Fabry Disease

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Keywords
Fabry disease · α-Galactosidase A · Globotriaosylceramide · Enzyme replacement therapy · Substrate reduction therapy · Chaperone · Gene therapy

4.1 Case Report

A 41-year-old male visited our hospital with proteinuria and hearing loss. The patient is Japanese, had never experienced pain attacks in the extremities, and sweats normally. He did not present with chest pains and palpitations and often had diarrhea without abdominal pain. Proteinuria was diagnosed when he was 25 years old, and he was then diagnosed with chronic nephritis. At 30 years of age, he experienced recurrent sudden-onset deafness, sometimes with tinnitus. He had hypertension, hypercholesterolemia, and hyperuricemia. His electrocardiogram revealed arrhythmia, and he had a panic disorder. He gave up smoking when he was 30 years old. His mother had cardiac involvement with Fabry disease but no history of kidney diseases. She also had a c.465 T > A (amino acid: D155E) heterozygous mutation in *α-galactosidase A* of white blood cells. Physical examination revealed his body mass index was 22.5 kg/m^2 and had regular heartbeats, but no hepatosplenomegaly, no angiokeratoma, no rash, and no any neurological sign. In his blood counts, there were normal white blood cells and platelet but no anemia. His serum creatinine 0.97 mg/dL (normal; 0.65–1.07), estimated glomerular filtration rate (eGFR) 69 mL/min/1.73 m^2 (normal; >90) and cystatin C 0.90 mg/L (normal; 0.63–0.95), LDL-cholesterol 98 mg/dL (65–139), and triglyceride 188 mg/dL (normal; 40–149) were almost normal, but brain natriuretic peptide 55.3 pg/mL(normal; <18.5) was slightly elevated. He exhibited proteinuria (3+) and quantitative urine protein/creatinine ratio 3.8 g/g Cr (normal; <0.3) but not hematuria. Serum α-galactosidase A activity 2.82 nmol/hr/mg protein in his white blood cells was extremely low (normal control: 155.85–222.59). Thin-layer chromatography of his urinary lipids indicated globotriaosylceramide (Gb3) positive (Fig. 4.1). Gene analysis identified c.465 T > A (amino acid: D155E) hemizygous mutation in *α-galactosidase A* of his white blood cells.

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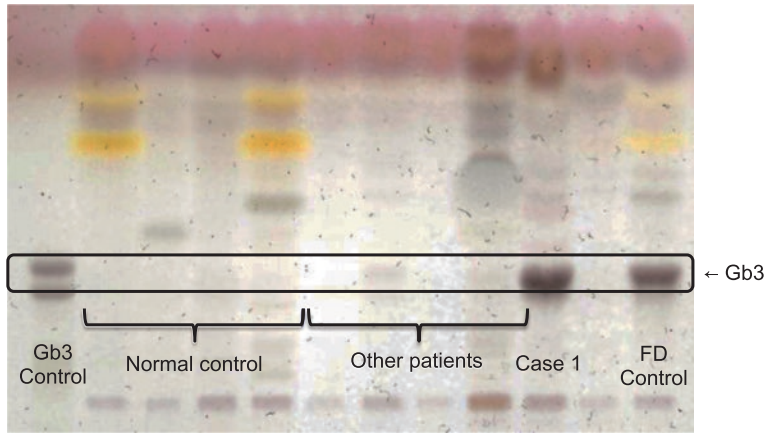

Fig. 4.1 Analysis of patient urine samples for presence of Gb3 by thin-layer chromatography.
The band from urine sample of case 1 patient via thin-layer chromatography indicates the presence of Gb3, as well as Gb3 control marker (far left lane) and Fabry disease (FD) patient's positive control (far right lane), whereas the Gb3 band is not detected in urine samples of normal healthy controls and patients with other diseases. *FD* Fabry disease, *Gb3* globotriaosylceramide

4.2 Diagnosis

Fabry disease (FD) is an inherited lysosomal disorder caused by a genetic deficiency in α-galactosidase A (*GLA*). A deficiency in GLA leads to the accumulation of globotriaosylceramide (Gb3) mainly in the kidney, heart, and brain (Desnick et al. 2017). Usually, FD is divided into three phenotypes: classical type, late-onset type, and heterozygote female type. The symptoms of classical-type FD include pain in the extremities and hypohidrosis that begin when the patient is of school age as well as kidney disease, heart disease, and cerebrovascular disease at middle age (Figs. 4.2 and 4.3).

The average age of onset is 9 and 13 years for males and females, respectively. The average age of diagnosis is 23 and 32 years for males and females, respectively. The diagnostic delays are due to non-specific symptoms. The main symptoms of classical-type FD in males are neurological pain (62%), skin symptoms including hypohidrosis (31%), gastroenterological symptoms (19%), and renal signs (17%); in females, this includes neurological pain (41%), gastroenterological symptoms (13%), and skin symptoms (12%) (Eng et al. 2007).

Because patients with FD exhibit pain in their fingers and toes, they are often misdiagnosed with collagen diseases, fibromyalgia, or mental disorders. Some patients with FD are also misdiagnosed with other cardiomyopathies by echocardiogram and electrocardiogram findings, or with chronic nephritis, such as in the case report described above. Specific techniques used in magnetic resonance imaging (MRI), such as T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences, often show multiple high intensities of the brain in FD patients (Fig. 4.3c). This finding may lead to a misdiagnosis of multiple sclerosis. Many patients with FD may not have been correctly diagnosed at the onset of initial symptoms. The most important point to be able to make a precise diagnosis is to suspect FD from unspecific findings such as finger pain, hypohidrosis, and cardiomyopathy.

To make a definitive diagnosis, male patients should be evaluated for their GLA enzyme activity in plasma or white blood cells, and the amount of Gb3 in their urine. Many female patients have normal blood GLA activity in plasma or white blood cells and normal amounts of Gb3 in urine, thus requiring a genetic analysis of the *GLA* locus. Another pitfall is the existence of pseudo-deficiency alleles, which have a mutation that may alter or reduce protein function, but do not result in disease. Thus, even in male patients, a genetic analysis is required if GLA

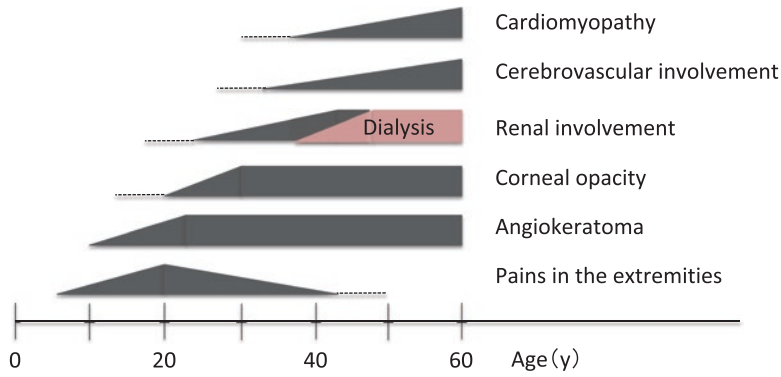

Fig. 4.2 Clinical course of classical type of Fabry disease.
Classical type of Fabry disease (FD) presents clinical symptoms with advancing age. First, patients with FD experience pains in extremities at roughly 4 years old and then angiokeratoma, corneal opacity, and proteinuria at young adulthood, cardiomyopathy and cerebrovascular involvement at middle age, and finally renal pathology requiring hemodialysis

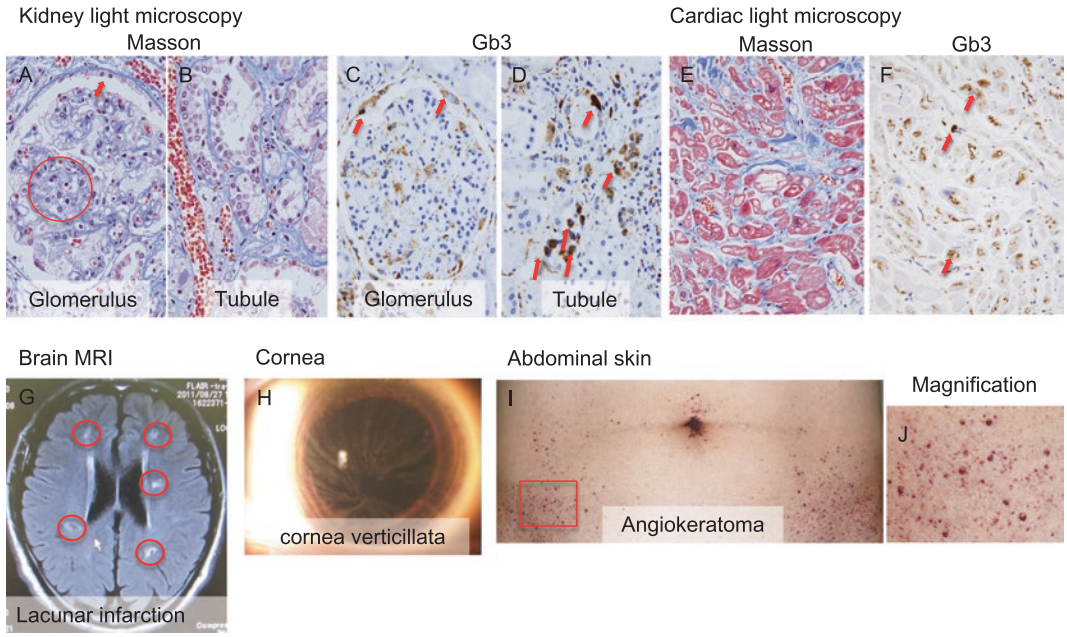

Fig. 4.3 Specific examinations for Fabry disease.
The kidneys from FD male patient at 66 years old examined by light microscopy show glomerulus (**a**, **c**), tubules (**b**, **d**), and cardiomyocytes (**e**, **f**). He had received ERT for 6 years, but he had had still Gb3 accumulation of tissues, because of acquiring high titer of immunoglobulin G against GLA. The histological findings (**a**–**f**) are a typical FD. Masson staining of glomerulus (**a**) shows enlarged epithelial cells and Bowman's capsules (red arrows) and an increased number of stromal cells (red circle). Tubules show almost normal structure by Masson staining (**b**). Gb3 staining shows the accumulation, brown spots (red arrows) mainly in epithelial cells of the glomerulus (**c**) and in distal and collecting tubules (**d**). Light microscopy of cardiac tissue (**e**, **f**), from the patient, shows almost all enlarged myocardial fibers (**e**), and light brown staining of cardiomyocytes (red arrows) indicates the accumulation of Gb3 (**f**). Brain magnetic resonance imaging (MRI) that consisted of fluid-attenuated inversion recovery (FLAIR) sequence shows several small high-intensity spots in the deep brain parenchyma (red circles), which indicate lacunar infarctions (**g**). Slit lamp examination shows the corneal verticillata (**h**). The red spots on the skin indicate angiokeratoma from 40s FD male patient (**i**). The magnification is from red square of the left picture (**j**). *Gb3* globotriaosylceramide

activity is moderately decreased. The E66Q mutation in the *GLA* gene is thought to be a functional polymorphism, and there is no abnormal accumulation of Gb3 in tissues, even if the E66Q mutation reduces the enzymatic activity of GLA (Kobayashi et al. 2012).

4.3 Biochemical and Molecular Perspectives

FD is an inherited metabolic disorder caused by gene mutations in *GLA*, which is one of over 40 lysosomal enzymes. Decreased GLA activity leads to the accumulation of Gb3 in lysosomes of various tissues including the kidney and heart, and Gb3 accumulation causes lacunar infarctions of the brain, cornea verticillata, and autonomic nerve (Fig. 4.3).

GLA is located on Xq22.1, spans 13 kb, includes 7 exons and 6 introns, and encodes 398 amino acids. More than 600 gene mutations in *GLA* have been described, including splicing mutations, nonsense mutations, missense mutations, and deletions. Genotype and phenotype correlations of FD are unclear, resulting in the same mutation from the same family with different phenotypes. The main substrate of GLA is Gb3, which consists of ceramide with three monosaccharides (Fig. 4.4). Other substrates include galabiosylceramide and blood group B substances. GLA degrades the terminal galactose of Gb3 in an acidic lysosome. Mutant GLAs are unstable biochemically, and huge deletions in *GLA* also cause unstable RNA transcripts (Fig. 4.5a). Furthermore, mutant GLAs can result in the accumulation of Gb3 in lysosomes (Fig. 4.5b) and can affect various tissues. Skewed X-inactivation might explain the variable clinical presentation in female patients heterozygous for FD (Gubler et al. 1978). Random inactivation occurs on one X chromosome per cell. If X-inactivation occurs on the X chromosome carrying the wild type *GLA* allele, the mutant *GLA* allele remains active and can lead to very low GLA activity. In contrast, if the X chromosome carrying the mutant *GLA* allele is inactivated, GLA activity is normal. Each organ has a mosaicism of cells with normal or mutant GLA protein (Fig. 4.5c).

Table shows the clinical characterization of FD. Pain in the extremities, caused by Gb3 accumulation in dorsal root ganglions, begins from childhood and regresses spontaneously after middle age. Figure 4.3i and j shows typical angiokeratoma on a patient's skin. Gb3 accumulation also occurs in cardiac myocytes and affects the conducting system of the heart, resulting in cardiac ventricular hypertrophy and arrhythmia (Fig. 4.3e, f). Cardiac-type FD is documented in 3% of patients with left ventricular hypertrophy (LVH) with unknown etiology. The kidney is the main organ affected in FD. Gb3 accumulation occurs in various kidney cells (Fig. 4.3a–d). Proteinuria is a common finding in young adults, and eGFR progressively decreases with age. Untreated patients will require renal transplantation. Specific findings in the kidney include Gb3 accumulation in various cell types such as podocytes, mesangial cells, and renal tubular cells (Fig. 4.3a–d). Recently, fused foot processes in podocytes have been reported (Kanai et al. 2011) (Table 4.1).

4.4 Therapy and Prevention

It is very important for the clinical management of FD to relieve the excruciating pain and prevent the progression of the disease in the kidney, heart, and brain.

4.4.1 Pain Relief

Carbamazepine and phenytoin are more effective than other drugs for the pain experienced by patients with FD, which has been evidenced in a systematic review (Schuller et al. 2016).

4.4.2 Enzyme Replacement Therapy

To remove the accumulated Gb3, replacements with normal enzymes have been attempted in vitro and in vivo. Lysosomal enzymes are

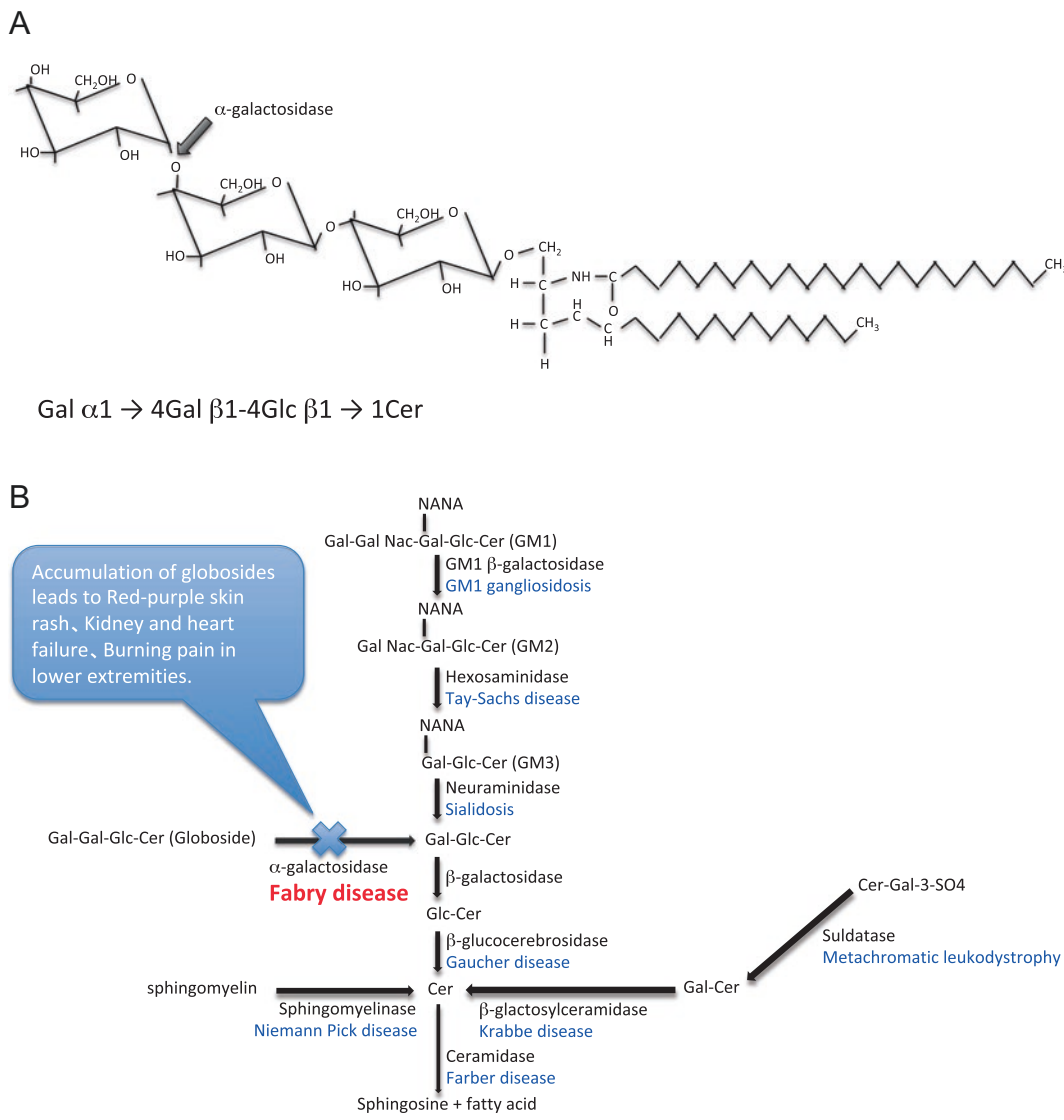

Fig. 4.4 Structure of Gb3 and GLA cleavage site and metabolic pathway of glycolipids.
Globotriaosylceramide (Gb3) has one glucose and two galactose molecules bound to ceramide (**a**) In normal cells, Gb3 is degraded by normal GLA at the terminal galactose, and the metabolic pathway of Gb3 is continuously active. Patients with Fabry disease cannot degrade the terminal galactose, resulting in an accumulation of Gb3. Gb3 is present in many cell types, including endothelial cells and thus can affect the kidney, heart, and brain. The map (**b**) shows the metabolic pathway of glycolipids and enzyme-specific lysosomal diseases. The process to metabolize Gb3 to sphingosine depends on several lysosomal enzymes to degrade glycolipids. *GLA* α-galactosidase A, *Gal* galactose, *Glc* glucose, *Cer* ceramide

taken up into cells and can function in the lysosomes. This is called "cross correction." In 1973, a partially purified enzyme from human placental tissue was intravenously administered to patients with FD (Brady et al. 1973). The substrates in their plasma decreased to approximately 50% in 45 min. Subsequently, independent Phase III clinical trials with recombinant human GLA have been performed. Currently, two recombinant human GLAs for the treatment of FD are available. One is produced in Chinese hamster ovary

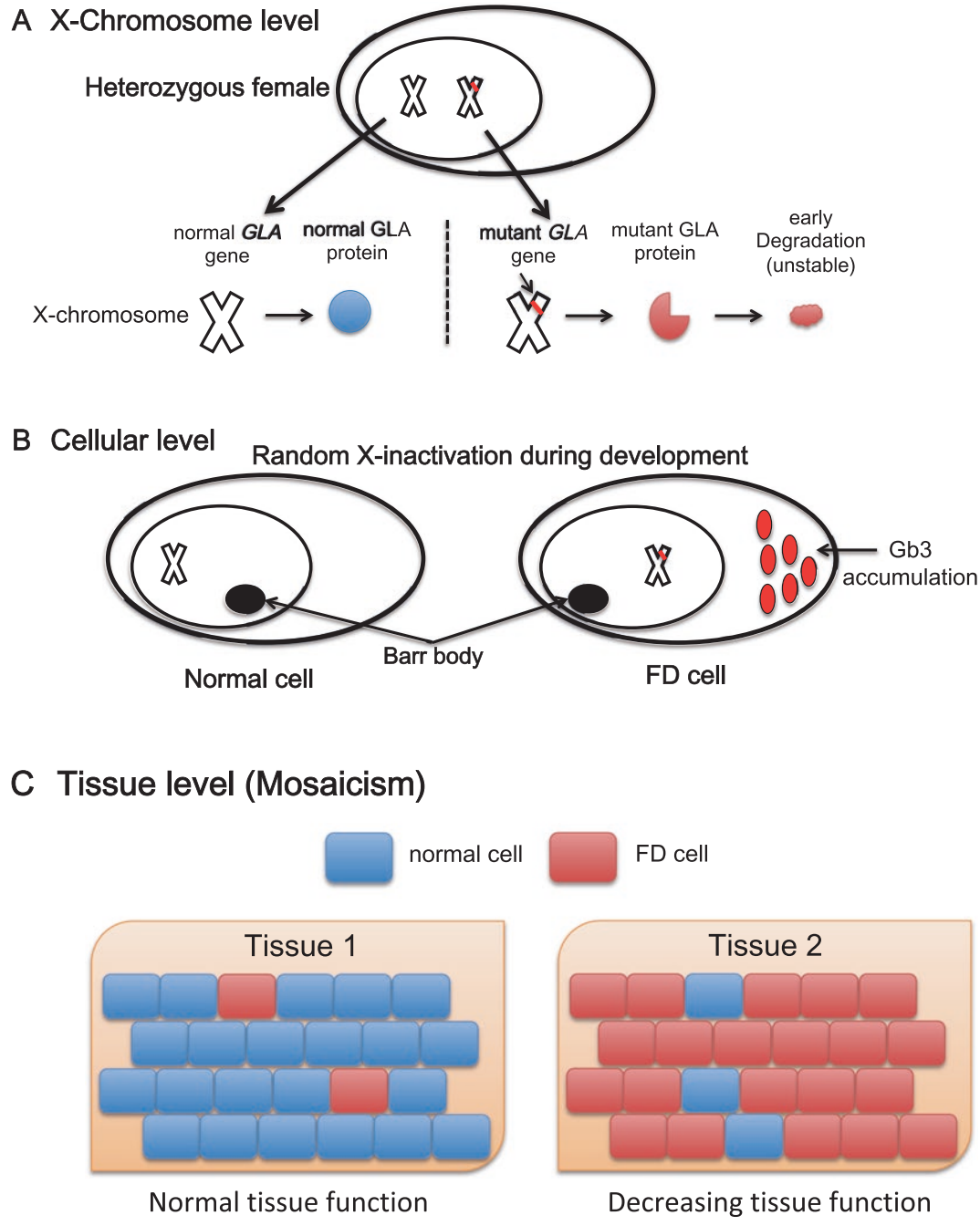

Fig. 4.5 Pathogenesis in females heterozygous for Fabry disease as suggested by Lyon's hypothesis.
Normal GLA protein is produced only by the normal *GLA* gene (left). Mutant *GLA* gene produces unstable GLA protein, which is degraded prematurely (right) (**a**). Lyon's hypothesis states that in a heterozygous female, X-inactivation causes either normal or mutant GLA to be produced per cell (**b**) In certain tissues in females heterozygous for Fabry disease, random X-inactivation results in chimeras consisting of normal cells producing normal GLA protein and mutant cells producing mutant GLA protein. The ratio of these chimeras results in various phenotypes for female patients with Fabry disease (C). *GLA* α-galactosidase A, *FD* Fabry disease

Table 4.1 Clinical characterization of Fabry disease

Classification	Classical type	Renal variant	Cardiac variant	Cerebrovascular variant
Onset age (y)	4~8	25	40 <	20~55
Death age	41	?	60 <	?
Hypohidrosis	++	±	–	+
Pain in the extremities	++	±	–	+
Angiokeratoma	++	±	–	±
Corneal clouding	++	±	–	?
Cardiac involvement	LVH	LVH	LVH	±
	MI		MI	
Cerebrovascular involvement	Brain infarction	?	–	Brain infarction
Renal disturbance	RF	RF	Proteinuria	+
Residual GLA activity	< 1%	< 5%	< 10%	?

Table shows clinical characterization of Fabry disease (FD) depending on classification. The ages in table are approximate ages. *LVH* left ventricular hypertrophy, *MI* myocardial infarction, *RF* renal failure, *GLA* α-galactosidase A

(CHO) cells (agalsidase beta) and the other in human fibroblasts (agalsidase alpha).

These clinical trials with open-label extensions demonstrated the frequency of immunoglobulin G (IgG) anti-GLA antibody formation among patients with enzyme replacement therapy; i.e., 89.7% of the patients with FD were treated with agalsidase beta (Wilcox et al. 2004) and 56.0% of patients with agalsidase alpha (Schiffmann et al. 2006). An experimental study also demonstrated that the serum, from patients with FD included IgG against a therapeutic enzyme, reduced GLA activity in Fabry fibroblast cells and Fabry mice tissues (Ohashi et al. 2008). Another study described immunologic tolerance for the therapeutic protein observed in some patients. Female patients with FD were significantly more tolerant than men; i.e., 58% female patients versus 11% male. Patients with FD that have nonsense mutations in *GLA* were more likely to develop anti-GLA antibodies than were patients with missense mutations. The frequency of infusion-associated reactions for enzyme replacement therapy in males was more than that in females (26% vs 11%, respectively) (Wilcox et al. 2012).

4.4.3 Pharmacological Chaperones

Certain missense mutations produced active mutant enzymatic proteins that are unstable and easily degraded in the endoplasmic reticulum (ER). However, these mutant proteins can be stabilized by small molecules. Migalastat, a pharmacological chaperone of GLA, binds to the active site of GLA and stabilizes certain mutant enzymes. Such mutant enzymes are then translocated to the lysosome, escaping degradation in the ER, and eventually degrade accumulated substrates in the lysosome. Clinical trials for migalastat demonstrated that eGFR was stabilized and LVH was improved in patients with amenable mutations. Moreover, the severity of diarrhea, reflexes, and indigestion was also decreased in treated patients. Based on these observations, migalastat has been approved for use in the European Union. Each patient's mutation is tested by HEK (HEK: human embryonic kidney) assay, that the mutation is transfected into HEK cells, to determine whether the patient has amenable mutations, before migalastat treatment. Although migalastat is only effective in patients who carry amenable mutations the advantage of migalastat is that it can be administered orally, without any serious adverse event (Germain et al. 2016).

4.4.4 Substrate Reduction Therapy

In substrate reduction therapy, the oral administration of Genz-682452, an antagonist of glucosylceramide synthase, results in selective inhibition of glucosylceramide synthase and reduced tissue levels of Gb3 and lyso-Gb3 in

Fabry mice while also delaying the loss of the thermal nociceptive response. Moreover, Genz-682452, but not recombinant enzymes, can cross the blood-brain barrier and reduce the levels of accumulated glycosphingolipids in treated murine brains. A combination therapy of substrate reduction and enzyme replacement may give additive therapeutic merits to patients with FD (Ashe et al. 2015).

4.4.5 Gene Therapy

Several experimental gene therapies for lysosomal storage diseases have been performed. NOD/SCID-Fabry mice were generated, and ex vivo gene therapies were examined in these mice. Human CD34+ cells, a marker for hematopoietic stem and progenitor cells, that were transduced with lentiviral vectors carrying human *GLA*/IRES-human CD25 were transplanted into the model mice. This transplant experiment demonstrated that plasma GLA activity was significantly elevated and Gb3 accumulation in various tissues was significantly reduced (Pacienza et al. 2012). Phase I clinical trial of gene therapy for FD has started at the University of Calgary in Canada (Press release, University of Calgary).

End-of-Chapter Questions

(1) A young male patient presents in your clinic with pain in the extremities as well as hypohidrosis. What should you look for to make a definitive diagnosis for Fabry disease?
(2) Is genetic analysis of *GLA* required for definitive diagnosis of females with symptoms of Fabry disease?
(3) Fabry disease is X-linked. What is the mechanism by which female patients develop Fabry disease?
(4) If only one treatment can improve Fabry patients completely, what would be that treatment?

Answers

1. While some symptoms or combination of symptoms can lead to a diagnosis of Fabry disease, most of the symptoms are not definitive, such as pain in the extremities. For definitive diagnoses, in vitro functional assays should be done on whole blood (or white blood cells) patient samples for GLA activity, and urine should be tested for presence of Gb3.
2. Yes, because GLA activity from a female patient with Fabry disease cannot be distinguished from a normal control individual. A female, heterozygous at the *GLA* locus, will likely still produce functional enzyme in an in vitro assay from half the cells in the sample. Only an analysis at the gene sequence level can a possible Fabry disease diagnosis be confirmed for a female.
3. Females will likely have tissue mosaicism, giving a variable disease presentation and progression from patient to patient. As random X-inactivation occurs during development, some cells will inactivate the normal allele, resulting in Fabry disease cells that will constitute some of the cells in the resulting tissue. The degree of symptoms will depend on the degree of lyonization.
4. Theoretically, a one-time treatment using gene therapy can improve patients with Fabry disease by addition of a healthy exogenous allele of the *GLA* gene. Successful gene therapy would result in production of functional enzyme by all cells treated ex vivo and would reduce excess Gb3. A limitation would be that only cells treated ex vivo would produce functional enzyme, not all cells in the patient. Therefore, there may be residual symptomology.

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