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## Fabry Disease: from Molecular Diagnosis to Enzyme Therapy

Yeong-Hau H. Lien, MD, PhD

Fabry disease is a rare genetic disease due to a deficiency of the lysosomal hydrolase  $\alpha$ -galactosidase A ( $\alpha$ -Gal A) [1]. The recent advance of knowledge in Fabry disease can be applied to other lysosomal diseases and enhance our understanding in the pathogenesis and management of those once considered miserable and untreatable diseases. Unlike other lysosomal diseases, Fabry disease involves multiple organ systems and may have an onset at a wide range of age [2], therefore, it may be encountered by a variety of medical professionals, such as:

- Nephrologists for proteinuria and chronic renal failure
- Dermatologists for angiokeratoma
- Cardiologists for cardiomyopathy, valvular disease, heart failure, and arrhythmia
- Neurologists for neuropathic pain and strokes
- Ophthalmologists for corneal opacity and cataracts
- Pediatricians for febrile illness, exercise intolerance
- Primary care physicians for pain, weakness, heat and cold intolerance
- Otolaryngologists for hearing loss and tinnitus
- Rheumatologists for joint pain and fever

The diagnosis of Fabry disease has been a challenge to physicians. The misdiagnoses have been included:

- Systemic lupus erythematosus
- Focal glomerulosclerosis
- Familial or idiopathic cardiomyopathy
- Coronary artery disease
- Strokes
- Multiple sclerosis
- Fibromyalgia
- Erythromelalgia
- Raynaud's syndrome
- Rheumatic fever
- Rheumatoid arthritis
- Neurosis
- Growing pain
- Malingering

Fabry disease is an X-linked disease, thus it manifests primarily in affected hemizygous males and to some extent in heterozygous (carrier) females. Traditionally, the diagnosis is made by measuring  $\alpha$ -Gal A activity in plasma, leukocytes, or skin fibroblasts. However, female carriers can have  $\alpha$ -Gal A activity ranged from barely detectable to normal [2]. For this reason, recently, molecular diagnosis or mutation analysis has emerged as an important diagnostic tool for Fabry disease. We routinely perform direct sequencing on RT-PCR product using mRNA isolated from 4-ml whole blood. We identified mutations in 10 unrelated families including one novel mutation (R301G) and 9 previously reported mutations (P40S, R112C, N215S, R301Q, R220X, R227X, 777delA, 1188delC and IVS1-1G>C). From 22 individuals at risk (most of them have heterozygote mother) of 6 unrelated

families, we diagnosed 4 male patients and 7 female carriers [3]. The same technique can be used for prenatal diagnosis.

Knowledge of the patient's mutation and its molecular consequences may have practical relevance. Currently, efforts to establish genotype/phenotype correlations have been limited, because most Fabry disease patients have private mutations. Worldwide Fabry registry studies that follow Fabry disease patients with known genotypes 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 Fabry disease. Genotypes with nonsense and frame-shift mutations, which cause a premature termination of protein synthesis, are associated with classic Fabry disease. Many missense mutations involving catalytic sites, dimerization sites, or protein folding also correlate to classic Fabry disease. So far, only 18 genotypes have been reported to be associated with cardiac variant Fabry disease, in which patients only have cardiomyopathy and proteinuria. All except one (an in-frame shift with 3-nucleotide deletion) are missense mutations. Several of them cause protein instability due to improper protein folding, and one alters the glycosylation site (N215S). Interestingly, 4 genotypes (R112H, R301Q, G328R and R404del) have been reported to be associated with cardiac variant Fabry disease in one family, but classic Fabry disease in the other [2].

The most exciting development in Fabry disease is the availability of enzyme replacement therapy [4,5]. Two products have been developed independently: Fabrazyme (Genzyme) and Replagal (Transkaryotic Therapies). Both have been available in Europe and other countries, but not in the United States. Fabrazyme is manufactured in Chinese hamster ovarian cells, while Replagal in human fibroblasts. Chemically, the glycosylation patterns are different between the two. Fabrazyme is given at 1.0 mg/kg over 4 hours, and Replagal 0.2 mg/kg over 40 min, every other week. As for safety profiles, both cause infusion reactions such as fever, chills, headache and rigors, which are not uncommon in other types of protein therapies. Both induce antibodies against  $\alpha$ -Gal A, but the development of antibodies does not appear to affect therapies and the titers of antibodies seem to decrease over time. The initial studies show that Fabrazyme significantly reduces globotriaosylceramide levels (undegraded  $\alpha$ -Gal A substrate) in plasma and endothelial cells, but have no significant clinical effects on renal function, pain relief and quality of life [4]. Replagal appears to reduce neuropathic pain and preserve renal function [5]. Clinical trials are ongoing for both enzymes and will provide more information on the efficacy and safety properties.

Although the enzyme replacement therapy is promising, the requirement of frequent infusions and the enormous cost for life-long therapy make gene therapy an attractive alternative solution. Gene therapy has been tried on a  $\alpha$ -galactosidase A knockout mouse model. The adenoviral vector based gene therapy was able to increase  $\alpha$ -galactosidase A activity transiently. The globotriaosylceramide storage in tissues was reduced to near normal range for up to 6 months [6]. In our lab, we tested whether RNA/DNA chimeric oligonucleotides can be used to correct point mutation in Fabry cells. Our preliminary data showed that the point mutations in the  $\alpha$ -galactosidase A gene can be converted with this approach in cultured Fabry cells [7] and suggested that it may be a promising modality for Fabry disease.

In conclusion, Fabry disease is a fascinating disease, which can mimic many other diseases. Molecular diagnosis for Fabry disease is available and useful for early and prenatal diagnosis as well as detection of female carriers. Enzyme replacement therapy is promising and will be available in the

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United States soon. Gene therapy is currently under development and may be the ideal therapy for Fabry disease in the future.

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*Y. H. Howard Lien, M.D./Ph.D. is Professor of Medicine, Renal Section, of University of Arizona College of Medicine, Tucson, AZ.*