal de São Paulo, São Paulo, Brazil
[3] Departament of Psychobiology, Universidade Federal de São Paulo, São Paulo, Brazil

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Corresponding Author:
Sandra O. Kyosen, Centro de Referência em Erros Inatos do Metabolismo – CREIM, Rua Coronel Lisboa, Universidade Federal de São Paulo, 957, CEP: 04020-041, São Paulo, Brazil.
Emails: skyosen.dped@epm.br; skyosen.dped@gmail.com

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manifestations. Mutations known as ''classic'' have an earlier onset of symptoms, and usually cause a more severe clinical phenotype. On the other hand, ''nonclassic'' usually shows slower disease progression with later onset of symptoms but patients can also present severe organ damage.[8]

The treating physician has to be aware of the different kinds of mutations and their clinical implications. Nonsense and frameshift mutations are supposed to cause a severe phenotype, since these mutations cause important damage to protein structure (misfolding), leading to a highly dysfunctional protein.[9] Mutations without previous description in literature require further investigation not only by performing brain and heart magnetic resonance imaging (MRI) but also by considering renal biopsy to evaluate which organs might be affected. There are also controversial mutations, like the p.D313Y and intronic variants, described in literature as pathogenic by some authors[10] and as benign by other authors.[11] The treating physician should always rely on the patients' clinical manifestations and examination, rather than the genotype, when deciding whether to begin enzyme replacement therapy (ERT).[12]

Although there has been specific treatment over the last 15 years for FD, patients still face the challenge to have an early diagnosis that will allow a timely treatment initiation to avoid irreversible damage to organs. Treating physicians still have the challenge to decide what would be the ideal time to treat the later onset variants patients, and researchers of molecular alterations face the challenge of the unknown effect of *GLA* variants. We will try to address some of these challenges.

Biochemical Diagnosis

Determination of α-gal A activity is usually the first laboratory test for diagnosing FD in index patients; if there is a known familial *GLA* mutation, a straightforward DNA analysis is indicated. Many biological materials can be used for the analysis of α-gal A activity, but the most common are plasma, leukocytes, fibroblasts, and DBS. The assay in leukocytes is regarded as the ''gold standard'' and DBS assays are widely used for screening purposes.

As an X-linked disorder, male patients can be reliably diagnosed with FD if presenting low or undetectable α-gal A activity; results from DBS analysis below the normal range often require a confirmatory test on leukocytes and, if low α-gal A activity is also detected in this material, FD is confirmed. *GLA* sequencing for mutation identification is recommended for index cases, which could provide important insights for prognosis and treatment, as well as for genetic counseling but is not mandatory for diagnosis.

On the other hand, analysis of α-gal A activity in heterozygous females is usually not conclusive for Fabry diagnosis, as women may present normal enzyme levels in different samples due to random X-inactivation. Therefore, in those cases, identification of a pathogenic *GLA* mutation is mandatory to define the diagnosis and carrier status of a female patient. Additional complementary tests such as measuring Gb3 and its metabolites (mainly lyso-Gb3) in urine/plasma and biopsies for histology/electron microscopy are also recommended.[13]

Lyso-Gb3 is similar to Gb3, only lacking the fatty acid chain.[14] Levels of plasma lyso-Gb3 have been correlated with disease severity as well as with response to ERT.[15,16] In male patients, lyso-Gb3 is a reliable diagnostic tool to distinguish between classical and nonclassical FD from non-FD participants. However, in some cases of female patients, the levels of lyso-Gb3 overlap with controls, suggesting that increased lyso-Gb3 values are very suggestive of FD, but normal values cannot exclude FD.[17] Urinary lyso-Gb3 levels and its analogs are lower than plasma, but it has been demonstrated that male patients present higher levels of these markers when compared to females, and none of these markers are found in healthy individuals.[18] In addition, increases in lyso-Gb3/creatinine ratio correlates with Gb3 concentration, types of GLA mutation, gender, and ERT status,[19] emphasizing the diagnostic value of this biomarker.

Challenges in Biochemical Diagnosis

At our laboratory (LEIM-UNIFESP), DBS and leukocytes assays are performed to determine α-gal A activity through fluorometric methods using 4-methylumbelliferyl α-D-galactopyranoside as substrate and α-N-acetylgalactosamine as α-galactosidase B inhibitor.[20] These fluorometric assays are the most commonly used for Fabry diagnosis, but the analysis of α-gal A activity in DBS through tandem mass spectrometry has also become available.[21–23]

Since renal disease is a common feature among patients with FD, individuals undergoing hemodialysis (with no other known diagnosis) have been considered a high-risk population for FD, with an overall prevalence of 0.62%, ranging from 0% to 1.2% worldwide (reviewed by van der Tol et al).[24] We performed a pilot screening protocol in which DBS samples from Brazilian hemodialysis centers were sent to LEIM-UNIFESP for α-gal A activity analysis (this study was approved by the ethical research committee from Universidade Federal de São Paulo, CEP #0384/05). We received 6277 DBS samples from male patients at hemodialysis centers located in all regions of Brazil, and α-gal A activity was below the normal range in 320/6277 (5%) of samples.

As a primary screening protocol, patients with low α-gal A activity in DBS were requested to send leukocyte samples to confirm the diagnosis. From 320 patients with low enzyme activity in DBS, only 176 (55%) actually sent new samples for determination of α-gal A activity in leukocytes, probably due to transportation issues, which is common in large countries like Brazil—this type of sample must be transported under refrigeration, in contrast to DBS, which can be transported at room temperature or even sent by regular mail. From these, only 20 (11.4%) showed α-gal A activity in leukocytes below the normal range. We then requested samples for molecular analysis to identify GLA mutations, but only 18 samples were received and mutations were identified in 8 patients. Thus, the observed prevalence of FD among Brazilian hemodialysis

patients found in this pilot study was 0.1%. It is important to note that 45% of patients with low α-gal A activity in DBS did not send leukocyte samples and, regarding molecular analysis, only GLA coding regions were analyzed in this study, which could explain why some patients presented low α-gal A activity in leukocytes and had no mutations identified. Therefore, it is possible that the observed FD prevalence may still be underestimated (unpublished data).

The cutoff value of α-gal A activity in DBS used in this screening protocol (2.5 μmol/L blood/h) was determined on the basis of a prior validation of the DBS assay in a group of healthy Brazilian volunteers.[20] We decided to set a slightly high cutoff value to ensure that no patient with FD was missed in this initial analysis. However, since there was a high rate of false positives in DBS, we decided to change our cutoff to 2.2 μmol/L blood/h after a receiver–operating characteristic curve analysis, maintaining the assay sensitivity at 100%. In addition, another important alteration for the next screening protocol is that we will no longer request samples of leukocyte assays due to the high transportation costs; patients with low α-gal A activity in DBS will have DNA extracted from the DBS card and sent directly to molecular analysis, which is described in the next session.

As quality control for FD diagnosis, another lysosomal enzyme should be tested in the same DBS. The assay most commonly used for this purpose is evaluation of β-galactosidase, which should be within the normal range to ensure sample quality. Our reference range for β-galactosidase activity was previously determined through analysis of healthy Brazilian volunteers' samples,[20] and for α-gal A evaluation, only DBS samples with β-galactosidase activity within the normal range were used in this study.

Screening and Case Finding

The FD has signs and symptoms peculiar in its clinical presentation, generally poorly understood by physicians and misunderstood by them due to their lack of knowledge about this disorder; thus, it usually takes several years from the onset of symptoms until the definite diagnosis is established, as is the case in most rare disorders. The timely diagnosis of FD remains a challenge.[25] In a study of 126 Brazilian patients enrolled in the Fabry Registry (61 males and 65 females), the median time between the onset of symptoms and diagnosis was 20.3 years in males and 14.3 years in females; the median age of diagnosis was 31.9 years in males and 27.1 years in females.[26]

Screening is defined as testing of apparently well people to find those at increased risk of having a disease or disorder that should be medically important, with a known natural history and an effective intervention must exist; looking for additional illness in those with medical problems is termed case-finding, and screening is limited to those apparently well.[27]

Newborn screening (NBS) for FD has gained some interest since effective treatment became available.[28,29] In NBS studies, it was found that 1:3092 Italian male neonates had deficient α-gal A activities and specific mutations[30]; 1:2388 Taiwanese male neonates tested positive on enzyme assays and molecular tests,[31] and 1:3024 Japanese neonates (both genders) also tested positive on α-gal A activity assays and *GLA* gene analyses.[32] The challenge in NBS for FD is that further studies are necessary to determine the natural history of the later onset mutations to define the optimal timing for therapeutic intervention.

Timely treatment is very important to prevent irreversible damage of target organs as shown in a study with 1044 adults (641 men and 403 women) enrolled in the Fabry Registry, in which many patients had advanced disease by the time of ERT initiation—among the patients who started ERT <40 years of age (n = 526), 37% already had left ventricular hypertrophy (LVH) and 24% had an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2. When analyzing the population that started ERT at 40 years of age or more (n = 518), 33% had an eGFR <60 mL/min/1.73 m^2 and 79% LVH.[33]

In a systematic review of 20 case-finding studies, the overall prevalence of FD in men on dialysis was 0.33% and in women was 0.1%; the combined prevalence in renal transplant was 0.38% in men and 0% in women; in patients with LVH, the overall prevalence ranged from 0.9% to 3.9% in men and 1.1% to 11.8% in women, depending on the selection of study population and study method.[34]

Kitagawa and colleagues proposed that the measurement of GL-3 in urine by tandem mass spectrometry could be a reliable screening method in hemizygotic patients.[35]

We still have to overcome some challenges in screening or case-finding studies, such as the fact that enzyme assay is not suitable for screening females, thus the screening for mutations on the *GLA* gene can be performed in this population; however, this is a laborious method because FD does not have common mutations and full sequencing of the *GLA* gene is necessary, and there are some variants that have unknown effect.[24,36]

Molecular Diagnosis

The human *GLA* gene is organized into 7 exons encompassing over 12 kb; the exons range in length from 92 to 291 bp. There are several possible regulatory elements in the 5′-flanking region such as, ''CCAAT'' box, enhancer elements, and sequences corresponding to the activator protein 1, octanucleotide, and ''core'' enhancer element but no typical ''TATA'' box. Moreover, the *GLA* gene has an unmethylated CpG-rich island upstream of the initiation codon.[37] The messenger RNA encodes a protein of 429 amino acids, including the N-terminal signal peptide of 31 amino acids.[37–39] After cleavage of the signal peptide, posttranslational modifications occur in the Golgi and lysosomes and a mature protein is formed.[40]

Mutations in the *GLA* gene have been identified using DNA sequencing. The 7 exons of *GLA* gene are amplified by polymerase chain reaction. Sense and antisense oligonucleotide primers are synthesized based on the sequences flanking the 7 exons of the *GLA* gene. Sequences are compared with the reference sequence NG_007119 (http://www.ncbi.nih.gov) and confirmed by reverse strand sequencing. To date, more than

840 mutations have been reported in the *GLA* gene in the Human Gene Mutation Database,[41] that are believed to cause FD. Of these, 589 are missense and nonsense mutations, 42 are splicing substitutions, 4 are regulatory substitutions, 114 are small deletions, 40 are small insertions, 11 are small indels, 35 are gross indels, 4 are gross insertions, and 6 are complex rearrangements.

In a study conducted in our laboratory with 568 individuals from 102 families with suspected FD, we found 51 families presenting 38 different alterations in the *GLA* gene, among which 19 were not previously reported in the literature.[42] Most mutations are detected in only 1 family, and it is necessary to determine the physiological impact of these new mutations. In order to predict the possible impact of the new mutations on the structure and function of a human protein, different silico predictors are used.

In addition, in our sample we found an intronic haplotype with 4 variants described in individuals with suspected FD. One variant, the c.-10C>T (rs2071225), was found in the untranslated region of exon 1. It is described in the literature as polymorphism and appears in 12% of the population.[43] (The single nucleotide polymorphism database [dbSNP] can be found at http://www.ncbi.nlm.nih.gov/SNP.) The variants c.370-77_370-81delCAGCC (rs5903184), c.640-16A>G (rs2071397), and c.1000-22C>T (rs2071228) are also found in intronic and are described in the literature as nonpathogenic polymorphisms (dbSNP). Although these variants are described as nonpathogenic, the impact of these changes together is unknown. Considering that these regions are not routinely evaluated by gene sequencing, the prevalence of FD may be underestimated.[44]

Challenges in GLA *Gene Variants*

Most of the disease-causing mutations are de novo (private mutations) restricted to 1 single family or patient.[1,2,45] Because of the frequency of novel mutations, it is necessary to sequence the entire *GLA* gene and flanking regions to identify the FD mutation in a family. Also, as previously reported by Laney et al in 2013, several familial mutations were identified by comparative genomic hybridization (CGH) array. They were caused by a microdeletion or microduplication in the *GLA* gene, where conventional genomic sequencing did not identify a point mutation.[46]

The genotype–phenotype correlation in FD is difficult to determine due to several causes. First, unlike other metabolic disorders, there are a high number of de novo mutations, implying that the majority of families carry different mutations.[9,47] Another cause that impedes the correlation is the clinical variability among patients carrying the same mutation, even among patients in the same family. Recently, it has been hypothesized that part of the phenotype can be modified by nongenetic features, such as accumulation of the misfolded defective enzyme.[9]

In 2004, Garman and Garboczi[48] mapped *GLA* mutations onto a crystallographic model of the structure of α-gal A enzyme and correlated genotype and phenotype by a meta-analysis of the phenotypes reported in the literature.[9] It appears that missense mutations that cause a mild phenotype affect areas more distant from the enzyme active site. On the other hand, missense mutations associated with severe FD affect areas that are near the active site, leading to a more dysfunctional or inactive enzyme.[9,48]

Nonsense mutations that lead to a premature stop codon usually result in an inactive protein or do not result in any proteic product (''null'' allele). Missense mutations, on the other hand, may severely reduce enzyme activity, but there may be some residual activity.[49] Patients with residual enzyme activity have been described as exhibiting a later onset of renal cardiac and neurovascular involvement, a decreased prevalence of neuropathic pain, and milder/later onset of other symptoms compared with those individuals with no residual enzyme activity.[9,49]

Several mutations are currently being reported as of unknown significance, causing controversy in literature, showing that the diagnostic of FD in patients with nonspecific symptoms such as LVH and a nonclassical phenotype can be difficult. Misdiagnoses may occur in patients presenting with GLA mutations and isolated nonspecific findings, such as LVH. In these cases, genetic and enzyme analyses are often not sufficient to diagnose FD. A structured diagnostic approach is mandatory, including electron microscopy of a biopsy of an affected organ.[50]

Previous articles discuss the pathogenicity of a mutation in an attempt not to misdiagnose a patient as having FD, starting a very expensive treatment without needing it.

The D313Y genotype, previously reported in literature as a benign mutation not clinically relevant toFD,[11] has been reported in 2016 as having a significant impact on health-related quality of life in respective individual patients; this mutation might represent a cofounding risk factor for certain isolated symptoms triggering a specific mild clinical variant of FD.[51]

The p.A143T and p.R112H variants are other controversial mutations. The p.A143T variant has previously been reported to be associated with a renal variant of FD.[30] Later, Terryn et al reported patients with the p.A143T variant as having no storage in biopsies with light microscopy. They concluded that the variant is most likely nonpathogenic. Additionally, individuals with the p.A143T variant have no increase in plasma Gb3 and lyso-Gb3.[52]

When facing a new or controversial mutation, patients presenting any symptomatology should be thoroughly investigated to evaluate organic damage, performing brain and heart MRI, nephrologic evaluation, and laboratory assessment. In cases of single-organ impairment, a targeted biopsy showing signs of deposit can confirm or discard the diagnostic and should be performed in order to correctly indicate ERT.

Indications for Treatment

Many questions about the optimal time to initiate FD treatment are still present in clinical practice. The main studies guide the

physician who has the autonomy of therapeutic decision as he or she often already knows the family history and the complications involved.

Information about the evolution of the disease is not always available, so some suggestions can be applied. First, the differentiation according to gender should be made by the team that treats a patient with FD. Another feature to be considered is whether the disease presents itself in classical or nonclassical form, as described above. In most consensus and expert opinions on FD, those with classical or nonclassical disease should be treated as soon as the first target organ impairment signals are noted, such as in the kidney, heart, or central nervous system.[6,53–55] In Brazil, for all ages greater than or equal to 18, treatment should be indicated if the following are present:

- microalbuminuria, proteinuria, or renal failure,
- cardiac hypertrophy, even without signs of fibrosis or cardiac arrhythmia signals (such as sinus bradycardia or cardiac repolarization modifications),
- white matter lesions or transient ischemic attack,
- gastrointestinal symptoms (GIS) with chronic diarrhea or abdominal pain (GIS may be due to neuropathic and myopathy changes leading to symptoms of dysmotility[56]), or
- acroparesthesia, even if controlled with analgesics, since the neuropathic pain is associated with glycosphingolipid accumulation in small fiber neuropathy, and the combination of acroparesthesia and mild glomerular endothelial cell deposits and arteriopathy may constitute a clinical and morphological combination heralding a potentially progressive renal disease.[57,58]

In relation to a glomerular filtration rate indicating hyperfiltration was excluded as an essential criterion for treatment of FD because it is an unspecific and unclear clinical relevance finding, even considered a confounding factor for some nephrologists.[55]

Another topic to be discussed is the indication of treatment for children. An ERT is safe for this age-group.[59] The initiation of treatment among this group is also controversial. Studies show a lower accumulation of kidney substrate, especially if ERT starts before the age of 10. Recent research has shown that early treatment slows disease progression.[57,60–62]

When the diagnosis is made by neonatal or family screening or its mutation is not a classic one, medical decisions should be based on clinical information of other family members if available. However, an intravenous treatment can be initiated early, before symptoms can decrease school performance and lead children to being labeled chronically ill.[63] Thus, the decision to treat should be careful, involving the patient, family, and health-care team.[59] A good doctor–patient relationship is the key to success and the decision to start ERT must be evaluated on a case-by-case basis by physicians.[54]

The treatment can be considered mainly for boys between 8 and 10 years old with classical FD. There are renal deposit findings before microalbuminuria begins.[59,64] In children with nonclassic disease, the current recommendation is frequent monitoring with different medical specialties. That treatment should be promptly initiated when symptoms emerge or in the presence of an abnormal renal biopsy.[59]

Conclusion

The challenges in the diagnosis and indications for treatment of FD are part of today's clinical practice. Thus far, we have learned that the enzymatic assay is diagnostic for men, while women most often need the molecular study to have a definite diagnosis. The enzyme activity assay in DBS has its established use in case studies in high-risk populations due to the stability of the enzyme in this kind of sample and ease of shipping. The effects of controversial mutations in exons or multiple intronic are not yet fully understood and seem to have individual variations. In this situation, the disease staging of the patient will give us the answer if the ERT is indicated or not. We believe today that treatment is indicated in children with acroparesthesia due to the relationship that seems to exist with their presence and renal impairment. In conclusion, FD is still being studied and in the medium-/long-term, we will have some answers and probably more questions.

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References

1. Desnick RJ, Ioannou YA, Eng CM. α-Galactosidase A deficiency: Fabry disease. In: Valle D, Beaudet AL, Vogelstein B, Kinzler KW, Antonarakis SE, Ballabio A, Gibson K, Mitchell G, eds. *The Online Metabolic and Molecular Bases of Inherited Disease*. New York, NY: McGraw-Hill; 2014. http://ommbid.mhmedical.com/content.aspx?bookid=971&Sectionid=62644837. Accessed September 22, 2016.
2. Germain DP. Fabry disease. *Orphanet J Rare Dis*. 2010;5:30.
3. Glass RB, Astrin KH, Norton KI, et al. Fabry disease: renal sonographic and magnetic resonance imaging findings in affected males and carrier females with the classic and cardiac variant phenotypes. *J Comput Assist Tomogr*. 2004;28(2):158-168.
4. 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(3):788-794.
5. Wang RY, Lelis A, Mirocha J, Wilcox WR. Heterozygous Fabry women are not just carriers, but have a significant burden of disease and impaired quality of life. *Genet Med*. 2007;9(1):34-45.
6. Wilcox WR, Oliveira JP, Hopkin RJ, et al; Fabry Registry. Females with Fabry disease frequently have major organ involvement: lessons from the Fabry Registry. *Mol Genet Metab*. 2008; 93(2):112-128.

7. Weidemann F, Niemann M, Sommer C, Beer M, Breunig F, Wanner C. Interdisciplinary approach towards female patients with Fabry disease. *Eur J Clin Invest*. 2012;42(4):455-462.
8. Schaefer E, Mehta A, Gal A. Genotype and phenotype in Fabry disease: analysis of the Fabry Outcome Survey. *Acta Paediatr Suppl*. 2005;94(447):87-92; discussion 79.
9. Mehta A, Beck M, Sunder-Plassmann G, eds. *Fabry Disease: Perspectives From 5 Years of FOS*. Oxford, UK: Oxford PharmaGenesis; 2006.
10. Lenders M, Duning T, Schelleckes M, et al. Multifocal white matter lesions associated with the D313Y mutation of the alpha-galactosidase A gene. *PLoS One*. 2013;8(2):e55565.
11. Niemann M, Rolfs A, Giese A, et al. Lyso-Gb3 indicates that the alpha-galactosidase A mutation D313Y is not clinically relevant for Fabry disease. *JIMD Rep*. 2013;7:99-102.
12. Tolouian R, Salameh H. Treat the patient not the lab value. *NDT Plus*. 2010;3(1):81-83.
13. Gal A, Hughes DA, Winchester B. Toward a consensus in the laboratory diagnostics of Fabry disease – recommendations of a European expert group. *J Inherit Metab Dis*. 2011;34(2):509-514.
14. Aerts JM, Groener JE, Kuiper S, et al. Elevated globotriaosylsphingosine is a hallmark of Fabry disease. *Proc Natl Acad Sci U S A*. 2008;105(8):2812-2817.
15. Rombach SM, Dekker N, Bouwman MG, et al. Plasma globotriaosylsphingosine: diagnostic value and relation to clinical manifestations of Fabry disease. *Biochim Biophys Acta*. 2010;1802(9): 741-748.
16. van Breemen MJ, Rombach SM, Dekker N, et al. Reduction of elevated plasma globotriaosylsphingosine in patients with classic Fabry disease following enzyme replacement therapy. *Biochim Biophys Acta*. 2011;1812(1):70-76.
17. Smid BE, van der Tol L, Biegstraaten M, Linthorst GE, Hollak CE, Poorthuis BJ. Plasma globotriaosylsphingosine in relation to phenotypes of Fabry disease. *J Med Genet*. 2015;52(4):262-268.
18. Auray-Blais C, Boutin M, Gagnon R, Dupont FO, Lavoie P, Clarke JT. Urinary globotriaosylsphingosine-related biomarkers for Fabry disease targeted by metabolomics. *Anal Chem*. 2012; 84(6):2745-2753.
19. Auray-Blais C, Ntwari A, Clarke JT, et al. How well does urinary lyso-Gb3 function as a biomarker in Fabry disease? *Clin Chim Acta*. 2010;411(23-24):1906-1914.
20. Müller KB, Rodrigues MDB, Pereira VG, Martins AM, D'Almeida V. Reference values for lysosomal enzymes activities using dried blood spots samples – a Brazilian experience. *Diagn Pathol*. 2010;5:65.
21. Li Y, Scott CR, Chamoles NA, et al. Direct multiplex assay of lysosomal enzymes in dried blood spots for newborn screening. *Clin Chem*. 2004;50(10):1785-1796.
22. Zhang XK, Elbin CS, Turecek F, et al. Multiplex lysosomal enzyme activity assay on dried blood spots using tandem mass spectrometry. *Methods mol biol (Clifton, NJ)*. 2010;603: 339-350.
23. Tortorelli S, Turgeon CT, Gavrilov DK, et al. Simultaneous testing for 6 lysosomal storage disorders and X-adrenoleukodystrophy in dried blood spots by tandem mass spectrometry. *Clin Chem*. 2016; 62(9):1248-1254.
24. van der Tol L, Smid BE, Poorthuis BJ, et al. A systematic review on screening for Fabry disease: prevalence of individuals with genetic variants of unknown significance. *J Med Genet*. 2014;51(1):1-9.
25. Grunfeld JP. How to improve the early diagnosis of Fabry's disease? *Kidney Int*. 2003;64(3):1136-1137.
26. Martins AM, Kyosen SO, Garrote J, et al. Demographic characterization of Brazilian patients enrolled in the Fabry Registry. *Genet Mol Res*. 2013;12(1):136-142.
27. Grimes DA, Schulz KF. Uses and abuses of screening tests. *Lancet*. 2002;359(9309):881-884.
28. Eng CM, Guffon N, Wilcox WR, et al; International Collaborative Fabry Disease Study Group. Safety and efficacy of recombinant human alpha-galactosidase A – replacement therapy in Fabry's disease. *N Engl J Med*. 2001;345(1):9-16.
29. Schiffmann R, Kopp JB, Austin HA III, et al. Enzyme replacement therapy in Fabry disease: a randomized controlled trial. *JAMA*. 2001;285(21):2743-2749.
30. Spada M, Pagliardini S, Yasuda M, et al. High incidence of later-onset fabry disease revealed by newborn screening. *Am J Hum Genet*. 2006;79(1):31-40.
31. Hwu WL, Chien YH, Lee NC, et al. 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.
32. Inoue T, Hattori K, Ihara K, Ishii A, Nakamura K, Hirose S. Newborn screening for Fabry disease in Japan: prevalence and genotypes of Fabry disease in a pilot study. *J Hum Genet*. 2013; 58(8):548-552.
33. Ortiz A, Abiose A, Bichet DG, et al. Time to treatment benefit for adult patients with Fabry disease receiving agalsidase beta: data from the Fabry Registry. *J Med Genet*. 2016;53(7):495-502.
34. Linthorst GE, Bouwman MG, Wijburg FA, Aerts JM, Poorthuis BJ, Hollak CE. Screening for Fabry disease in high-risk populations: a systematic review. *J Med Genet*. 2010;47(4):217-222.
35. Kitagawa T, Ishige N, Suzuki K, et al. Non-invasive screening method for Fabry disease by measuring globotriaosylceramide in whole urine samples using tandem mass spectrometry. *Mol Genet Metab*. 2005;85(3):196-202.
36. Linthorst GE, Vedder AC, Aerts JM, Hollak CE. Screening for Fabry disease using whole blood spots fails to identify one-third of female carriers. *Clin Chim Acta*. 2005;353(1-2):201-203.
37. Bishop DF, Kornreich R, Desnick RJ. Structural organization of the human alpha-galactosidase A gene: further evidence for the absence of a 3′ untranslated region. *Proc Natl Acad Sci U S A*. 1988;85(11):3903-3907.
38. Bishop DF, Calhoun DH, Bernstein HS, Hantzopoulos P, Quinn M, Desnick RJ. Human alpha-galactosidase A: nucleotide sequence of a cDNA clone encoding the mature enzyme. *Proc Natl Acad Sci U S A*. 1986;83(13):4859-4863.
39. Blom D, Speijer D, Linthorst GE, Donker-Koopman WG, Strijland A, Aerts JM. Recombinant enzyme therapy for Fabry disease: absence of editing of human alpha-galactosidase A mRNA. *Am J Hum Genet*. 2003;72(1):23-31.
40. Eng CM, Desnick RJ. Molecular basis of Fabry disease: mutations and polymorphisms in the human alpha-galactosidase A gene. *Hum Mutat*. 1994;3(2):103-111.

41. Stenson PD, Mort M, Ball EV, et al. The Human Gene Mutation Database: 2008 update. *Genome Med*. 2009;1(1):13.
42. Turaca LT, Pessoa JG, Motta FL, et al. New mutations in the GLA gene in Brazilian families with Fabry disease. *J Hum Genet*. 2012; 57(6):347-351.
43. Geer LY, Marchler-Bauer A, Geer RC, et al. The NCBI BioSystems database. *Nucleic Acids Res*. 2010;38(Database issue): D492-D496. Epub ahead of print October 23, 2009.
44. Gervas-Arruga J, Cebolla JJ, Irun P, et al. Increased glycolipid storage produced by the inheritance of a complex intronic haplotype in the alpha-galactosidase A (GLA) gene. *BMC Genet*. 2015; 16:109.
45. Ashton-Prolla P, Tong B, Shabbeer J, Astrin KH, Eng CM, Desnick RJ. Fabry disease: twenty-two novel mutations in the alpha-galactosidase A gene and genotype/phenotype correlations in severely and mildly affected hemizygotes and heterozygotes. *J Invest Med*. 2000;48(4):227-235.
46. Laney DA, Bennett RL, Clarke V, et al. Fabry disease practice guidelines: recommendations of the National Society of Genetic Counselors. *J Genet Couns*. 2013;22(5):555-564.
47. Schafer E, Baron K, Widmer U, et al. Thirty-four novel mutations of the GLA gene in 121 patients with Fabry disease. *Hum Mutat*. 2005;25(4):412.
48. Garman SC, Garboczi DN. The molecular defect leading to Fabry disease: structure of human alpha-galactosidase. *J Mol Biol*. 2004; 337(2):319-335.
49. Branton MH, Schiffmann R, Sabnis SG, et al. Natural history of Fabry renal disease: influence of alpha-galactosidase A activity and genetic mutations on clinical course. *Medicine*. 2002;81(2): 122-138.
50. Smid BE, Hollak CE, Poorthuis BJ, et al. Diagnostic dilemmas in Fabry disease: a case series study on GLA mutations of unknown clinical significance. *Clin Genet*. 2015;88(2):161-166.
51. Oder D, Uceyler N, Liu D, et al. Organ manifestations and long-term outcome of Fabry disease in patients with the GLA haplotype D313Y. *BMJ Open*. 2016;6(4):e010422.
52. Terryn W, Vanholder R, Hemelsoet D, et al. Questioning the pathogenic role of the GLA p.Ala143Thr ''mutation'' in Fabry disease: implications for screening studies and ERT. *JIMD Rep*. 2013;8:101-108.
53. Desnick RJ, Brady R, Barranger J, et al. Fabry disease, an underrecognized multisystemic disorder: expert recommendations for diagnosis, management, and enzyme replacement therapy. *Ann Intern Med*. 2003;138(4):338-346.
54. Martins AM, D'Almeida V, Kyosen SO, et al. Guidelines to diagnosis and monitoring of Fabry disease and review of treatment experiences. *J Pediatr*. 2009;155(suppl 4):S19-S31.
55. Biegstraaten M, Arngrimsson R, Barbey F, et al. Recommendations for initiation and cessation of enzyme replacement therapy in patients with Fabry disease: the European Fabry Working Group consensus document. *Orphanet J Rare Dis*. 2015;10:36.
56. Zar-Kessler C, Karaa A, Sims KB, Clarke V, Kuo B. Understanding the gastrointestinal manifestations of Fabry disease: promoting prompt diagnosis. *Therap Adv Gastroenterol*. 2016;9(4):626-634.
57. Tondel C, Bostad L, Hirth A, Svarstad E. Renal biopsy findings in children and adolescents with Fabry disease and minimal albuminuria. *Am J Kidney Dis*. 2008;51(5):767-776.
58. Bertelsen AK, Tondel C, Krohn J, et al. Small fibre neuropathy in Fabry disease. *J Neurol*. 2013;260(3):917-919.
59. Hopkin RJ, Jefferies JL, Laney DA, et al. The management and treatment of children with Fabry disease: A United States-based perspective. *Mol Genet Metab*. 2016;117(2):104-113.
60. Wang RY, Bodamer OA, Watson MS, Wilcox WR; ACMG Work Group on Diagnostic Confirmation of Lysosomal Storage Diseases. Lysosomal storage diseases: diagnostic confirmation and management of presymptomatic individuals. *Genet Med*. 2011; 13(5):457-484.
61. Tondel C, Bostad L, Larsen KK, et al. Agalsidase benefits renal histology in young patients with Fabry disease. *J Am Soc Nephrol*. 2013;24(1):137-148.
62. Tondel C, Kanai T, Larsen KK, et al. Foot process effacement is an early marker of nephropathy in young classic Fabry patients without albuminuria. *Nephron*. 2015;129(1):16-21.
63. Bugescu N, Naylor PE, Hudson K, Aoki CD, Cordova MJ, Packman W. The psychosocial impact of Fabry disease on pediatric patients. *J Pediatr Genet*. 2016;5(3):141-149.
64. Wijburg FA, Benichou B, Bichet DG, et al. Characterization of early disease status in treatment-naive male paediatric patients with Fabry disease enrolled in a randomized clinical trial. *PloS One*. 2015;10(5):e0124987.