

Cardiovascular Manifestations of Fabry Disease and the Novel Therapeutic Strategies

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Abstract

Fabry disease is an inherited lysosomal storage disorder characterized by a pathological intracellular glycosphingolipid deposition. The disease is caused by a deficit in the lysosomal enzyme α -galactosidase A, the gene for which is located in the X chromosomal region Xq 22. Globotriaosylceramide (Gb3) accumulate progressively in multi-organ vulnerable cells throughout the body, including cardiovascular, renal, and cerebrovascular systems. The present manuscript is to review cardiovascular and renal manifestations of Fabry disease and the new diagnostic procedures for earlier detection and the therapeutic assessments of this disease. We are applying noninvasive cardiovascular and microcirculation analysis methods and novel cardiac biomarkers. Novel therapeutic strategies for this disease have been developing in recent years, which include the clinically introduced enzyme infusion replacement therapy and experimentally developing gene-transfer therapy. We have reported that AAV-mediated muscle-directed gene transfer is very effective for long-term systemic delivery of α -gal A (25% of normal mice enzyme activity), resulting in complete clearance of multi-organs Gb3 accumulation. Echocardiographic and immunohistochemical examination demonstrated structural improvement of cardiac hypertrophy. When and to whom the novel therapeutic strategies should be applied to obtain the maximum efficacy and safety remain to be established.

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Key words: Fabry disease, cardiac hypertrophy, endothelial function, troponin, B-type natriuretic peptide, gene therapy, enzyme replacement therapy

Introduction

Fabry disease is an inherent lysosomal storage disorder, and the disease is caused by a defective activity of lysosomal enzyme α -gal A (α -galactosidase

A), the gene for which is located in the X chromosomal region Xq 22^{1,2}. Globotriaosylceramide (Gb3), the major substrate of the deficient α -gal A, accumulate progressively in multi-organ vulnerable cells, tissues, and organs throughout the body, including cardiovascular, renal, and cerebrovascular system.

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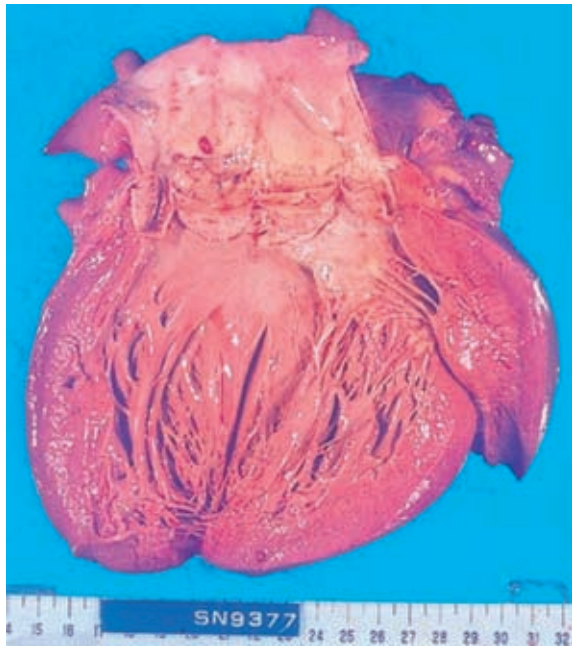


Fig. 1 Hypertrophy of left ventricle (61 years old male Fabry disease, autopsy case)

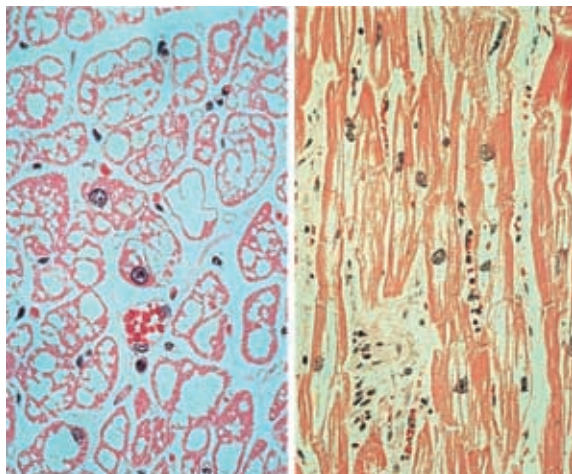


Fig. 2 Left ventricular myocardium shows hypertrophic and extensively vacuolated cardiomyocytes. (Hematoxylin and eosin, $\times 250$) (61 years old male Fabry disease, autopsy case)

Affected cell types include endothelial cells, renal epithelial cells, pericytes, and peripheral neuronal cells, that are more evident in hemizygous males, and present characteristic skin lesions (angiokeratoma corporis diffusum universale), neurological symptoms (acroparesthesia), ocular features (corneal opacities), cardiac involvement (left ventricular hypertrophy and conduction

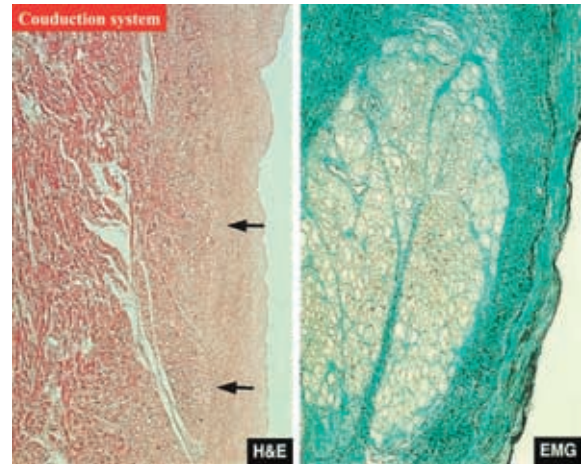


Fig. 3 Conduction system of the heart; engulftment by glycolipid material and vacuolization. (left Hematoxylin and eosin, right EMG stain) (61 years old male Fabry disease, autopsy case)

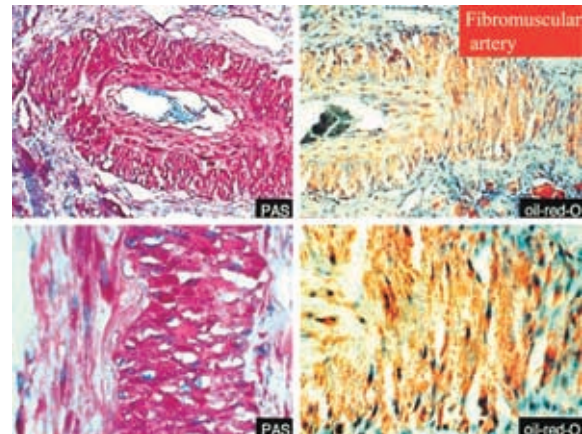


Fig. 4 Extensively vacuolated smooth muscle cells of mesenteric artery which were occupied by glycolipid material (PAS positive and oil-red positive). (61 years old male Fabry disease, autopsy case)

abnormality), cerebrovascular manifestations (thrombosis and hemorrhage), and kidney involvements (renal failure).

Most male patients with this disease present three clinical phases. The first phase (childhood and adolescence) is characterized by myalgia, arthralgia, acroparesthesia, fever, cutaneous angiokeratoma, and development of corneal opacities. The second phase is characterized mainly by appearance of renal involvement. The third phase is complicated by worsening renal impairment and involvement of the cardiovascular and cerebrovascular systems.

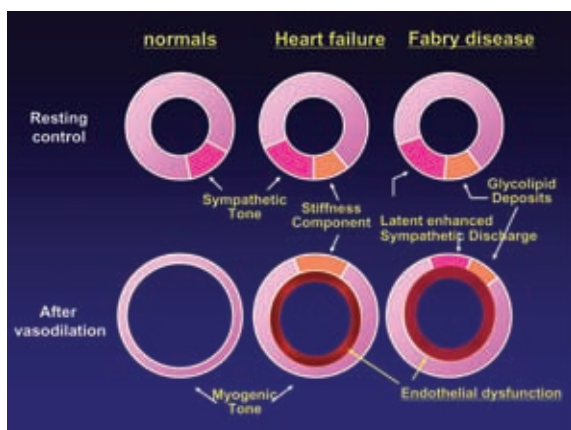


Fig. 5 Previous plethysmographic study indicated the presence of vasoconstrictive process in both resistance and capacitance vessels in cutaneous and skeletal muscular vascular beds. A limited response in the cutaneous circulation to vasodilation procedure was also evidenced. These data are interpreted as that latent enhanced sympathoadrenal discharge and endothelial dysfunction play an important role in the disturbed pathophysiology in peripheral circulation of Fabry disease¹⁶.

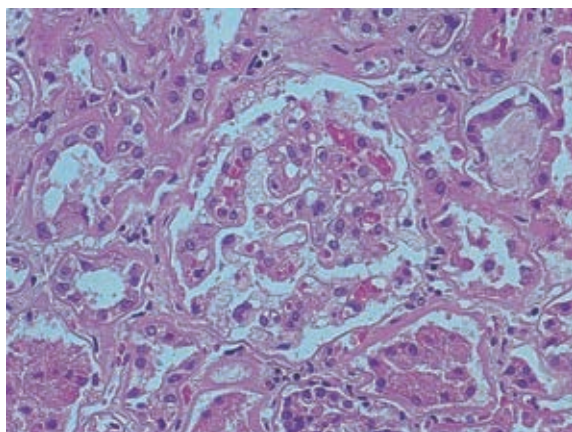


Fig. 6 Extensively vacuolated glomerular epithelial cells and tubular cells of the kidney. (Hematoxylin and eosin stain) (61 years old male Fabry disease, autopsy case)

Progression to end-stage renal disease is common in hemizygous male patients in the fourth to fifth decade of life, and critical events occur because of cerebral and/or cardiovascular complications in patients undergoing chronic dialysis therapy. However the clinical manifestations are sometimes varied, the residual activity of α -gal A and different gene mutations might explain the different phenotypes of this disease such as cardiac variant,

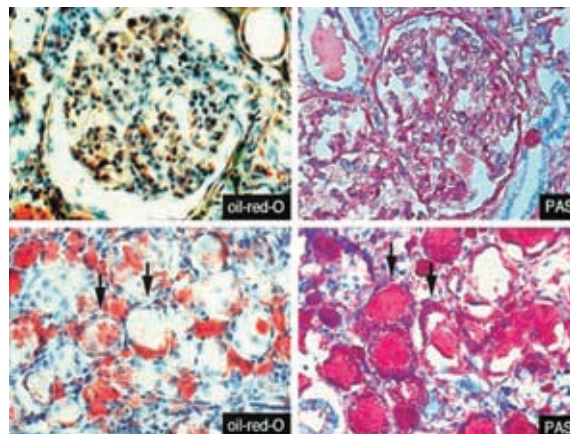


Fig. 7 Vacuolated glomerular epithelial cells and tubular cell which were occupied by glycolipid material (PAS positive and oil-red positive). (61 years old male Fabry disease, autopsy case)

renal variant, or other types^{3,4}.

The present manuscript reviews cardiovascular manifestations of Fabry disease and the diagnostic procedures for earlier detection and therapeutic assessments of the disease, and furthermore reviews novel therapeutic strategies developed in recent years.

Cardiovascular Manifestations of Fabry disease

Common cardiac manifestations include progressive left ventricular hypertrophy (**Fig. 1 and 2**) usually with normal systolic function but mild to moderate impairment of diastolic function, vasospastic or stenotic coronary artery disease leading to angina pectoris and myocardial infarction, valvular disease especially mitral insufficiency due to valvular accumulation of Gb3, conduction abnormalities or arrhythmias; AV-block or short PR-interval, cardiac sudden death associated with Gb3 accumulation in the AV-conduction system (**Fig. 3**), and congestive diastolic heart failure⁵. Some of the cardiac structural changes associated with Fabry disease may not be associated with significant symptomatic condition or functional disturbances, but would serve as useful markers of disease progression and severity.

Although this disease has been considered an x-linked recessive disorder, affected female patients are increasingly recognized, suggesting that this

disease may follow an x-linked dominant rather than x-linked recessive transmission⁶. In a recent study, cardiac involvement was detected in 56% of heterozygous female patients <38 years of age, in 86% of female patients >38 years of age, and almost all female patients >45 years of age⁵. In contrast to that Fabry disease has been recognized as the cause of left ventricular hypertrophy in 6% of men with late-onset hypertrophic cardiomyopathy (HCM), this disease may account for up to 12% of women with late-onset HCM⁷. The higher percentage of myocardial cells involved in the disease suggested a nonrandom or skewed inactivation of the wild-type X chromosome. Skewed X inactivation, a phenomenon by which the same X chromosome is silenced in most of or all the cells of a tissue, has been shown to be common in normal female subjects, and the X-inactivation pattern can vary widely between different tissues⁸⁻¹⁰. This variability makes inaccurate the extrapolation of the X-inactivation of the status from one tissue to another and explains the nearly normal values of blood α -gal A activity in most of the female patients despite the higher prevalence of isolated cardiac involvement compared with hemizygous male patients.

Altered vasomotor activity has been reported as a clinically prominent feature of this disorder, severe extremities pain, paresthesia, or Raynaud-like vasospasm being induced by changes in temperature. Regarding vascular manifestations, where deposition of Gb3 extensively involves in the lysosomes of vascular endothelial and smooth muscle cells (**Fig. 4**), we previously investigated peripheral hemodynamics using segmental pulse volume and venous occlusion plethysmography and thermal probes¹¹⁻¹⁵ in patients with Fabry disease¹⁶. Forearm vascular resistance in Fabry disease patients was significantly higher than that in normal subjects, and forearm venous capacitance was significantly lower. Segmental pulse volume amplitudes showed no significant difference in any segment (upper arm, wrist, thigh, above and below knee, and the calf) between the two groups. However, finger and toe blood flow, finger and toe pulse volume, and temperature in the resting state were all significantly lower than those in normal subjects,

and finger and toe blood flow and pulse volume after vasodilation procedures was significantly lower than those in normal subjects despite equal elevation of digital temperature obtained after the vasodilation procedure in both groups. These findings indicate the presence of vasoconstrictive process in both resistance and capacitance vessels in cutaneous and skeletal muscular vascular beds. A limited response in the cutaneous circulation to vasodilation procedure was also evidenced¹⁵. These data are interpreted as that latent enhanced sympathoadrenal discharge and endothelial dysfunction play an important role in the disturbed pathophysiology in peripheral circulation in this disorder (**Fig. 5**).

Findings in the medium-sized arterial remodeling (increase in radial and carotid artery intima-media thickness and distensibility)¹⁷ or arterial wall properties and Womersley flow (no structural or mechanical abnormality in radial artery)¹⁸ are conflicting. However findings in the endothelial functional abnormalities are coincidence. Investigation of endothelial nitric oxide synthase (eNOS) genetic variations in addition to α -gal A gene mutations in the disease process has revealed that more than half of the patients with Fabry disease carry the eNOS genetic variations; the Glu298Asp variant in exon 7 (approximately 68%) and/or the intron 4 (4b/a) polymorphism (approximately 68%)¹⁹. Further study of the cutaneous microcirculation as a reflection of endothelial functional abnormalities, using the Doppler flow spectroscopy we developed²⁰ will be warranted.

Renal Manifestations of Fabry disease

Renal manifestations develop in the late teens to early twenties with lipiduria, proteinuria with minimum hematuria, nephritic syndrome, hypertension, and progressive renal insufficiency. Proteinuria, isosthenuria, azotemia, and elevation of serum creatinine levels are common findings and suggest renal involvement of Fabry disease. Appearance of "mulberry body" which arises from lipiduria in the urine sediments, is strongly

suggestive of Fabry disease and extremely useful for the diagnosis and management of this disease.

Renal failure is a common manifestation in hemizygous male patients in the 3rd to 5th decade, and critical events develop around the 5th decade of life because of severe cardiovascular or cerebrovascular complications²¹. In the kidney, accumulation of Gb3 occurs in the endothelial cells of every vessel, in the epithelial cells of every tubular segment, and further in the whole glomerular cells (**Fig. 6 and 7**). The broad spectrum of renal lesions is a characteristic pathophysiological continuum with progressive impairment of renal function related to continuous intracellular accumulation of Gb3. Focal and global glomerulosclerosis are later features. Significant increases in patients' urinary excretion of vitronectin receptor (integrin alpha V beta 3) and expression of vitronectin receptor in patients' kidney tissues suggest pathophysiological involvement of integrins in the progression of renal injury in this disease²².

Detection and Assessment of Cardiovascular Involvements

Left ventricular hypertrophy on electrocardiogram is the most common feature in patients with Fabry disease. Fabry disease has been recognized as the cause of left ventricular hypertrophy in 6% of men with late-onset hypertrophic cardiomyopathy (HCM), and in up to 12% of women with late-onset HCM⁷. The mechanism of development of cardiac hypertrophy in Fabry disease is different from other forms of infiltrative cardiomyopathies. For instance, in cardiac amyloidosis, extensive interstitial infiltration is encountered, whereas in Fabry disease, infiltration is caused by intracellular deposits of Gb3. So reduction of tissue Gb3 levels per se is the most important condition for the treatment of Fabry disease. We have reported that 10% of the normal α -gal A activity was enough to clear up the accumulation of Gb3 in the Fabry mouse heart. However, such enzyme level seems not to be enough to clear up Gb3 in patients with cardiac type Fabry disease.

Short PR-interval, AV-block, intraventricular

conduction disturbance, bundle branch block, or occurrence of arrhythmias (sick sinus syndrome, atrial fibrillation, paroxysmal supraventricular tachycardia, etc.) are also observed in this disease. Although there has been little investigation of the proportion of Fabry disease in patients with implanted cardiac pacemakers, not so few patients with this disease might be missed out and classified as undetermined etiology.

Assessment of cardiac structure and function by echocardiography is essential. The study by means of tissue Doppler analysis in the pulsed Doppler mode of Fabry patients with biopsy-proven cardiac involvement showed a reduction of both diastolic and systolic myocardial velocities recorded at septal and lateral corners of mitral annulus²³. Tissue Doppler abnormalities were present not only in patients with left ventricular hypertrophy but also in younger patients with normal wall thickness and represent earlier sign of myocardial damage. Interestingly tissue Doppler imaging seems to be useful in detecting cardiac involvement in heterozygous female carriers with no systemic manifestations of Fabry disease. Tissue Doppler imagings can provide a preclinical diagnosis of Fabry cardiomyopathy, allowing early institution of enzyme replacement therapy.

Enzyme Replacement Therapy

Recently, a novel therapeutic approach of enzyme replacement therapy (ERT) with recombinant α -gal A was introduced. A multicenter randomized double-blind clinical trial in the United States showed that ERT (every 2 weeks for 20 weeks) cleared microvascular deposits of Gb3 from the kidneys, skin, and the heart, and plasma levels of Gb3 (mean value; decline from 14.5 ng/microl to undetectable) were directly correlated with clearance of the microvascular clearance²⁴. Another randomized double-blind trial evaluated effect of ERT (every other week for 24 weeks) on neuropathic pain, renal function, and renal pathology, and demonstrated a significant decline in neuropathic pain severity score, significant increase in creatinine clearance, and significant decrease in glomeruli with

mesangial widening or glomeruli with segmental sclerosis²⁵. In this trial Plasma Gb3 level declined 50% (from 2.1 to 5.6 nmol/ml). In Japanese open ravel trial, twelve patients were assessed before and during the intravenous injection of α -gal A (every another week 12 times 22 weeks). ERT showed continuous decline in plasma and urinary Gb3 levels and decline of Gb3 in kidney biopsy tissues, but renal function remained unchanged in this trial²⁶. Decline in left ventricular volume evaluated by echocardiography, nuclear magnetic resonance imaging, ultrasonic strain rate imaging, and/or electrocardiogram observed in several trials suggested reversibility of cardiac involvement following ERT in this disease²⁷⁻²⁹. We are trying to apply novel cardiac biomarkers³⁰⁻³⁵ to detect and assess the cardiac involvement and therapeutic efficacy in both hemizygous and heterozygous Fabry patients. Now, three male Fabry patients are undergoing ERT with good response in our institute.

Gene Transfer Therapy

A serious limitation in ERT is the short half-life of enzyme molecules, and repeated administration of large amounts of the enzyme is required for a long-term period. Another novel therapeutic strategy is gene-mediated enzyme replacement therapy in which the expression vector for α -gal A is inserted into the patient's cells instead of direct infusion of α -gal A enzyme. Bone marrow cells were transduced with a retroviral vector containing the α -gal A gene and were transplanted into sublethally irradiated Fabry mice³⁶. Increased α -gal A activity and decreased Gb3 storage were observed in multiple organs of recipient animals. In this study, bone marrow cells were used as a reservoir for systemic and continuous secretion of α -gal A. Unfortunately, the efficiency of retroviral mediated gene transfer into human bone marrow cells is still too low to apply this novel strategy into clinical trials. Since retroviral vectors can transduce only dividing cells, it is difficult to find other target cells susceptible to retroviral mediated gene transfer. Another possibility is the use of adeno-associated virus (AAV) vectors, which are able to transduce non-

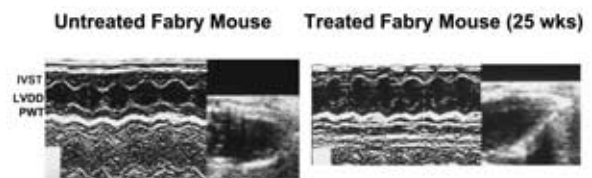


Fig. 8 Echocardiographic evaluation showed significant decreases in intraventricular septal wall thickness in the treated group compared with the untreated group at 25 weeks post gene-transfer treatment.

dividing cells and achieve long-term expression of a therapeutic gene from either integrated or episomal vector genome. The AAV vector carrying the α -gal A gene was delivered to the liver via the hepatic portal vein resulting in long-term correction of both the enzyme activity and the Gb3 storage in the Fabry mice³⁷. However, the safety of intravenous injection of viral vectors must be carefully evaluated prior to clinical application.

We recently reported a simple and clinically applicable strategy for gene-based enzyme replacement of Fabry disease³⁸. The AAV vector was directly injected into the quadriceps muscle of the Fabry mice, resulting in long-term expression of α -gal A (25% of normal mice enzyme activity for 30 weeks observation) and complete clearance of multi-organs Gb3 accumulation. Reduction of Gb3 levels was also confirmed by immunohistochemical and electronmicroscopic analyses. Echocardiographic examination of treated mice demonstrated structural improvement of cardiac hypertrophy 25 weeks after the treatment (**Fig. 8**). We demonstrated that AAV-mediated muscle-directed gene transfer is very effective for long-term systemic delivery of α -gal A. When the gene transfer therapy should be started to obtain the maximum efficacy and safety is remained to be established.

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