ANDERSON FABRY DISEASE

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Abstract

Anderson Fabry Disease (AFD) is a rare X-linked disorder caused by mutations in Alpha Galactosidase gene (GLA) that encodes alpha-galactosidase (α-Gal) enzyme. Mutations in the GLA gene affect synthesis, trafficking, folding, degradation and enzymatic activity of α-Gal resulting in progressive intracellular accumulation of globotriaosylceramide (Gb3). Accumulation of α-Gal substrates in cells and organs causes AFD, which can manifest with classic multiorgan involvement (heart, kidney, brain, eye, skin, ear, peripheral nerves, and gastrointestinal system) or with late-onset milder phenotypic variants demonstrating major manifestations at the cardiac, renal and nervous system levels. Hemizygous males show the most severe and earliest phenotypes; heterozygous females show later onset and milder phenotypes. Major potentially fatal complications are renal and cardiac failure and cryptogenic stroke. The heart is involved in up to 70% of AFD patients often mimicking sarcomeric hypertrophic cardiomyopathy. Data on the long-term evolution of the disease in untreated patients demonstrate that men have a reduced life expectancy and an increased risk of developing complications. Early diagnosis and administration of enzyme replacement therapy control disease progression and prevent organ transplantation.

Anderson Fabry Disease (AFD) is a rare X-linked disorder caused by defects of alpha-galactosidase (α-Gal) enzyme. Mutations in the Alpha Galactosidase gene (GLA), which encodes for α-Gal, affect synthesis, trafficking, folding, degradation and enzymatic activity of alpha-galactosidase resulting in progressive intracellular accumulation of globotriaosylceramide (Gb3). Intracellular Gb3 and related glycosphingo lipids accumulation leads to organ/tis-

sue damage potentially affecting cardiovascular, renal, gastrointestinal, cerebrovascular, neurologic, auditory, ocular and cutaneous systems. Recent evidences support the hypothesis of a tissue-specific, mutation-dependent "affinity" for Gb3 storage [1]. The AFD clinical phenotype is characterized by variability in the age of onset and severity and can be severe and early in classic forms of AFD or mild and later in variant forms. Replicated evidences demonstrate that carriers of certain mutations in the GLA gene develop preferential, albeit non-exclusive, cardiac, renal and neurologic phenotypes: e.g. p.(Asn215Ser) and p.(Phe113Ile) are invariably associated with late onset Hypertrophic CardioMyopathy-like (HCM-like) phenotype [2,3].

Hemizygous male with the classic form of AFD demonstrate low or absent enzyme activity. The patients typically develop signs and symptoms in childhood or adolescence (delayed puberty and growth, gastrointestinal symptoms, corneal opacities, angiokeratomas, acroparesthesias/neuropathic pain). Thickening of the left ventricular wall, renal failure, vascular complications, cryptogenic stroke and Transient Ischemic Attack (TIA) are features that become noticeable only during adulthood. Heterozygous female with the classic form of AFD may experience later onset of the disease and typically exhibit heterogeneous and milder phenotypes. They may have normal residual enzyme activity limiting the role of testing α-galactosidase activity as diagnostic assay. The non-classic AFD-patients have symptoms mostly limited to single organs and their phenotypes are termed cardiac, renal and neurological variants. The non-classical variants of the disease lead to difficult clinical dilemmas increasing the risk of misdiagnosis.

Major complications of AFD are renal failure, HCM-like cardiac involvement and cryptogenic stroke. Data on the long-term evolution of the disease in untreated patients provide limited information: a few studies performed before introduction of Enzyme Replacement Therapy (ERT) in 2001 had shown that men have a reduced life expectancy and an increased risk of developing complications [4,5].

Inheritance and family scenarios

Family history and clinical traits in the proband and relatives may be highly informative. AFD is an X-linked disorder: the GLA gene maps in Xq22.1 (MIM*300644). This means that all hemizygous men are affected while their daughters are obligate heterozygous carriers and their sons are non-carrier and non-affected: in fact, hemizygous fathers cannot pass the disease to their male children. Vice versa, there is a 50% probability for male children of heterozygous women to be affected. Affected young adult men and middle-aged women are more likely to be referred to a cardiologist for medical advice [6]. Usually symptoms at presentation are atypical, with the former diagnosed incidentally with Left Ventricular (LV) hypertrophy and the latter referred for palpitations and incidental discovery of mild concentric LV hypertrophy. Paternal history is usually negative for AFD young adult men. Conversely, an history of LV hypertrophy or renal failure is common among fathers of AFD middle-aged women. Maternal history of AFD young adult men may be positive for non-specific gastrointestinal or neurological problems. Presence of cryptogenic strokes in the maternal lineage of a young adult man referred for

LV hypertrophy should be alerting especially when associated with long-lasting non-specific symptoms such as pain attacks and depression. Other typical clinical scenarios in which a cardiologist should suspect AFD could be the case of a young boy presenting with short PR at the ECG and an history of tiredness, lethargy and episodes of limb and abdominal pain or the case of a PFO-negative transesophageal echocardiography in a middle aged woman referred for cryptogenic strokes.

Diagnostic work-up

The ideal diagnostic work-up starts with the clinical suspect of the disease. In classical AFD, the cardiac phenotype is usually associated with non-cardiac manifestations such as renal dysfunction, TIA or cryptogenic stroke. Fabry facies is unusual and more common in male than in female patients; when present, it is characterized by prominent supraorbital ridges, bushy eyebrows, widened nasal bridge and bulbous nasal tip, recessed forehead, shallow midface, full lips, coarse features, prognathism, and posteriorly rotated ears. Skin angiokeratomas are prevalently (but not exclusively) located in typical "bathing suit" areas; labial and proximal nail fold telangiectasia can be present. Deep exploration of the clinical history usually highlights abdominal crises of pain starting in infancy, and heat intolerance, with acral painful episodes. In atypical or variant forms of AFD (cardiac, renal, nervous), the phenotype is characterized by prevalent involvement of one organ, which makes the clinical diagnostic hypothesis difficult to be formulated. However, deep phenotyping may demonstrate abnormalities of other organs/tissues. Multidisciplinary evaluation including ophthalmology, cardiology, neurology, nephrology, dermatology is usually activated when the first clinician that observes the patient suspects a systemic disease. Therefore the clinical skill is a major contributor to clinically based diagnostic hypothesis.

However, the majority of AFD cases are diagnosed in the context of large genetic screening of patients presenting with either HCM (cardiology setting) or renal failure (nephrology setting) or cryptogenic stroke (neurology setting), when the most common causes of the three above conditions are excluded. This strategy gives a diagnostic yield of 0.12% in high-risk subgroups of patients; when all specialties pertinent to the disease are included in the screening programs of high-risk patients (adding dermatology, ophthalmology and gastroenterology to the more common cardiology, nephrology and neurology settings) the diagnostic yield increases to 1.8% and falls to 1.2% after exclusion of uncertain genetic variants [1].

The Fabry Heart

Irrespective of symptoms, the cardiologic diagnostic work-up includes cardiology visit, baseline ECG, imaging [2D-TransThoracic Echocardiography (TTE) and Cardiac Magnetic Resonance (CMR) when needed] and evaluation of possible arrhythmias. The heart is involved in up to 70% of patients. AFD cardiac manifestations often mimic HCM, for which no specific treatment exists. A disease-oriented diagnostic mindset is essential to clinically suspect

AFD and highlight differences of AFD heart from sarcomeric HCM. The MOGE(S) cardiomyopathy classification system [7] (that considers cardiac morpho-functional phenotype together with extra-cardiac organ involvement, familial inheritance pattern and etiological description) can guide the clinical evaluation and allows annotation of cardiac and non-cardiac phenotypic traits [1].

Electrocardiography

A short PR interval may be one of the first signs of cardiac involvement and has been shown to be due in particular to shortening of P-wave duration [8]. Short PR interval could be found in 20 to 40% of adult AFD patients and in about 30% of pediatric AFD patients. Atrio-ventricular and intra-ventricular intervals may increase with age and may become prolonged in advanced phases of the disease, more likely reflecting a progressively increasing disease burden and age-related degenerative process. Electrocardiographic parameters may change together with macroscopic myocardial changes (i.e. prolonging PR with left atrial enlargement due to left ventricular hypertrophy with diastolic dysfunction) losing their diagnostic relevance in the differential diagnosis of the left ventricular hypertrophy. The use of PR interval minus P-wave duration in lead II has been proposed as an index useful to overcome the impaired diagnostic value of PR duration in AFD patients with enlarged left atrial dimension [9]. The specificity of this latter index remains to be confirmed in larger series. In adults, ECG signs of left ventricular hypertrophy are present in up to 60% of men and 18% of women and can be associated with repolarization abnormalities. These changes are observed in lateral leads (V5 and V6) in patients with LV hypertrophy and Late Gadolinium Enhancement (LGE) (sign of focal fibrosis) in CMR as well as in patients without echocardiographic signs of left ventricular hypertrophy and no evidence of focal scarring. Abnormal low voltages on ECG (i.e. the total sum of QRS amplitude in DI, DII, DIII <1.5 mV) are not typical of AFD patients and this sign could be useful to consider the diagnosis unlikely [10].

Ambulatory ECG monitoring

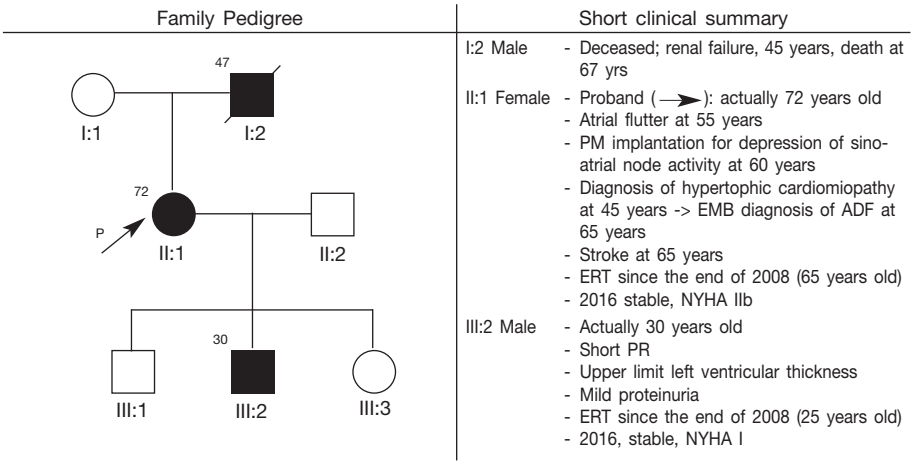
The most common arrhythmia in patients with AFD is sinus bradycardia, followed by ectopic rhythm. Increasing age has been demonstrated to be associated with progressive sinus and atrio-ventricular node disease necessitating a close monitoring for bradyarrhythmias and the implantation of a pacemaker [11]. Therefore, bradyarrhythmias are common in adult male patients, most of all in the late phase of the disease. Arrhythmias, including atrial fibrillation and ventricular tachycardia, occur in about 30-40% of AFD patients; of note they can be encountered in patients with preserved left ventricular function and in the absence of left ventricular hypertrophy or valve disease [12].

Non-sustained ventricular tachycardia and nonspecific intra-ventricular conduction disturbances are detectable in almost all affected adults. The recurrence of arrhythmias is independent of the presence of LV hypertrophy. Sudden cardiac death related to ventricular arrhythmia is uncommonly observed but remains the most life-threatening condition even if a rather more important role for bradycardia has been proposed.

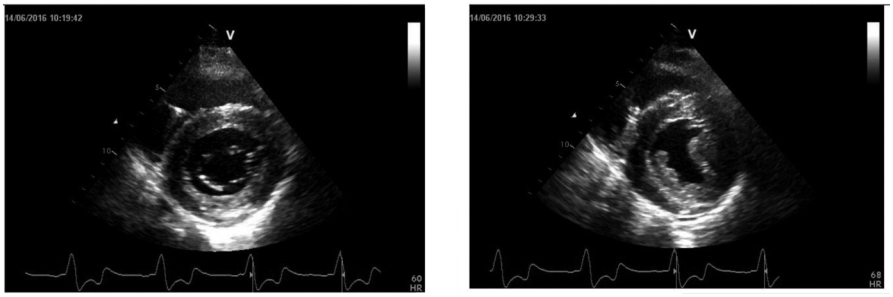
Echocardiography

Left ventricle mild to moderate concentric hypertrophy (14 to 20 mm) is the most common finding in affected adult patients (fig. 1). Among AFD patients with concentric LV hypertrophy the prominence of papillary muscles and the ratio between papillary muscle size to LV circumference have been proposed as echocardiographic marker for diagnosing AFD. Their combination has yielded a sensitivity of 75% and a specificity of 86% for diagnosing AFD with LV hypertrophy [13]. LV remodeling or hypertrophy has been demonstrated in male and female patients since adolescence. Anyway, even in classic AFD male patients (those with the most severe phenotype), the maximum diastolic interventricular septum thickness is unlikely to be over 15 mm below 20 years

Family Pedigree and clinical data, ECG and 2D-TTe of II:1



MOGE(S) Nosology
II:1 - MH(15MM) OH(AF+PM)+C+E(CV)+N(STROKE) GXL EG-*GLA*[p.Tyr184Asp] Sc-IIb
III:2 - ME[H](10mm) OH(PR 80 ms)+E(CV) + K (Proteinuria) GXL EG-*GLA*[p.Tyr184Asp] SA-I
I:2 - MH(N-Obs) OH + K (CRF) +E(CV) GXL EG-*GLA*[p.Tyr184Asp]



II:1 Echocardiographic parasternal short-axis of the same patient. *To notice:* concentric hypertrophy (maximum thickness 15 mm), thickened mitral valve leaflets, prominent papillary muscles.

Fig. 1. The figure shows the pedigree of a family with classic AFD, the summary of clinical phenotypes in affected members as summarized by the MOGE(S) nosology system and the ECG and 2D-TTE images of family member II:1.

of age. For this reason severe LV hypertrophy (>15 mm) below 20 years of age could be a feature that make the diagnosis of AFD unlikely. Progressive systolic and diastolic dysfunction becomes evident with increasing age. Systolic and diastolic tissue Doppler imaging indexes show early reductions before the onset of myocardial hypertrophy; usually the longitudinal performance is impaired before the radial one. In up to 50% of patients with hypertrophy basal LV posterior wall shows selective hypokinesia and a corresponding thinning (4 to 7 mm). Aortic and mitral valve are mildly involved in the disease and may show thickened and stiffer leaflets in 20% of children and in about 50% of adults. LV mass may be increased in up to 76% of patients and in almost all women aged >45 years.

CMR

CMR provides information on LV hypertrophy, trabecular and papillary anatomy, and may detect delayed enhancement that is related to the presence of ventricular scarring. Sub-endocardium is usually spared by DE and in most of DE-positive patients it is distributed in the infero-postero-lateral LV wall. DE may be the substrate for electrical re-entry and sudden arrhythmic death. It has been proposed that focal fibrosis may reflect in homogeneous LV wall stress or relative myocardial ischemia in the presence of patent epicardial coronary arteries.

EndoMyocardial Biopsy (EMB)

The endo-myocardial biopsy provides a definite diagnosis in both classic forms and cardiac variants. The light microscopy study shows optically empty myocytes that correspond to the effects of tissue processing on intracellular sphingo lipids. The ultrastructural study shows characteristic osmiophiliclamellated bodies in the absence of medication use inducing FD-like storage (i.e. amiodarone or chloroquine) [1,6]. Gb3 deposits are specifically labeled by anti-Gb3 antibodies (fig. 2) [1]. These findings demonstrate that LV hypertrophy is, at least partly, the result of intramyocyte accumulation of glycosphingolipids. The accumulation of globotriaosylceramide in the myocardium can occur in patients with borderline LV hypertrophy offering proof of evidence of early myocardial involvement. Histology of the heart has been recognized as the gold standard for a definite diagnosis of AFD in cases with an uncertain, non-classical phenotype in which enzymatic or genetic testing cannot provide a definite diagnosis [10].

Extracardiac traits

In presence of cardiac signs and symptoms mentioned above and typical extra cardiac features (tab. I) the clinical suspicion of AFD should be high. Among others, cornea verticillata has shown to be highly specific when a iatrogenic cause (i.e. choloquine and amoidarone) is excluded [1]. Definitive diagnosis should be based on genetic testing, enzyme activity and tissue studies demonstrating Gb3 accumulation. If pathological evaluation is not available, genetic results should be interpreted with the support of enzyme assays in male subjects and with imaging or functional test useful to demonstrate organ/tis-

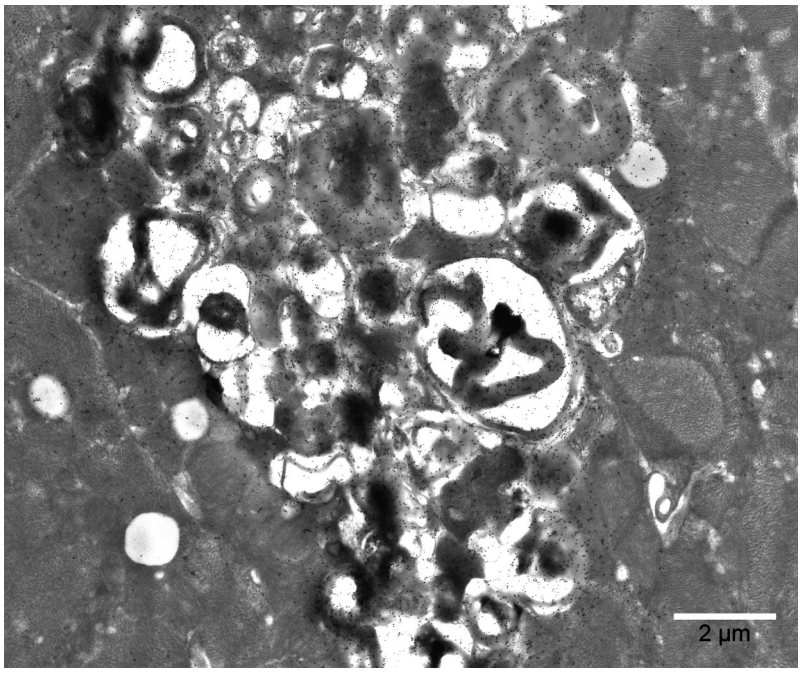


Fig. 2. Electron micrographs from the endomyocardial biopsy of a patient with cardiac variant AFD caused by the p.(Asn215Ser) mutation. The dark osmiophilicintra myocyte bodies are specifically immune labeled by anti-Gb3 antibodies (small gold particles).

Table I - AFD – Extracardiac Features.

Ophtalmologic	Cornea verticillata, posterior subcapsular cataract, visual impairment.
Renal/urinary	Hematuria, microalbuminuria, proteinuria, renal failure
Cutaneous	Angiokeratoma, teleangiectasia, hyperhidrosis, hypohidrosis, anhidrosis
Audiologic	Hearing impairment, sudden deafness, tinnitus, vertigo
Endocrine	Delayed puberty, growth delay
Gastrointestinal	Abdominal pain, constipation, diarrhea, nausea, vomiting
General feeling	Cold intolerance, heat intolerance, fever attacks
Muscoloskeletal	Body pain, joint pain, joint stiffness, limb weakness, muscle pain
Neurologic	Peripheral: chronic pain, pain attacks, postural hypotension Central: stroke, transient ischemic attacks
Psychiatric	Depression
Respiratory	Asthma
Vascular	Lymphedema

sue involvement. The diagnosis of AFD is certain in patients who carry a known pathogenic GLA mutation. Tissue studies are needed to demonstrate Gb3 accumulation when an atypical GLA variant or a novel mutation is found in a patient with signs of organ involvement. In silico analyses might contribute but it has been demonstrated that can't be conclusive. Integrated interpretation of genetic, pathologic and clinical data should provide a precise evidence of causa-

lity or reasons for exclusion. Actually, AFD is a treatable disease: the earlier the enzymatic replacement therapy is begun, the greater the potential for benefit: the correct diagnosis is mandatory to prevent irreversible organ damage.

Treatment

The treatment of AFD is currently based on the regular IV administration of ERT [after 2001, with two possible formulations: agalsidase alfa (Replagal®; Shire HGT) or agalsidase beta (Fabrazyme®; Genzyme Corp)], which is indicated in patients with proven clinical and genetic diagnosis. Beneficial effects have been demonstrated on symptoms (such as pain), cardiovascular function and renal function without convincing evidence for an effect on neurological events [14]. In a recent study including 211 adults and seven children (range of treatment duration, 0 to 9.7 and 0 to 4.2 years respectively), time on ERT was associated with a statistically significant small linear decrease in left ventricular mass index ($p=0.01$); a reduction in the risk of proteinuria after adjusting for angiotensin-converting enzyme inhibitors and angiotensin receptor blockers ($p<0.001$) and a small increase in the estimated glomerular filtration rate in men and women without pre-treatment proteinuria ($p=0.01$ and $p<0.001$, respectively). However, the same benefits were not observed in children [15]. Overall, the effectiveness of ERT for adults and children with Fabry disease remains difficult to assess: major reasons are the lack of control groups and objective markers able to measure the benefits of ERT. Tissue biopsy could highly contribute to demonstrate the effects of ERT on tissue deposits of Gb3 but this would imply serial invasive procedures on heart and kidney, while remaining impossible at the nervous level. Surrogate markers are being used, such as dosage of Gb3 and LysoGb3, but they do not inform about organ benefits. Serial imaging and functional studies contribute to evaluate the effects of ERT.

A novel oral pharmacological chaperone treatment (Migalastat, Galafold®) is an emerging alternative to intravenous ERT: it stabilizes specific mutant forms of α-Gal (amenable mutations) to facilitate normal lysosomal trafficking [16]. Stem cell therapy is a future possible option requiring further developments.

Heart and kidney transplantation has been performed in the past in unrecognized AFD patients, clinically diagnosed with end-stage cardiomyopathy or end-stage renal failure. When these patients are diagnosed after heart or kidney transplantation treatment with ERT is indicated to limit further progression of the Gb3 accumulation in non-transplanted organs/tissue. Supportive treatments, both cardiologic and nephrological are usually maintained. Pregnant women can continue ERT treatment during pregnancy.

Conclusion

AFD is a rare X-linked lysosomal storage disease that is characterized by the involvement of multiple organs and tissues. Enzyme replacement therapy is available since 2001. The disease is formally included in the list of rare diseases (ICD-10: E75.2; ORPHA324; RCG080; MIM#301500). The diagnosis is typically late and is done years (up to decades) after the onset of symptoms.

Early diagnosis provides the basis for ERT that controls disease progression. End-stage renal failure or heart failure may lead to the need of organ transplantation that could be eventually avoided in timely diagnosed and treated patients.

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