Biochemical and Molecular Genetic Basis of Fabry Disease

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Anderson-Fabry disease (AFD) is an inborn error of glycosphingolipid metabolism that is due to a deficiency of the lysosomal hydrolase α-galactosidase A (α-Gal A) (1). It is an X-linked lysosomal storage disorder (LSD) associated with multisystemic involvement resulting from the accumulation of neutral glycosphingolipids (mostly ceramide trihexoside [CTH]) in various organs (2). The disease manifests primarily in affected hemizygous males and to some extent in heterozygous (carrier) females. After a brief overview of the clinical features, this section of the supplement focuses on the biochemical and genetic basis of AFD.

Overview of Clinical Manifestations

The clinical features of AFD include corneal and lenticular opacities (Figure 1), acroparesthesias, angiokeratomas (Figure 2), hypohidrosis, and major end organ disease (with involvement of the kidneys, heart, and brain) (3,4). Acroparesthesia constitutes the earliest major source of morbidity during the first two decades of life and often remains undiagnosed unless other manifestations or a positive family history provide diagnostic clues. Most affected males have proteinuria and ultimately develop renal failure. Details on the renal aspects of AFD follow in subsequent sections. The clinical course can also be complicated by cardiac and cerebrovascular disease, which combined with renal failure, lead to early mortality. The clinical course in heterozygous (carrier) women with AFD indicates a later onset of symptoms and milder progression, although some women may have presentations not unlike that seen in the classically affected male. The median survival is 50 yr (interquartile range, 40 to 56) for affected males and 70 yr (57 to 78) for carrier females (3,4).

Fabry disease is a rare pan-ethnic disorder with an estimated frequency of 1 in 117,000 male births (5). However, recent studies suggest that the incidence may be underestimated, as certain patients with residual enzyme activity (5 to 35% of normal levels) have disease characterized predominantly by cardiac involvement. Indeed, in one study that involved screening of patients with left ventricular hypertrophy, 3% were found to have an underlying α-Gal A deficiency (6). In addition to ventricular hypertrophy, arrhythmias and valvular involvement were also noted (see Kampmann et al. in this issue). Although there was no indication of chronic renal insufficiency, several of the patients were found to have proteinuria.

The diagnosis of AFD among patients without a positive family history has been a challenge to nephrologists and an even greater dilemma for other physicians who may see these patients before the onset of renal failure. Indeed, there is often significant delay in the diagnosis of AFD, with mean age at diagnosis for males of 21.9 yr (3). The renal manifestations usually occur during adolescence or adulthood. Most renal related symptoms and signs are nonspecific. Polyuria due to concentration defects may be the earliest renal symptom, but it is often ignored by patients or physicians. Proteinuria, usually in the non-nephrotic range, and chronic renal insufficiency are the most common reasons for a referral to nephrologists. Birefringent oval fat body with a Maltese cross pattern can be seen in the urine sediment under polarized light, but this finding is not specific to AFD. Renal biopsy is frequently not considered because of the lack of active urine sediment or nephrotic syndrome. Thus, the clues for AFD are derived mainly from recognition of the associated extrarenal manifestations, which include recurrent burning pain in the hands and feet (acroparesthesia), heat intolerance, lack of sweating, and characteristic skin lesions—individual punctate, dark red spots or angiokeratomas in a “bathing suit” distribution (Figure 2). Incidental findings of corneal opacity from slit-lamp microscopic examinations by ophthalmologists or optometrists can be another sign (Figure 1). In adults, unexplained left ventricular hypertrophy, arrhythmia, and stroke-like symptoms such as hemiparesis, vertigo, diplopia, and others should also raise the index of suspicion for AFD.

The Metabolic Defect

Glycosphingolipids (GSLs) are components of the plasma membrane that are degraded in the lysosome after internalization through the endocytic pathway. Their sequential catabolism requires the concerted action of several hydrolyzing enzyme and various cofactors (e.g., sphingolipid activator proteins). Deficiency of these enzymes or their relevant cofactors lead to distinct clinical entities (e.g., Tay-Sachs, Sandhoff, G_M1 gangliosidosis, Fabry, Gaucher), associated with characteristic manifestations that reflect the tissue-specific sites of storage of the incompletely degraded substrates (7).
Deficient activity of α-Gal A in AFD patients leads to the progressive accumulation of GSLs with terminal α-galactosyl residues (including blood group B, B1, Pk, and P1 antigen), derived primarily from the turnover of cells in the kidneys, liver, lungs, and erythrocytes (2). Endogenous metabolism is a major source of substrate accumulation in avascular sites such as cornea and in neural cells, which presumably are protected from the increased circulating levels of CTH by the blood-brain barrier.

Lipid deposits are prominent in epithelial cells of the glomeruli and tubules of the kidney, in cardiac myocytes, ganglion cells of the autonomic system, and the cornea. Other tissue target sites of pathology include endothelial, perithelial, and smooth-muscle cells of blood vessels and, to a lesser degree, histiocytic and reticular cells of connective tissue. The deposited GSLs accumulate within extended multivesicular bodies or, in more advanced stages, as free intracytoplasmic masses, which may lead to cellular dysfunction or degeneration, or necrosis as a consequence of ischemia due to vascular occlusion.

Plasma GSLs are synthesized primarily in the liver and incorporated into lipoprotein particles (i.e., LDL and HDL) in the systemic circulation. Turnover times for plasma GSLs are calculated to be from 4 to 8 d, and the turnover rates are from 1 to 6 μmol/d (8). Although storage occurs in hepatocytes, hepatic dysfunction or enlargement is not a feature of AFD. Kinetic studies suggest that about 25% of the plasma GSL pool is newly synthesized each day, and a portion is derived from the turnover of senescent erythrocytes (2). The rate of exchange of plasma GSLs with those found in plasma membranes or stored in disease cells has not been determined. These observations have to be taken into account when assessing the significance of changes in plasma CTH as a surrogate measure of stored substrate and clinical response to enzyme therapy. About 75% of the urinary cells shed by an affected individual are derived from desquamated lipid-laden distal tubular epithelial cells (9). This observation suggests that changes in urinary CTH concentration may be a more sensitive and specific measure of tissue (i.e., kidney) and body CTH burden.

The Molecular Basis

The α-Gal A protein is encoded by a 12-kb gene mapped to the long arm (Xq22.1 region) of the X chromosome (2). The native α-Gal A enzyme is a glycoprotein of approximately 101 kD with a homodimeric structure. Multiple natural forms are observed on isoelectric focusing of purified preparations from plasma and various tissues, resulting from variations in the amount of sialic acid on the carbohydrate chains. Negatively charged sialic acid residues are presumably responsible for the prolonged circulatory half-life of enzyme administered intravenously to AFD patients. Thus the type and number of complex sugar residues may be a factor in determining which organs acquire sufficient enzyme concentration and functional activity after exogenous administration.

The α-Gal A gene is comprised of seven exons (Figure 3).
Molecular analyses have demonstrated that a wide variety of molecular lesions can cause AFD; approximately 57% of disease alleles are missense mutations, 11% nonsense mutations, 18% partial gene deletions, 6% insertion, and 6% RNA processing defects due to aberrant splicing (2). Mutations are found in all seven exons (10,11,12). Most mutations are private, i.e., confined to a single AFD family. There are several mutations reported in more than one unrelated family, but many of these occur at CpG dinucleotides, a known mutation hot spot due to the deamination of methylcytosine to thymidine (13). The α-Gal A gene defect may be associated with no detectable enzyme activity or protein (as a result of an unstable mRNA transcript), no enzymatic activity but detectable levels of enzyme protein (mutations that involve the catalytic site or result in improper folding of the protein), and measurable residual α-Gal A activity (mutations that alter protein folding, substrate binding, or rate of turnover).

Knowledge of the patient’s mutation and its molecular consequences may have practical relevance. Most patients with no detectable protein (i.e., immunologic cross-reacting material [CRIM]–negative) may be at higher risk for immune-mediated adverse events, although the experience noted in the clinical trials reported to date suggests that most patients (including CRIM-negative individuals) tolerate their infusions and that some of the patients who have seroconverted have shown a decline in antibody titers over time. Furthermore, none of the antibodies detected have been shown to neutralize the activity of the enzyme. As the studies involved a small number of patients, the relationship of antibody formation to genotype could not be established. However, greater experience with treatment of more AFD patients should elucidate the significance of genotype information and whether antibody formation should remain a safety or therapeutic concern.

It has recently been demonstrated that certain mutations associated with residual α-Gal A activity may be ameliorated by binding of certain reversible competitive inhibitors of the enzyme with the mutant protein. These inhibitors (e.g., galactose, 1-deoxygalactonojirimycin) are speculated to act as “chemical chaperones” that enhance the stability of the mutant enzyme by promoting the proper folding, dimerization, and processing of the enzyme (14). Additional studies are required to define the role of this approach in the treatment of atypical AFD patients with residual α-Gal A activity.

Identification of the mutation in a given AFD family permits precise diagnosis of other family members (and their proper assignment as carriers or affected). This may be accomplished by sequencing techniques and the use of a mutation-specific restriction endonuclease digestion or the use of oligonucleotide probes corresponding to the normal and mutant gene sequences.

**Genotype/Phenotype Correlation**

Efforts to establish genotype/phenotype correlations have been limited, because most AFD patients have private mutations. Worldwide registry studies of AFD patients with known genotypes followed for a prolonged period of time would provide important information to establish genotype/phenotype correlation. Prediction of the clinical phenotype on the basis of type or location of a molecular lesion is also premature, as information on structure-function relationships is incomplete. Most genotypes (93%) are associated with classic AFD (2). Genotypes with nonsense and frameshift mutations, which cause a premature termination of protein synthesis, are associated with classic AFD. Many missense mutations involving catalytic sites, dimerization sites, or protein folding also correlate with classic AFD. So far, only 18 genotypes have been reported to be associated with the cardiac variant AFD; all except one (an in-frame shift with three-nucleotide deletion) are missense mutations. Several of them cause protein instability due to improper protein folding, and one alters the glycosylation site (N215S). Interestingly, four genotypes (R112H, R301Q, G328R, and G404del) have been reported to be associated with cardiac variant AFD in one family but classic AFD in the other (2).

Investigators recently found the presence of detectable residual α-gal A activity in peripheral leukocytes to be associated with a later onset of chronic renal insufficiency, lower renal CTH content, and lower scores for renal histologic damage (15). They also noted that conservative missense mutations were associated with longer renal survival compared with nonconservative missense or other mutations.

The basis of phenotypic variation in AFD is not entirely clear, although it may be partly attributed to the heterogeneity in underlying causal mutations and perhaps the patient’s blood type as well. Patients with blood group B and AB (with α-galactosyl terminated residues on their cell membranes) may have a greater body substrate burden and have a more aggressive disease course when compared with patients with blood group A or O. This hypothesis has not
been systematically studied, and additional information on the natural history of AFD is necessary to better appreciate the various factors that may influence disease expression.

**Genetic Counseling**

The diagnosis of Fabry disease should prompt screening of family members who may be at risk for the disease or carriers of the trait, which segregates as an X-linked disorder. Although manifest primarily in hemizygous males, the condition can be expressed in heterozygous (carrier) women presumably as the result of nonrandom chromosome X-inactivation. Although several AFD-causing gene defects (>200) have been identified, molecular testing often requires analysis of the α-Gal A gene sequence, as most affected families tend to have private mutations.

In symptomatic individuals, the diagnosis can be established on the basis of low activity of α-Gal A in plasma, leukocytes, or cultured skin fibroblasts (16). There is significant overlap in the α-Gal A activity levels of carrier females and the general population; thus, α-Gal A activity may not reliably distinguish the affected (carrier) from unaffected (non-carrier) women. Among patients with a family history, the diagnosis can be confirmed by molecular testing on the basis of demonstration of an α-Gal A mutation in cases in which the causal defect is known or by linkage analysis when the specific gene defect is not established.

Among affected women, quantitation of urinary sediment glycolipids (by HPLC) may be used for diagnostic confirmation, although this assay is not routinely available. Analyses of GSLs in urinary sediment among affected women reveal increased total glycolipid fraction (10- to 100-fold), and elevated CTH (2- to 70-fold) and digalactosylceramide levels (17). Heterozygote detection may also be accomplished by the histologic finding of lipid-laden cells in biopsied skin and tissues or in the urinary sediment.

AFD can be detected prenatally on the basis of analyses of α-Gal A activity or identification of the causal α-Gal A mutation in cells obtained by chorionic villus sampling or amniocentesis. Linkage analysis may be performed as an indirect method in families where the causal gene defect has not been identified but the relevant haplotype information is established. This approach may also be used to assign carrier status among women in families at risk, but it may be limited by the presence of recombination between the marker and relevant genes. Genetic counseling regarding prognosis is complicated by clinical heterogeneity.

The availability of enzyme therapy for AFD (see Branton et al. in this issue) necessitates the identification of disease markers that may prove to be valuable in monitoring response, or predictive of disease progression and lead to earlier consideration of therapy. Additional information is also required on affected males and carrier females to ascertain the need for and appropriate time to initiate treatment to achieve the best possible response, ideally before established end-stage histologic changes. Further biochemical and molecular studies may lead to new insights into novel therapeutic approaches, including substrate synthesis inhibitors, chemical chaperones, and gene therapy (18,19).

**References**