IUBMB *Life*, 61(11): 1043–1050, November 2009

Critical Review

Fabry Disease: Treatment and Diagnosis

Paula A. Rozenfeld
LISIN, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina

Summary

Fabry disease is an X-linked lysosomal disorder that results from a deficiency of the lysosomal enzyme α-galactosidase A leading to accumulation of glycolipids, mainly globotriaosylceramide in the cells from different tissues. Classical Fabry disease affects various organs. Clinical manifestations start at early age and include angiokeratoma, acroparesthesia, hypohydrosis, heat/exercise intolerance, gastrointestinal pain, diarrhea, and fever. The main complications of Fabry disease are more prominent after the age of 30 when kidney, heart, and/or cerebrovascular disorders appear. Most of the heterozygous females are symptomatic. Enzyme replacement therapy (ERT) is the only specific treatment for Fabry disease. The beneficial effect of ERT on different organs/systems has been extensively evaluated. Quality of life of patients receiving ERT is improved. Enzyme replacement stabilizes or slows the decline in renal function and reduces left ventricular hypertrophy. Fabry disease may be underdiagnosed because of nonspecific and multiorgan symptoms. Different screening strategies have been carried out in different at-risk populations in order to detect undiagnosed Fabry patients. An increasing knowledge about Fabry disease within the medical community increases the chances of patients to receive a timely diagnosis and, consequently, to access the appropriate therapy. © 2009 IUBMB
IUBMB *Life*, 61(11): 1043–1050, 2009

Keywords Fabry disease; lysosomal storage disorders; α-galactosidase A; globotriaosylceramide; enzyme replacement therapy; diagnosis.

INTRODUCTION

Fabry disease (OMIM 301500) is an X-linked disorder of glycosphingolipid catabolism that results from a deficiency of the lysosomal enzyme α-galactosidase A (α-D-galactoside galactohydrolase, EC 3.2.1.22; α-Gal A) (*1*, *2*). Patients with absolute deficiency of α-Gal A activity present the classical Fabry phenotype, whereas those with residual activity have a milder or later-onset variant. This defect leads to the accumulation of the enzyme substrates, mainly globotriaosylceramide (Gal α 1–4Gal β 1–4Glc β 1-1Cer; Gb3; or ceramide trihexoside) in lysosomes of a variety of different cell types throughout the body (*3*). It was initially described as "angiokeratoma corporis diffusum" by the dermatologists Johannes Fabry (*4*) and William Anderson (*5*) in 1898.

INCIDENCE

The incidence of Fabry disease has been estimated to range from 1/40,000 to 1/117,000 live births for males (*6*, *7*). No ethnic predilection has been observed. Fabry disease may be underdiagnosed because of nonspecific and multiorgan symptoms. Moreover, it is supposed that many heterozygotes remain undiagnosed, due to the less severe clinical manifestations of female patients.

A recent report on a screening test for newborns in Italy has found an incidence of α-Gal A deficiency of 1/3,100 newborn males (*8*). However, all but one of the newborns identified in this study had mutations predicting the later-onset phenotype. Taking into account the unique case with the classical phenotype associated-mutation, the incidence of this phenotype would be 1/37,000, consistent with reported estimates (*7*). Therefore, the estimated ratio of later-onset:classical phenotypes would be 11:1.

CLINICAL MANIFESTATIONS

Lysosomal storage diseases (LSD) usually present a wide spectrum of clinical severity. Fabry disease is not an exception. The most severe presentation accounts for the classical disease, which affects various organs. Patients with the classical form have low or very little GLA activity. Milder or later-onset variants with manifestations circumscribed mainly to one organ, such as, the "cardiac variant" and "the renal variant," have been also described (*9*, *10*). Patients with this mild presentation

Received 19 June 2009; accepted 10 August 2009
Address correspondence to: Dra Paula Rozenfeld, LISIN, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 y 115, 1900 La Plata, Argentina. Tel.: 0054-221-4250497; Fax: 0054-221-4226947. E-mail: paurozen@biol.unlp.edu.ar

ISSN 1521-6543 print/ISSN 1521-6551 online
DOI: 10.1002/iub.257

have residual α-galactosidase A activity (between 1 and 20% of normal levels).

Cardiac Variant

Clinical manifestations are limited to heart and present in the sixth or seventh decade of life. This variant is characterized by left ventricular concentric hypertrophy, and by the lack of angiokeratoma, acroparesthesias, hypohydrosis, or corneal opacities. Proteinuria can be present but renal function is generally not affected. Mutations detected in cardiac variant patients' are missense mutations or intronic lesions that reduced mRNA levels (*10*).

Renal Variant

Patients develop end-stage renal disease at ages similar to those of classically affected patients, but lack the other classical manifestations. Most of these cases are initially diagnosed as chronic glomerulonephritis (*9*).

Classical Fabry Disease

Patients with classical Fabry disease show signs and symptoms affecting various organs. In affected patients with classical Fabry disease, manifestations start at early age, during the first decade of life. Symptoms, such as, acroparesthesia, hypohydrosis, heat/exercise intolerance, gastrointestinal pain, diarrhea, and fever (*11–13*).

The typical skin manifestation of Fabry disease, the angiokeratomas present in adolescence and are mainly found in bathing trunk area and umbilicus (*14*). They can be found also in oral mucosa, fingers, and thorax. Corneal opacity (cornea verticillata) is a frequent sign in Fabry disease, but does not reduce vision, and is generally detected by trained ophthalmologists (*15*).

Male Fabry patients show facial minor dysmorphic features, such as, periorbital fullness, prominent supraorbital ridges, bushy eyebrows, pronounced nasal angle, generous nose/bulbous nasal tip, shallow midface, full lips, prominent nasal bridge, broad alar base, and posteriorly rotated ears (*16*, *17*).

Auditory and vestibular symptoms includes tinnitus, hearing loss, and vertigo (*18*). Patients also suffer from cefalea. Psychiatric studies revealed a high incidence of severe depression (*19*) that is related to the degree to which symptoms interfered with normal life.

The main complications of Fabry disease are more prominent after the age of 30 when kidney, heart and/or cerebrovascular disorders appear (*20*). These manifestations are the most frequent cause of death and reduce life expectancy by 20 years compared with the normal population. The earliest manifestation of renal involvement is microalbuminuria or proteinuria. During the third decade of life, a progressive reduction of glomerular filtration rate develops (*21*). The decline in renal function ultimately leads to requirement of dialysis or kidney transplantation. Cardiac complications are diverse, and includes concentric hypertrophic miocardiopathy, diastolic dysfunction, and valvular or conduction defects (*22*). Magnetic resonance imaging frequently reveals white matter lesions (*23*), and cerebrovascular events, such as, transient ischemic attack or stroke occur, sometimes before the development of renal or cardiac events (*24*).

Heterozygotes

Fabry disease is an X-linked disorder, neither recessive nor dominant (*25*). The penetrance of Fabry disease in females is quite high, with at least 70% of females showing clinical manifestations of the disease (*26*). For this reason, when referring to females with Fabry disease, the term "carrier" should be avoided and replaced by the term "heterozygotes." According to the X-linked inheritance of Fabry disease, the number of female cases is likely to double that of male cases. However, registries of Fabry disease usually contain a similar number of female and male patients enrolled. This suggests the existence of a substantial number of undiagnosed heterozygous, whose data are missing. For this reason, data from females enrolled in registries should not be interpreted as representing the whole universe of heterozygous.

Disease expression in females depends on the chromosome X inactivation pattern. As a result of the process of chromosome X inactivation (*27*), females show a mosaic of cells expressing genes from maternal origin or paternal origin. Cells with normal α-Gal A activity cannot correct the deficiency of the enzyme in cells expressing mutant α-Gal A, and both type of cells contribute to the organism (*26*). The ratio between normal cells and mutant cells determines the phenotype of the female patient. Even females with random X inactivation patterns (*28*) are symptomatic. Unfavorably skewed X inactivation may result in a high severity of disease manifestations (*29*, *30*). Age at onset of symptoms in females is generally older and more variable than in males. Disease manifestations in heterozygotes are more diverse than in males. Severe complications in females are most often associated to heart disease and stroke (*31*). Life expectancy of heterozygotes is 70 years, 15 years shorter than that of the general female population (*20*).

TREATMENT

Symptomatic treatment was the only possible therapy for Fabry disease until 2001, when a specific treatment by enzyme replacement therapy (ERT) became available (*32*, *33*). This treatment is based on a biweekly intravenous infusion of the recombinant human α-galactosidase A. Two drugs are now commercially available: agalsidase alpha (Replagal, Shire HGT) and agalsidase beta (Fabrazyme, Genzyme) which are based on enzyme preparations produced in human fibroblasts and CHO cell line, respectively. Primary structure of both polypeptides is the same, but the glycosylation pattern may vary according to the species from which the cell line is derived. Studies of both proteins suggest that they are biochemically, structurally, and functionally equivalent (*34*). A study comparing both enzymes at the same dose did not reveal any difference in clinical effect (*35*).

The beneficial effect of ERT on different organs/systems has been extensively evaluated. Quality of life of patients receiving ERT is improved and pain severity is reduced (*36*). Gastrointestinal manifestations including abdominal pain are alleviated by ERT (*37*). Enzyme replacement stabilizes or slows the decline in renal function (*38*, *39*) as opposed to a decline of glomerular filtration rate of 7 mL/min/year in nontreated patients (*21*). However, proteinuria does not seem to be improved (*40*). Studies in kidney transplanted patients show that ERT is safe and effective to reduce the progression of extra-renal manifestations of Fabry disease (*41*, *42*). A benefit on cardiac disease has been also reported, with a reduction in left ventricular mass in patients with left ventricular hypertrophy (*43*). Agalsidase alpha has been shown to stabilize, and possibly improve vestibular function (*44*) and hearing in Fabry patients who have not already progressed to severe hearing loss (*45*). However, patients suffering from cerebrovascular disease have not shown a significant improvement after treatment (*46*).

In general, therapies based on the infusion of recombinant proteins induce a specific immune response due to the immunogenicity of the infused polypeptide. Regarding safety and tolerability, infusion reactions occur in 10–50% of patients receiving agalsidase alfa or beta, respectively. These reactions usually consist of fever, redness, rhinitis, and/or rigors. IgG antibodies develop in 55–80% of patients (*32*, *33*). IgE has been reported in patients receiving agalsidase beta (*47*), but not with agalsidase alfa. Treatment related adverse events can be avoided by reducing the infusion rate and/or by premedication with antihistamines and/or corticosteroids. Antibodies against the enzyme seem to impair the efficacy of ERT in some patients (*48*), but more long-term studies should be carried out to confirm these findings.

Home treatment is feasible and safe, and constitutes clear benefit for patients because it produces less alteration of their usual activities and improves compliance with this life-long ERT (*49*). Patients receive ERT at hospital under careful medical supervision for a period of 3–6 months to check for any adverse events or complications, and then they can be transferred to a home infusion setting (*50*).

Because the experience with ERT for Fabry disease is limited to the last 8 years, there are no absolute conclusions about how ERT is able to change the natural history of Fabry disease. Moreover, there are yet no answers to the question about the optimal age to start ERT. Taking into account that studies in adult patients suggest that ERT could prevent or slow down the progression of tissues alterations, it may be hypothesized that initiation of ERT at an early age would reduce the massive Gb3 deposition that leads to irreversible organ damage (*51*). However, studies evaluating this concept have not been reported yet.

PATHOPHYSIOLOGY

Alterations observed in Fabry disease are primarily due to the deficiency of α-Gal A, and the consequent accumulation of glycolipids in lysosomes. However, lysosomes are not only the site of Gb3 accumulation. Using immunodetection, Gb3 was shown to be distributed in other cellular structures including ER, cell membrane, and nucleus (*52*). The distribution of Gb3 accumulation was heterogeneous among different organs/tissues, with the stronger staining found in heart and kidney, the organs that accumulate the greatest amounts of Gb3.

Gb3, also known as CD77, is a differentiation antigen expressed on B cells in the germinal centers (*53*). These cells undergo rapid and spontaneous apoptosis when isolated and cultured *in vitro* (*54*).

The anomalous accumulation of Gb3 in Fabry disease could be associated to different mechanisms leading to disease. The exact molecular mechanism that translates the primary insult into cell damage remains to be determined. Other concurrent pathological mechanisms could be elicited, contributing to the phenotypic expression of the disease (*55*). Dr. Schiffmann's group has focused its studies on vascular disorder of Fabry disease, and has demonstrated the presence of abnormalities in blood flow, vessel wall, and blood components (*56*). In terms of alterations of blood components, they have detected an inflammation-activated state in endothelium and leukocytes in Fabry disease (*57*, *58*).

Other studies have focused on possible proinflammatory aspects of Fabry disease, since the suggestion of the existence of a proinflammatory status in Fabry disease (*59*). There are reports showing the coexistence of Fabry disease and autoimmune disorders (*60*, *61*). Abnormalities in the number of immune cell subsets in Fabry patients have been recently described (*62*). The gene NAIP (Neuronal apoptosis inhibitory protein) associated with inflammation was found to be upregulated in Fabry children (*63*). Exposure of endothelial cells to Gb3 resulted in increased intracellular production of reactive oxygen species (*57*).

Another compound related to Gb3, lyso Gb3, was recently found to be increased in plasma from Fabry patients. This compound has been shown to induce smooth muscle proliferation, which could account for the hypertrophy of the wall of blood vessels (*64*).

The phenotypic spectrum of Fabry disease could be due to interactions with other genes that could modify the clinical expression. This was demonstrated for certain polymorphisms in genes coding for endothelial nitric oxide synthase, interleukin-6, and factor V Leiden (*65*, *66*).

More studies to detect pathophysiological mechanisms of Fabry disease are encouraged in order to understand how the primary defect translates into organ damage and clinical manifestations. This knowledge would also be beneficial to understand the effect of specific therapy and to improve further its efficacy.

DIAGNOSIS

Diagnosis of Index Cases. Fabry disease is difficult to diagnose in clinical practice. Disease manifestations in childhood

are diverse and unspecific, involve various organs, and are easily confused with other pathologies, including rheumatic diseases. A previous misdiagnosis is not uncommon (*67*). Fabry diagnosis has been described as an "odyssey": Fabry patients visit an average of 10 different medical specialists along 10 years before achieving confirmatory diagnosis. These facts lead to a delay of 10 years between age at symptoms onset and age at diagnosis. Suspicion of the disease in index cases often comes from nephrologists, dermatologists or geneticists, among others. For this reason, dissemination of knowledge about Fabry disease, its diagnosis and the necessity to treat the patient using a multidisciplinary biomedical approach is crucial for improving Fabry disease diagnosis (*68*).

Generally, index cases detected by suspicion based on clinical manifestations are affected males. When a new Fabry male is diagnosed a new Fabry family is detected. After the diagnosis of an index case, a screening of the relatives allows confirmatory diagnosis of other affected members.

Confirmatory Diagnosis. As with other LSD, the diagnosis of Fabry disease is generally carried out in a reference specialized lab. Implementation of the diagnostic methods requires a complete knowledge and grasp of the pathologies by the professionals involved. This requirement adds another obstacle to the ready access to a confirmatory diagnosis.

After disease suspicion by a physician, confirmation of diagnosis in hemizygous males is made by demonstrating absent or reduced GLA enzymatic activity. Because heterozygous females can have low or normal GLA activity values, diagnosis confirmation in females is not based on enzymatic activity determination (*69*). The determination of GLA enzymatic activity is carried out on blood leukocytes or cultured skin fibroblasts. Recently, determination of GLA activity on dried blood filter paper was introduced (*70*). This method has proven to be reliable with high sensitivity and specificity to diagnose male Fabry patients. Blood samples collected in filter paper can be shipped to the reference laboratory by ordinary mail during the ensuing month without a significant loss of enzymatic activity. The implementation of this method allows testing samples from patients that are far away from the reference labs, thus improving patient access to diagnosis.

Genetic testing to identify the disease-causing mutation is also a valuable and complementary assay. The detection of a given mutation in the index male case leads to testing the presence of the same mutation in female relatives who may be heterozygotes. Genetic testing is the only reliable method to confirm diagnosis in females, because the enzymatic activity assay in heterozygotes is inconclusive (*71*).

Fabry disease-causing mutations are private, meaning that almost each family has its own mutation (*72*). There are no frequent mutations as is found, for example, in Gaucher disease (*73*). More than 400 mutations leading to Fabry disease have been described in the literature (Human Gene Mutation Database, http//www.hgmd.org). For this reason, genetic testing is carried out by direct sequencing of the gene coding for GLA (*74*). GLA gene is formed by seven exons, and the 1,393 bp full-length cDNA codes for a precursor peptide of 429 amino acids including the 31 residues' signal peptide (*75*). Alterations in the gene includes large rearrangements, splicing defects, insertions/deletions, and point mutations. The most common alterations are point mutations, including nonsense and missense ones. Mutations occurring at different positions in each of the seven exons have been described (*76*).

Genotype-Phenotype Correlation

Although different types of mutations have been reported in Fabry disease, the genotype-phenotype correlation is generally poor (*3*). Moreover, the same mutation may cause different phenotypes in patients from the same family and different mutations may cause the same phenotype. After the resolution of the 3D structure of α-galactosidase A (*76*), a few reports concerning correlations between genotype and phenotype have been published (*77*). It is now recognized that phenotypes of even simple Mendelian disorders are influenced by complex genetic and environmental factors and that genotypes, rarely, absolutely predict phenotypes (*78*).

Screenings

Different screening strategies have been carried out in different at-risk populations in order to detect undiagnosed Fabry patients. Various studies included hemodialysis patients; with a frequency of confirmed diagnosis between 0.2 and 1.2% (*79*, *80*). Other studies have focused on patients with cryptogenic stroke or unexplained left ventricular hypertrophy with frequencies between 3 and 6% (*81*, *82*).

One important benefit from detecting a new Fabry patient through a screening strategy is the possibility of detection of other patients among relatives of the index case. The development of targeted protocols and the constitution of interdisciplinary groups for the identification of patients with Fabry disease are recommended to obtain a higher yield in the screening process.

Markers

No surrogate biomarker has yet been shown to reflect the global burden of Fabry disease activity, or to reflect the global response to ERT (*83*). The potential use of Gb3 as a marker of Fabry disease has been extensively evaluated by assaying its concentration in plasma and urinary sediment by different methods (*84*, *85*). Gb3 from urinary sediment comes from desquamated tubular cells and may reflect renal storage. The pattern of urinary sediment glycolipids from Fabry male patients revealed increased amounts of Gb3 (*86*), and the values from heterozygotes are between the ones from normal controls and hemizygotes. Moreover, urinary Gb3 excretion was found to correlate with type of mutation, sex, and treatment status (*87*).

Recently, high concentrations of globotriaosylsphingosine in plasma were found in male Fabry patients. Although no correla-

tion with age or disease severity was found, plasma levels were reduced in patients receiving ERT (*64*).

Prenatal and Newborn Screening

Precise heterozygote diagnosis by genetic testing allows to offer genetic counseling to couples at risk of having a Fabry disease-affected offspring. A heterozygous woman has a 50% probability to have affected boys or girls. After the couple receive this information, prenatal diagnosis could be offered together with orientation on advantages and risks of the procedure.

Prenatal diagnosis of Fabry disease could be performed using cultured amniocytes or direct and/or cultured chorionic villi (CV) (*88*). The recommended first diagnostic step for the genetic counseling of families with Fabry disease history is the fetal sex determination (*89*). After determining fetal sex, measurement of GLA activity and genetic testing are carried out. Direct studies of fresh CV provide the initial and rapid diagnosis. This initial diagnosis should be confirmed by studies on cultured CV, which also serves to rule out a possible maternal contamination in fresh CV. All prenatal diagnoses should be confirmed by analyzing cells or fetal tissues if gestation is interrupted or by analyzing plasma and/or leukocytes from cord blood after delivery (*90*). There is some controversy regarding prenatal testing in female fetuses. Heterozygozity can be efficiently confirmed by genetic testing but it is not possible to predict the clinical presentation.

Preimplantatory diagnosis could be done using blastomers (*90*). All preimplantation diagnoses should be confirmed by subsequent CV sampling or amniocentesis.

A debate still exists in relation to application of newborn screening not only for Fabry disease but also for all the LSD. Because LSDs have a low prevalence when considered individually, when they are considered as a group the combined estimated prevalence could be as high as 1/7,700 (*6*). The availability of therapy for Fabry disease opened the discussion in terms of newborn screening. Diagnosis of Fabry disease, as discussed earlier, could take several years. The chronic and progressive nature of the disease leads to an irreversible pathology. Early detection will enable early instauration of treatment for patients, before significant irreversible organ damage develops. On the other hand, some individuals remain asymptomatic until late age. At this time, as stated earlier, there is no evidence of a benefit from the early introduction of therapy. Clinical trials are in progress in order to evaluate this.

Attempts have been made to develop multiplex methods to analyze various LSDs in a single assay. One of these is based on an immunoassay to quantify the enzyme in terms of protein mass, taking into account that not only the enzymatic activity but also the number of enzyme molecules are reduced in Fabry disease (*91*). The detection of lysosomal enzyme activities in dried blood spots could also be multiplexed by using tandem mass spectrometry to detect the enzymatically generated product (*92*).

CONCLUSION

Fabry disease is an underdiagnosed pathology. Because of the advent of specific therapy, special attention has been given to this disease, and important knowledge has been obtained regarding its natural history, pathophysiology, and response to therapy. Many efforts are currently being made in order to improve diagnosis of this disease in different countries. An increasing knowledge about Fabry disease within the medical community increases the chances of patients to receive a timely diagnosis and, consequently, to access the appropriate therapy. In the near future, the increasing experience about Fabry disease treatment would shed light on the effect of such therapy on the clinical course of the disease. It could also suggest the optimal age for treatment onset, and could help to decide about the necessity of neonatal screening.

ACKNOWLEDGEMENTS

The author has received funds from Shire Human Genetic Therapies for research.

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