Anderson-Fabry, the histrionic disease: from genetics to clinical management

Franco Cecchi,1 Benedetta Tomberli,1,2 Amelia Morrone1,4

1Department of Clinical and Experimental Medicine; 2Department of Heart and Vessels, Referral Center for Cardiomyopathies, Careggi Hospital; 3Department of Neurosciences, Psychology, Pharmacology and Child Health, University of Florence; 4Molecular and Cell Biology Laboratory, Paediatric Neurology Unit, Neuroscience Department, Meyer Children’s Hospital, Florence, Italy

Abstract

Anderson-Fabry disease (AFD) is an X-linked lysosomal storage disorder of glycosphingolipid catabolism, due to deficiency or absence of a galactosidase A (α-gal A) enzyme. The disease may affect males and females, the latter with an average 10 years delay. Metabolites storage (mostly Gb3 and lyso-Gb3) leads to progressive cellular and multiorgan dysfunction, with either early and late onset variable clinical manifestations that usually reduce quality of life and life expectancy. Heart and kidney failure, stroke and sudden death are the most devastating complications. AFD is always been considered a very rare disease, although new epidemiologic data, based on newborn screening, showed that AFD prevalence is probably underestimated and much higher than previously reported, especially for late-onset atypical phenotypes. Currently, the diagnosis may be easier and simpler by evaluating α-gal A enzyme activity and genetic analysis for GLA gene mutations on dried blood spot. While a marked α-gal A deficiency leads to diagnosis of AFD in hemizygous males, the molecular analysis is mandatory in heterozygous females. However, referral to a center with an expert multidisciplinary team is highly advisable, in order to ensure careful management and treatment of patients, based also on accurate molecular and biochemical data interpretation. While long-term efficacy of enzyme replacement therapy (ERT) in advanced stage is still debated, increasing evidence shows greater efficacy of early treatment initiation. Concomitant, organ-specific therapy is also needed. New treatment approaches, such as chemical chaperone therapy, alone or in combination with ERT, are currently under investigation. The present review illustrates the major features of the disease, focusing also on biochemical and genetic aspects.

Introduction

Anderson-Fabry disease (AFD) (OMIM #301500) is named after two dermatologists, W. Anderson and J. Fabry, who separately described two cases of patients with angioedema corporis diffusum in 1898.1,2 Almost a century later AFD was showed to be a rare X linked multisystemic lysosomal storage disorder of glycosphingolipid catabolism, due to the deficiency of the lysosomal enzyme α galactosidase A (α-Gal A; EC 3.2.1.22).3,5 This deficiency leads to systemic accumulation of globotriaosylceramide (Gb3) and its deacylated Gb3, the globotriaosylphosphoglycerol (lyso-Gb3)7,8 throughout the body within the lysosomes and in the plasma. In symptomatic AFD male patients Gb3 and lyso-Gb3 are both increased, in plasma as well as in urine.6 However, symptomatic female patients often have Gb3 values within the normal range. Contrary to Gb3, lyso-Gb3 is absent in healthy controls and it is also markedly increased in symptomatic female patients. Furthermore, the absence of manifestations in male AFD children with no residual enzyme activity, and Gb3 accumulation within the cells, suggests that Gb3 may not be directly responsible for disease signs and symptoms, while lyso-Gb3 is likely to play a direct role in the pathogenesis of AFD.7

A specific therapy for AFD, enzyme replacement therapy (ERT), is available since 2001. The clinical and scientific interest in this rare disease is growing all over the world, with more than 2600 papers published up to date (Figure 1).

Lysosomes and α-galactosidase A

Glycosphingolipids are an essential part of the lipid bilayer in the intra and extracellular human membranes. The lysosomal enzyme α-Gal A is involved in the catabolism of glycosphingolipids within the lysosomes whereas it catalyzes the hydrolysis of α-galactosidic linkages of glycosphingolipids, glycoproteins and polysaccharides.1 This complex catabolic pathway allows a reutilization of the membrane components. Mutations in the GLA gene encoding the α-Gal A may affect the synthesis, processing, and stability of the enzyme, leading to the impairment of glycosphingolipids catabolism and to their progressive accumulation within the lysosomes. The storage of Gb3 and other toxic metabolites involves every cell of the human body and starts during the fetal life.5

However, cellular dysfunction and organ damage usually become evident with early signs and symptoms in infancy (classic phenotype), or decades later, in the late-onset variant.5

Genetic bases

The lysosomal enzyme α-GAL A is encoded by the GLA gene (MIM 300644) mapping on the X chromosome at locus Xq22.1 and organized in seven exons encompassing over 12 Kb.3 The GLA cDNA of 1290 bp encodes for a precursor protein of ~50kDa (429 amino acids), which is proteolytically cleaved into the lysosomal mature protein.8,9

The mature α-GAL A enzyme is a homodimeric glycoprotein of about 46 kDa (398 amino acids). Each monomer is composed of two domains. The domain 1 contains the active site (encompassing amino acids 32-330), at the center of the β strands in the (βαβ) barrel, while the domain 2 contains antiparallel β strands.12 At present 665 GLA gene mutations, most of...
them private and spread throughout all exons, have been reported (HGMD professional 2012.2), confirming a high genetic heterogeneity. Over 65% of the AFD causing genetic variants are missense or nonsense mutations, while 5% are splicing mutations, 22% small deletions/small insertions or regulatory mutations, and only 6% are gross deletion/insertion. While missense mutations not affecting the active site may lead to partial reduction of α-GAL A activity, no residual enzyme activity is expected for the remaining mutations.

AFD is transmitted as an X-linked disorder. Sons of affected males are always disease free, while their daughters are obligate heterozygotes. The heterozygous females can transmit their mutated allele to their male and female offspring. Thus in each pregnancy 50% of their sons can be hemizygotes and 50% of their daughters can be heterozygotes.

Although X-linked, penetrance and expressivity rate are high in both genders (100% in males and 70% in females). Heterozygous females are usually affected with a milder and late-onset disease (about 10 years later than males), but in a minority, symptoms and organ damage can be as severe and early as in males. Therefore it would be preferable to discontinue female carrier and X-linked recessive terms, as misleading. This huge variability of female phenotype can be partially explained by the random X inactivation process.

A genotype-phenotype correlation is often difficult, as most of the AFD mutations are private and unique within single families. However some common missense mutations, leading to residual α-GAL activity, have been linked mainly to specific organ involvement in AFD patients (e.g. N215S [c.644A>G→p.Asn215Ser] to hypertrophic cardiomyopathy). While missense mutations not affecting the active site may lead to partial reduction of α-GAL A activity, no residual enzyme activity is expected for the remaining mutations.

While recent bio-borns report an unexpectedly much higher incidence (Table 1). While the classic phenotype of AFD is actually rare, these data show that milder variant with late-onset phenotype may be much more frequent than expected. The late-onset subgroup comprises patients with disease onset much later in life than patients with classic AFD, usually in the fourth to six decades, and manifestations confined mainly to one organ system. Patients with atypical late-onset variants have residual enzyme activity, which ranges from 2 to 20% of normal values and results in a milder phenotype. However the correlation between residual enzyme activity and disease phenotype is not strong. The real strength of these newborn-screening initiatives goes well beyond the simple data itself. They shifted a historic paradigm of a rare disease, with multisystemic involvement in young males, to a more common disease, with a wide phenotype, sometimes with disease manifestation confined to one organ system.

Furthermore, screening for AFD among selected groups of patients, i.e. patients with juvenile cryptogenic stroke, renal failure or left ventricular hypertrophy, suggests that many patients are misdiagnosed. Indeed, the prevalence of AFD among patients with hypertrophic cardiomyopathy (HCM) ranges from 0.5% to 6%, depending on different selection criteria for age and gender, and diagnostic methods. It is about 1% if only males older than 35 years at diagnosis of HCM are selected.

AFD is a pan-ethnic disease, with few areas of high prevalence (i.e. Canada and West Virginia, USA), probably due to a founder effect.

### Epidemiology

AFD is rare disease, with a reported prevalence in the general population ranging from 1:17,000 to 1:467,000. However, recent biochemical and genetic screening data in newborns report an unexpectedly much higher incidence (Table 1). While the classic phenotype of AFD is actually rare, these data show that milder variant with late-onset phenotype may be much more frequent than expected. The late-onset subgroup comprises patients with disease onset much later in life than patients with classic AFD, usually in the fourth to six decades, and manifestations confined mainly to one organ system. Patients with atypical late-onset variants have residual enzyme activity, which ranges from 2 to 20% of normal values and results in a milder phenotype. However the correlation between residual enzyme activity and disease phenotype is not strong. The real strength of these newborn-screening initiatives goes well beyond the simple data itself. They shifted a historic paradigm of a rare disease, with multisystemic involvement in young males, to a more common disease, with a wide phenotype, sometimes with disease manifestation confined to one organ system.

Furthermore, screening for AFD among selected groups of patients, i.e. patients with juvenile cryptogenic stroke, renal failure or left ventricular hypertrophy, suggests that many patients are misdiagnosed. Indeed, the prevalence of AFD among patients with hypertrophic cardiomyopathy (HCM) ranges from 0.5% to 6%, depending on different selection criteria for age and gender, and diagnostic methods. It is about 1% if only males older than 35 years at diagnosis of HCM are selected.

AFD is a pan-ethnic disease, with few areas of high prevalence (i.e. Canada and West Virginia, USA), probably due to a founder effect.

### Clinical manifestations, clinical course and prognosis

AFD is a progressive disease, characterized by a wide variability of signs, symptoms and patterns, which, for the classical phenotype, are clearly different in three periods of life (Figure 2):

![Figure 1. The number of papers on Anderson-Fabry disease is significantly increased since the advent of enzyme replacement therapy, in 2001.](#)

Table 1. Epidemiology of Anderson-Fabry disease. Data from newborn screening based on α-Gal A activity and GLA gene analysis on dried blood spot.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Country</th>
<th>Total incidence</th>
<th>Male incidence</th>
<th>Classic phenotype</th>
<th>Late-onset phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Italy14</td>
<td>-</td>
<td>~1:3100</td>
<td>~1:37,000</td>
<td>~1:3400</td>
</tr>
<tr>
<td>M+F</td>
<td>Taiwan15</td>
<td>1:3200*</td>
<td>~1:1250*</td>
<td>~1:22,600 (M)*</td>
<td>~1:1400 (M)*</td>
</tr>
<tr>
<td>M+F</td>
<td>Taiwan15</td>
<td>1:2500*</td>
<td>~1:1400*</td>
<td>~1:57,000 (M)*</td>
<td>~1:1500 (M)*</td>
</tr>
<tr>
<td>M+F</td>
<td>Austria21</td>
<td>~1:3900</td>
<td>~1:17,000</td>
<td>~1:4100</td>
<td></td>
</tr>
</tbody>
</table>

M, male; F, female. *Newborn screening in Taiwan reveal a high incidence of the IVS4+919G>A cryptic splicing mutation, probably due to a founder effect. This mutation has been described in late-onset cardiac phenotype.14,15
- Childhood: although the pathologic accumulation of sphingolipids in various tissues throughout the body usually starts in the fetal life, early symptoms, mostly neuropathic pain, usually emerge in infancy.8
- Age 20 to 30: progressive cellular dysfunction, related to the slow but continuous intracellular storage, leads to organ damage and failure. Symptoms tend to progress, and the first, subclinical signs of organ involvement may be detected [e.g. proteinuria, electrocardiogram (ECG) changes and silent cerebral abnormalities].
- Over age 30: major organ involvement, such as renal, cerebrovascular and cardiovascular, may lead to disease progression and eventually to an end-stage phase.27 Average reduction of life expectancy is about 20 years in males and 10 in females.28

**Anderson-Fabry disease in the childhood: first signs and symptoms**

The first clinical symptom is usually neuropathic pain, which occurs in 70% of patients and usually emerges between age 4 and 12, earlier in males than in females.29 The pain is located at the extremities and can be either chronic or characterized by crisis. It may be triggered by exercise, fever, sun exposure, changes in temperatures or stress.30 The burning pain may be so intense to interfere with everyday life and substantially reduce quality of life.31 Neurologic examination is normal, as well as electromyography (EMG) and electroneurography.32 Both examinations only assess large nerve fibers function, while in AFD small nerve fibers are involved, and their reduced density on skin biopsy is sometimes required to diagnose AFD neuropathy.33 The neuropathic pain is difficult to manage with standard analgesic and may respond to narcotic analgesic (such as codeine or morphine) or anticonvulsant drugs (such as carbamazepine).34 Anhidrosis and hypohidrosis, a reduced or absent ability to sweat, can cause heat and exercise intolerance, often reported as recurrent fever after exercise by the patients and their parents. They may also trigger AFD pain crisis.35

Gastrointestinal involvement, characterized by abdominal pain, diarrhea, nausea, can also be reported during childhood as well as in the adult life.36

Retinal vessel tortuosity and cornea verticillata, a corneal lesion caused by Gb3 deposition, can also be detected in childhood.37 Vertigo, tinnitus and sudden hearing loss have been reported at an early stage and are thought to be related to small vessel involvement.38

Angiokeratoma is another early feature of AFD.39 Small purple skin lesions may be present in children and tend to increase in size and number with age. They can be found typically on the lower back, thighs, genital areas, umbilicus and sometimes on mucosae (Figure 3). Histologically they are characterized by vessel dilatation (angiomas) and hyperkeratosis. Major organ involvement, such as renal, cerebrovascular or cardiovascular is uncommon in children.40 Microalbuminuria and proteinuria, signs of early renal damage, may be detected in the second decade of life.41 Small white matter lesion on magnetic resonance imaging (MRI) may reveal an initial central nervous system involvement, as well as ECG changes of the heart. Although the early clinical picture is sugges-

---

**Figure 2. Symptoms onset and disease progression in classic Anderson-Fabry disease (AFD).** Signs and symptoms of AFD progressively increase with age. In infancy and adolescence neuropathic pain, gastrointestinal manifestations and angiokeratomas are the most frequent manifestations. Proteinuria, the first sign of kidney involvement, is also present in young affected males (Modified from Zarate and Hopkin).4

**Figure 3. Various aspects of angiokeratoma in Anderson-Fabry disease patients: angiokeratoma of the periungual and subungual area (panel A), of the palms, lips and umbilicus (panel B-D respectively) (Reprinted with permission of the publisher from Zampetti et al.39).**
tive of AFD, the diagnosis is rarely made in childhood and adolescence, with an average diagnostic delay of 10 years for both genders. Recognition of early and advanced clinical manifestations of AFD represents a real challenge for clinicians, due to variable clinical manifestations and symptoms that may resemble other common diseases.42

**Anderson-Fabry disease in the adult life**

The full spectrum of AFD phenotype slowly develops with age.

**Cardiac involvement**

Gb3 and related sphingolipids storage may occur in cardiomyocytes, cardiac valves, endothelial cells and conduction system.43 While ECG changes may be found in young patients, an overt cardiomyopathy with left ventricular hypertrophy (LVH), with diastolic dysfunction, preserved systolic function, often with mild valvular regurgitation emerges in the third or fourth decade of life in affected males, more rarely in a few females.44

**Conduction abnormalities and arrhythmias**

ECG changes are often present in AFD (Figure 4). A short PQ interval (<120 ms), described as a feature of AFD, is a non-specific and rather uncommon finding with a prevalence of 14%.45 Signs of LVH on ECG, along with repolarization changes (including deep T waves inversion) are frequent. They may be detected even in patients without a marked increase of left ventricular wall thickness at echocardiography.45,46 Bradycardia, sinus node disease, prolonged PQ intervals with variable degree of AV-block, and QRS broadening (particularly with a right bundle branch block pattern) are ECG alterations often seen later in life.46 PQ interval and QRS duration are directly proportional to age, suggesting a relationship to progressive storage of sphingolipids and development of fibrosis. Apoptosis and autonomic nervous system involvement might also play a role. Recently AV and sinus node disease were showed to be common in AFD patients.47 The prevalence of atrial fibrillation and non-sustained ventricular-tachycardia (VT) are higher in AFD patients when compared to the general population.48 Furthermore the risk of arrhythmias increases with age, while there are no gender-related differences. Sudden cardiac death may occur, usually in the end stage phase, although its prevalence is unknown.49 It may be triggered by episodes of sustained VT.

**Anderson-Fabry disease cardiomyopathy**

AFD cardiomyopathy is characterized by diastolic dysfunction and LVH, usually concentric and non-obstructive. Basal LV outflow tract obstruction (LVOTO) is rare, while exercise provokable LVOTO has been detected in 43% of patients,49 most of them with LV small cavity size.

LVH is usually mild, although male patients may also show severe and asymmetric septal or apical hypertrophy, both in classical and late-onset disease (Figure 5). Systolic function is usually preserved in most patients until advanced stage, but a few patients may gradually develop a systolic dysfunction, often leading to progressive refractory heart failure.50 The use of some echocardiographic technique, such as tissue doppler imaging or strain rate imaging, may detect early cardiac involvement before LVH develops.51,52 Replacement fibrosis, detected by cardiac MRI usually in the inferior or posterolateral wall, is considered a sign of disease progression (Figure 6).53 Myocardial fibrosis may also be present in female patients.
without LVH, suggesting that hypertrophy and fibrosis are not necessarily associated. The endocardial binary appearance (binary sign) was suggested as an echocardiographic hallmark of AFD cardiomyopathy; however, recent studies showed that these echo features can be detected also in patients with sarcomeric HCM. The binary sign is of limited utility in distinguishing the cause of LVH. Of note, right ventricular hypertrophy, is detected by MRI in about 2/3 of patients with LVH.

**Angina and coronary artery disease**

Chest discomfort and angina may be present, despite the absence of atherosclerotic coronary artery disease. Both are likely to be related to microvascular dysfunction (Figures 7 and 8). Coronary microvascular blood flow impairment can be detected in both genders, even without LVH. It can lead to chronic ischemia and myocardial replacement fibrosis.

**Other cardiac abnormalities**

The prevalence of mild aortic root enlargement is higher in male AFD patients, while exercise capacity is usually reduced, when measured by a cardiopulmonary exercise test.

**Kidney involvement**

Microalbuminuria and proteinuria, due to kidney impairment related to Gb3 accumulation and glomerular fibrosis, are usually present by the third decade of life, sometimes earlier when α-GAL enzyme activity is <1% (Figure 9). Proteinuria levels may vary widely, but tend to increase in severity with age and are inversely correlated to kidney function, based on glomerular filtration rate, which is often normal or even higher in young patients. After renal dysfunction has progressed to end-stage, dialysis and renal transplant may be necessary.

**Cerebrovascular involvement**

Transient ischemic attack and strokes are the most relevant and disabling complication of AFD and may significantly contribute to disease-related morbidity and mortality. Multifocal leukoencephalopathy is the typical lesions of AFD and results from small vessels involvement and microvascular dysfunction (Figure 10). The prevalence of strokes in AFD is about 7% in males and 5% in females, usually in the third and the fourth decade of life. However, the prevalence of cerebrovascular involvement rises up to 60-70% if minor signs or symptoms and abnormal brain MR image are considered. Psychiatric disorders, depression and dementia may be present and they can only be partially attributed to the cerebral microvascular disease. The severity of cerebrovascular disease may vary widely. Combining neurologic symptoms and intracranial ultrasound examination may be of limited utility in distinguishing the cause of LVH. Of note, right ventricular hypertrophy, is detected by MRI in about 2/3 of patients with LVH.

---

**Figure 6.** Myocardial fibrosis as a sign of disease progression. Panel A) cardiovascular magnetic resonance image showing an area of myocardial fibrosis involving the posterolateral wall (arrows in four-chamber horizontal long-axis view). Panel B) the extension of fibrosis is significantly increased, despite 6 years of enzyme replacement therapy (Reprinted with permission of the publisher from Imbriaco et al. 101).

**Figure 7.** Gb3 inclusions in the endothelium and cardiomyocytes. Panel A shows an intramural coronary artery in a patient with hypertrophic cardiomyopathy. Wall thickening is due primarily to severe thickening of medial wall, while the intima is mildly thickened. Panel B shows interstitial capillaries in the myocardium of a patient with Anderson-Fabry disease. Glycosphingolipid inclusions are present in the endothelium (arrow) and cardiomyocytes (arrowhead) (Reprinted with permission of the publisher from Camici e Crea 58).

**Figure 8.** Microvascular dysfunction detected by myocardial positron emission tomography. Male patients with Anderson-Fabry disease (AFD) show a marked reduction in myocardial blood flow (MBF) during adenosine-induced hyperaemia (ADO), compared to controls (Reprinted with permission of the publisher from Elliott et al. 59).
mental findings, CNS disease can be briefly classified into 3 categories: i) symptomatic patients with repeated cerebrovascular events, ii) patients with minor neurological manifestation such as vertigo, dizziness, hearing loss, headache and migraine; iii) asymptomatic patients with abnormal brain MRI findings.60

**Other findings**

Symptoms compatible with autonomic dysfunction, such as arterial hypotension, sinus bradycardia or gastrointestinal symptoms, have always been attributed to autonomic nervous system neuropathy,72 which is often neglected in the single patient.

Obstructive airway disease and osteopenia/osteoporosis in AFD patients have also been reported.73,74

**Diagnosis**

Once the clinical pictures rises a suspicion of AFD, biochemical and genetic tests are required to confirm the diagnosis.

**Biochemical diagnosis**

The detection of markedly reduced or absent α-gal A activity in leucocytes is sufficient to confirm AFD diagnosis,2 although it should be carefully evaluated with clinical and genetic molecular findings. While patients with a milder AFD phenotype usually have residual α-gal A activity, in classically affected young males enzyme activity it is very low or undetectable.25,26

α-gal A activity can be assessed in plasma. However, when reduced, its value needs to be confirmed in leukocytes or fibroblasts, in order to avoid diagnostic pitfalls, due to enzyme pseudodeficiency in plasma.27 The GLA gene sequence analysis should always be performed and correctly interpreted, in order to reveal polymorphisms, that potentially lead to an enzymatic pseudodeficiency.78 Biochemical diagnosis may be unreliable in heterozygous females, as they can have a normal or only mildly reduced α-gal A activity, due to random X-chromosome inactivation. Therefore, it is crucial to combine biochemical assays with appropriate molecular analyses of the GLA gene in order to attribute or exclude the AFD heterozygous status in females.73

More recently, screening tests on dried blood spot (DBS), using fluorescent methods, were introduced,88 together with multiplex assays of lysosomal enzymes by tandem mass spectrometry.91,92 The availability of multiplex technology using DBS on filter paper made possible newborn screening programs of treatable lysosomal storage diseases.93 DBS is simply transportable and represent an easier, faster and less expensive method for enzyme activity evaluation in selected at risk population. As false positive or negative results may occur, it is necessary to confirm a reduced residual enzyme activity by standard laboratory diagnostic procedures.93,94

**Genetic analysis**

Molecular analysis is usually performed in probands by direct sequencing analysis of the GLA gene coding regions, of its exons/introns boundaries and of the intronic region encompassing the known deep intronic mutations.86,87 Rarely, in males with low or no enzyme activity, and females with AFD clinical manifestations and normal or low enzyme activity, exon-based sequence analysis may fail to identify the disease-causing mutation. Such in these cases, further molecular and cellular laboratory investigations are needed to evaluate the GLA gene expression profile at mRNA and protein level. Both Ishii and Filoni identified, by mRNA analysis, deep intronic pathogenic mutations, which alter the GLA splicing regulation.86,87 In females, due to the presence of the wild-type allele, gross GLA gene rearrangements, such as deletion or insertion of entire exons, may be missed, unless specific laboratory technique, such as multiplex ligation-dependent probe amplification analysis and/or quantitative polymerase chain reaction amplification, are used.86,88

Additional investigations are also necessary in order to assess the pathogenicity of new variants. *In silico* and *in vitro* analysis, computational modeling, and functional studies are needed to prove or exclude the effect of the new identified variant as a disease-causing mutation.74 Co-segregation in male family members, tissue biopsies and determination of specific metabolites (i.e., Gb3 or lyso-Gb3) may also be necessary. Recently, several authors suggest to consider GLA gene mutations on the bases of their pathogenic significance, with variants predicted to have high (Class 1) or low probability to be disease-causing (Class 2, polymorphisms).89 Single nucleotide polymorphisms (SNPs) are exonic or intronic nucleotide variants detected with incidence higher than 1% in a healthy control population. They are usually interpreted as not disease-causing mutation. Several GLA gene polymorphisms, leading to molecular heterogeneity have been described (SNP, http://www.ncbi.nlm.nih.gov/SNP).75 Sometime exonic polymorphisms, including rare variants leading to aminoacid change, may be found in males with normal or decreased enzyme activity, but still well above the pathologic range of values. They are almost certainly non-pathogenic variants or *Class 2 mutations* and they include polymorphisms or sequence changes described as pseudodeficiency alleles, e.g. c.937 G>T (p.D313Y).90 Pseudodeficiency is a condition in which individuals show a reduced α-Gal A activity *in vitro*, but remain clinically healthy.

However, in a few patients, when the combination of clinical, histological, biochemical and functional data are still not clear-cut, the AFD diagnosis may remain uncertain91 (Figure 11).

**Role and significance of Gb3 and lyso-Gb3**

Biomarkers are chemical molecules that can be clinically useful to assess disease progression, therapeutic efficacy, as well as diagnostic confirmation. Although many attempts have been made in order to identify a biomarker for AFD, a valid and reliable molecule is still missing. Gb3 is the primary storage molecule and can be find inside cells and in biologic fluids, such as urine and plasma. For many years Gb3 has been considered a reliable marker for AFD, so that its reduction in tissue biopsies has served as a major criteria to prove the efficacy of the enzyme replacement therapy. With
time, new research investigations proved that Gb3 do not correlate with disease manifestations. Plasma Gb3 levels are abnormally high at a very young age (sometimes detectable even in utero), while clinical manifestations develop much later in life. Furthermore, Gb3 might be within the normal range in symptomatic female. The diagnostic value of this parameter has been questioned as well, since it can be detected in normal healthy subjects.

Globotriaosylsphingosine, also known as lyso-Gb3, is the deacylated form of Gb3. In contrast to Gb3, this relatively new biomarker is abnormally high in male and female patients with AFD while this is absent in control healthy subjects. Lyso-Gb3 may be a useful additional element for confirmation of AFD diagnosis in patients with GLA variant of unknown significance (Figure 11). Furthermore, lifetime exposure to lyso-Gb3 correlates with disease severity, even after adjustments for age, gender and cardiovascular risk factors, and suggests its pathophysiological role. However, the usefulness of lyso-Gb3 as a biomarker for assessing the response to ERT is still under investigation.

**Genetic counseling and multi-disciplinary approach**

Once biochemical and molecular genetic data are ascertained and/or available, their interpretation should be performed by a multi-disciplinary team with expertise in AFD, usually available in referral centers, in order evaluate the appropriate treatment strategy. Genetic counseling should then be offered to the proband, and his family members, who should undergo clinical screening.

Figure 10. Brain magnetic resonance imaging of an asymptomatic 54-year old female showing multifocal leukoencephalopathy (Reprinted with permission of the publisher from Buechner et al.68).

Figure 11. Diagnostic flow chart of Anderson-Fabry disease (AFD) in males and females: step-by-step approach. Panel A) males. 1) Clinical picture indicative of AFD, family history compatible with an X-linked transmission. 2) In males, normal values of $\alpha$-gal A activity exclude the diagnosis of AFD. Reduced (<20%) or absent enzyme activity values confirm the suspicion of AFD and require genetic testing. Biochemical analysis on dried blood spot (DBS) must be confirmed by standard laboratory procedures (i.e. whole blood analysis). 3) If no mutations are identified by genetic analysis, and the $\alpha$-gal A activity is low or undetectable, further laboratory analyses are required to confirm the diagnosis (e.g. mRNA and protein analysis, tissue biopsies). Comparably, the identification of new unknown variant requires laboratory analysis, along with co-segregation studies within the family. Panel B) females. 1) Clinical picture indicative of AFD, family history compatible with an X-linked transmission. 2) In females, the $\alpha$-gal A activity can be within the normal range. Therefore genetic analysis is mandatory to confirm the diagnosis; if no mutations are identified by genetic analysis, and the clinical suspicion is high, further laboratory analyses are needed (e.g. multiplex ligation-dependent probe amplification to detect deletions or insertions, mRNA and protein analysis, tissue biopsies). Comparably, the identification of new unknown variant requires laboratory analysis, along with co-segregation studies within the family. 3) Enzyme activity may be helpful in diagnosis and management of female AFD patients and should be measured after the genetic diagnosis is made. Biochemical analysis on DBS must be confirmed by standard laboratory procedures (i.e. whole blood analysis).
Management and follow-up

AFD is a multi-systemic disorder and a thorough evaluation of organ involvement is highly recommended.

Neurologic assessment comprises diagnosis and staging of neuropathic pain, through EMG and skin biopsy, if needed. Since leukoencephalopathy may be present even in young patients without symptoms and with a normal neurologic exam, brain MRI is required at initial assessment. It should be repeated at a 2-3 years interval depending on clinical picture.

Nephrologic assessment is based on microalbuminuria, 24 h proteinuria, renal function (creatinine level and estimated glomerular filtration rate), which should be routinely and repeatedly assessed. Ultrasound abdominal examination is also required for kidney morphology, as well as, in selected cases, renal biopsy.

Cardiologic evaluation

Patients should undergo ECG, color-doppler echocardiography, 24 h ambulatory ECG monitoring and exercise (possibly cardiorespiratory) test, in order to define cardiac involvement and future risk. Since arterial hypertension, although rare in these patients, may increase the risk of cerebrovascular and cardiovascular disease, blood pressure should be carefully controlled even with 24 h blood pressure monitoring. Pulmonary function tests are sometimes required in patients whose main symptom is dyspnea.

A rigorous control of conventional atherosclerotic risk factors is mandatory. Indeed, measurement of blood cholesterol, homocysteine level, and fasting glucose, should be performed once or twice a year, although it was suggested that statin treatment might be deleterious in AFD patients. Lifestyle change, especially smoking cessation, dietary modifications and mild physical activity, are also recommended.

Therapy

Since 2001 ERT is available and it has been validated as standard treatment for AFD patients in more than 45 countries. In Europe ERT is available in two commercial formulations: agalsidase α (Replagal®; Shire, Cambridge, MA, USA) and agalsidase β (Fabrazyme®; Genzyme Corp., Cambridge, MA, USA), but the former is not yet approved by Food and Drugs Administration in USA. Randomized, placebo-controlled clinical trials assessed safety and efficacy of both enzyme formulations. However, these trials enrolled small cohort of patients and, despite over 10 years of clinical practice, many questions about ERT long-term efficacy remain unanswered. Both preparations are infused intravenously once every other week, at different dose regimens (0.2 mg/kg for Replagal®; 1 mg/kg for Fabrazyme®). Patients should be treated with licensed recommended doses of agalsidase α and agalsidase β. Currently there is no level 1 scientific evidence showing superiority of one enzyme preparation over the other. Treatment failures may occur with both drugs and are probably related to age and advanced disease stage, because severe organ damage is often irreversible (Figure 6). Moreover patients can develop complications despite receiving optimal treatment, with concomitant therapy and ERT at licensed recommended dose.

There is increasing evidence that ERT is more effective when started in the early stage of disease, and early diagnosis is highly preferable. However, timing of ERT, especially in children and females, is still a matter of debate and may vary in different countries. Current expert opinions about timing of ERT are presented in Table 2, but real guidelines have never been written.

Concomitant treatment: appropriate organ-specific and adjuvant therapy must always be considered for all patients, along with ERT infusion, for symptom control and disease stabilization (Table 3).

Conclusions and future perspectives

AFD is a lysosomal storage disease with multiorgan involvement and reduced life expectancy. ERT is the only curative treatment available for AFD and a consensus of experts wrote this document, on October 2010, in order to support clinicians’ decisions during the period of enzyme replacement therapy (ERT) shortage. Although the present recommendations are not supposed to represent real treatment guidelines, they underline some important issues about the efficacy of ERT in advanced stage of Anderson-Fabry disease. Priority to receive ERT was based on disease severity, potential reversibility and rate of disease progression, while age and gender did not represent valid criteria (Modified from G.E. Linthorst et al. 2012).

Table 2. Priority stages for treatment in naïve male and female patients with Anderson-Fabry disease.

<table>
<thead>
<tr>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High priority</strong></td>
<td><strong>High priority</strong></td>
</tr>
<tr>
<td>Neuropathic pain unresponsive to optimal medical therapy</td>
<td>Neuropathic pain unresponsive to optimal medical therapy</td>
</tr>
<tr>
<td>Proteinuria &gt;250 mg/day</td>
<td>Proteinuria &gt;300 mg/day</td>
</tr>
<tr>
<td>Persistent microalbuminuria</td>
<td>Proteinuria &gt;300 mg/day</td>
</tr>
<tr>
<td>Normal or mild to moderate reduction of renal function (GFR 30-90 mL/min)</td>
<td>Proteinuria &gt;300 mg/day</td>
</tr>
<tr>
<td>Left ventricular mass index &gt;90th percentile (age adjusted)</td>
<td>LVH without extensive fibrosis (on MRI)</td>
</tr>
<tr>
<td>Age onset &gt;50 years</td>
<td>Cerebrovascular disease</td>
</tr>
<tr>
<td>TIA/stroke or white matter lesion on MRI</td>
<td>Disease onset &lt;50 years</td>
</tr>
</tbody>
</table>

**Intermediate priority**

<table>
<thead>
<tr>
<th>Intermediate priority</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age onset &gt;50 years</td>
</tr>
<tr>
<td>LVH with fibrosis</td>
</tr>
<tr>
<td>Severe renal dysfunction (GFR&lt;30 mL/min)</td>
</tr>
</tbody>
</table>

**Low priority**

<table>
<thead>
<tr>
<th>Low priority</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe cardiac or CNS disease (end stage)</td>
</tr>
<tr>
<td>Multiple organ failure</td>
</tr>
<tr>
<td>Other comorbidities with reduction of life expectancy &lt;1 year</td>
</tr>
<tr>
<td>Very mild multiorgan disease (e.g. normal renal function, no LVH, neuropathic pain well controlled by conventional medical therapy)</td>
</tr>
</tbody>
</table>
Table 3. Conventional medical treatment in Anderson-Fabry disease.

<table>
<thead>
<tr>
<th>Symptomatic management of Anderson-Fabry disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analgesics to control neuropathic pain</td>
</tr>
<tr>
<td>Avoidance of triggers of acute pain crisis</td>
</tr>
<tr>
<td>Anti-arrhythmic drugs</td>
</tr>
<tr>
<td>Rigorous control of any coronary risk factor (smoke, dyslipidemia, hypertension, hyperhomocysteinemia)</td>
</tr>
<tr>
<td>Long-term therapy to prevent organ damage</td>
</tr>
<tr>
<td>ACEI/ARB for proteinuria and kidney dysfunction</td>
</tr>
<tr>
<td>Anti-aggregrant (aspirin, clopidogrel) or anticoagulant (warfarin) to prevent TIA and strokes</td>
</tr>
<tr>
<td>End-stage disease management</td>
</tr>
<tr>
<td>Dialysis or kidney transplantation for renal failure</td>
</tr>
<tr>
<td>Heart transplantation for patient with refractory heart failure</td>
</tr>
</tbody>
</table>

ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; TIA, transient ischemic attack.

expectancy in many affected patients. AFD has always been considered a very rare disease. However, new screening data reported a much higher incidence. Currently, AFD diagnosis may be easier and simpler by evaluating \( \alpha \)-gal A enzyme activity and genetic analysis for \( GLA \) gene mutations on dried blood spot. A marked \( \alpha \)-gal A deficiency leads to diagnosis of AFD in hemizygous males, while molecular analysis is mandatory in heterozygous females. However, referral to a center with an expert multidisciplinary team is highly advisable, in order to ensure careful management and treatment of patients, based also on accurate molecular and biochemical data interpretation.

Enzyme replacement therapy in patients with early symptoms and initial organ disease, such as microalbuminuria and neuropathic pain, seem to be effective. Furthermore, new treatment approaches are currently under investigation, such as chemical chaperone therapy alone (phase III clinical studies; http://www.clinicaltrials.com) or in combination with ERT (preclinical studies). 102-104

While many aspects of this disease have been elucidated, further clinical and laboratory studies are still needed to clarify residual areas of doubt.

References

2. Fabry J. [Purpura papulosa haemorrhagica Hebrae]. Arch Dermatol Syphilit 1898;43:187-201. [In German].
5. Germain DP. Fabry disease. Orphanet J Rare Dis 2010;5:30-79.


