Chapter 12

Fabry Disease: Molecular Mechanisms and Emerging Therapeutic Approaches

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ABSTRACT:

Fabry Disease is a rare X-linked inherited metabolic disorder caused by mutations in the *GLA* gene, which result in deficient activity of the lysosomal enzyme α-galactosidase A. The enzymatic deficiency leads to progressive accumulation of glycosphingolipids, particularly globotriaosylceramide (Gb3), within lysosomes of various cell types. This accumulation contributes to multisystem involvement affecting the kidneys, heart, nervous system, and skin. Clinical manifestations may include neuropathic pain, angiokeratomas, renal dysfunction, cardiomyopathy, and cerebrovascular complications. Due to the variability of symptoms and lack of awareness, the disease is frequently underdiagnosed, especially in developing countries. Advances in molecular biology have improved the understanding of the underlying pathogenic mechanisms and facilitated the development of targeted therapeutic strategies. Current treatment primarily involves enzyme replacement therapy aimed at restoring α-galactosidase A activity and reducing substrate accumulation. In addition, emerging therapeutic approaches such as pharmacological

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chaperone therapy, gene therapy, and substrate reduction therapy are being investigated to provide more effective and long-term management options. This chapter discusses the molecular mechanisms underlying Fabry disease, its clinical manifestations, diagnostic approaches, and recent advances in therapeutic strategies, highlighting future perspectives for improved patient outcomes.

Keywords: *Fabry disease, α-galactosidase A deficiency, globotriaosylceramide (Gb3), enzyme replacement therapy, gene therapy.*

1. INTRODUCTION:

Fabry disease is an X-linked genetic disorder that impacts glycosphingolipid metabolism. The disorder arises from alterations in the galactosidase alpha (GLA) gene found on the X chromosome. The GLA gene encodes an enzyme known as α-galactosidase (AGAL), which is located in lysosomes. AGAL's role is to metabolize a sphingolipid known as globotriaosylceramide, referred to as Gb3 or GL3. Consequently, in Fabry disease, Gb3 is not broken down, leading to its accumulation in various tissues, including lysosomes. Lysosomes exist in various cell types in the heart (cardiomyocytes and fibroblasts), the kidneys (podocytes, tubular cells and glomerular endothelium), capillary endothelial cells, and neurons.

Endothelial cell growth and swelling are common in patients with this illness, which can result in heart disease, stroke, and renal failure in their third or fourth decade of life, or early death. The primary illness process is expected to start in infancy, while some experts suggest that it may have started during foetal life. Still, many people with Fabry disease do not exhibit any symptoms throughout their early

years of life, despite the evolution of many other lysosomal storage illnesses. Between the ages of three and ten, the first indications and symptoms that affect a child's overall condition and performance typically manifest in boys a few years earlier than in girls. Over time, cell damage and lysosomal accumulation worsen, impacting essential organs and eventually resulting in organ failure. End-stage kidney failure and cardiovascular consequences are the most serious and potentially fatal conditions.

Fabry disease was long thought to primarily affect men; women were only thought to be “carriers of the affected gene”. However, a number of studies show that women can exhibit a range of symptoms, from almost asymptomatic to the "classical" phenotype, with varying degrees of intensity and variability. Males typically have the "classical" phenotype, but cases with more severe cardiac involvement or renal manifestations.

Preventing permanent organ damage and stopping the disease's progression are the fundamental objectives of FD therapy. Enzyme replacement therapy (ERT) and the pharmacological chaperone migalastat are examples of disease-specific treatments that are essential. ERT has greatly improved the management of FD, reduced a number of symptoms and extended life expectancy by replacing or restoring inadequate enzyme activity. For FD patients with vulnerable mutations linked to low residual α-Gal A activity, migalastat, an oral chaperone, has been found to be especially helpful.

2. EPIDEMIOLOGY

In total, over fifty lysosomal storage diseases have been identified and described biochemically and genetically, including Fabry disease. Fabry disease is ranked second after Gaucher disease in terms of the

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frequency of these lysosomal storage disorders. The precise incidence of this illness is unknown, and because some people are not diagnosed, the prevalence may be underestimated in the existing data. Many individuals with Fabry disease do not receive an accurate diagnosis and their symptoms are mistakenly attributed to other disorders due to limited access of genetic testing.

The incidence of Fabry hemizygotes was reported to be 1:117 000. Although the incidence of heterozygotes was not indicated, a combined incidence of 1:58 000 can be calculated by projecting the finding in hemizygotes.

Other research revealed different numbers, but the methods employed to figure out them also differed. A notable high prevalence of the condition was discovered when attempting to calculate the incidence using newborn screening, such as in Italy, where one in 3100 people had the illness. One in 1500 newborn men had Fabry disease, according to another study, and the majority of them had the IVS4+919G>A mutation, which is thought to determine cardiac phenotype with later onset.

It was thought to look for individuals with Fabry disease among people with end-stage renal disease (ESRD) receiving haemodialysis because renal failure and cardiovascular illnesses are the most prevalent symptoms. The prevalence of Fabry disease in haemodialysis patients was found to be 0.22% in retrospective research involving 105 male patients. Based on similar assumptions, a Japanese study found that 1.2% of male dialysis patients had Fabry disease.

3. Genetic Basis:

A result of a mutation in the GLA gene, FD is a monogenic, recessive

inheritance condition associated with the X chromosome. This gene, which is found at location Xq22 on the long arm of the X chromosome, codes for the α-GAL enzyme. New mutations are uncommon, and the majority of instances are inherited. The disease has been linked to more than 900 distinct mutations.

Gb3 is broken down into galactose and lactosylceramide in the lysosomes by α-GAL, which has about 429 amino acids. As a result, GB3 accumulates in several tissues in FD patients. It has a preference for kidney podocytes and the vascular endothelium and smooth muscle cells of the cardiovascular system, which explains why these organs are more frequently affected by clinical symptoms.

The α-GAL gene is roughly 12 kb long and has seven exons. A variety of molecular mutations in this gene, including missense (57%), nonsense (11%), partial deletions (6%), insertions (6%), and abnormalities in RNA processing that result in aberrant splicing's (6%), can cause FD. Because diverse clinical symptoms might result from the same mutation, the relationship between genotype and phenotype is complicated. Both the blood group and environmental variables may be responsible for this. Due to an extra buildup of glycosphingolipids in the membrane of blood group B erythrocytes, patients with blood groups AB or B may exhibit more severe illness presentations.

4. PATHOPHYSIOLOGY:

Despite the accumulation of Gb3 during α-GalA deficiency is known to occur in lysosomes, not much information is known regarding the mechanisms that lead to cellular malfunction and ultimately to symptoms. Lipid-laden lysosomes may affect autophagic flux, including mitophagy, as with other inherited glycosphingolipidoses,

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which may contribute to the mitochondrial dysfunction seen in FD patients' fibroblasts. In a similar way the observed elevation of the unfolded protein response in cells of certain FD patients may indicate endoplasmic reticulum malfunction. Oxidative stress, inflammation, and fibrosis appear to be important pathogenic factors.

LysoGb3 has been proposed as a potential pathogenic factor in FD. For both classic male and female FD patients, lysoGb3 lifetime exposure was found to be significantly correlated with overall disease severity. In fact, lysoGb3 stimulates the growth of smooth muscle cells, which is consistent with the increased arterial stiffness and intima media thickness in FD. Additionally, it has been demonstrated that lysoGb3 destroys nociceptive neurones at the levels found in FD males, which is compatible with the reported pain in the extremities of classic FD males. The upper limb's thermal sensory limen and cold detection threshold were found to be highly correlated with lifetime exposure to lysoGb3. Following that, it is believed that lysoGb3 plays a role in glomerulus fibrosis and podocyte loss, two significant features of renal illness in FD patients. Finally, it has been discovered that lysoGb3 inhibits endothelial nitric oxide synthase (eNOS) at concentrations similar to those in FD patients, potentially contributing to the vasculopathy in FD.

5. Clinical Manifestations:

The build-up of Gb3 in the nervous systems tiny nerve fibres is what causes the initial symptoms to manifest in early childhood. One of the first signs of Fabry disease is pain, which is reported by 60–80% of boys and girls who are classically affected. Boys typically exhibit symptoms at younger ages. The Fabry disease is characterised by two forms of pain: (i) episodic crises, also referred to as "Fabry crises,"

which are defined as burning pain that starts in the extremities of the body (ii) persistent discomfort with burning sensations and paraesthesia in the extremities. Fabry crises can be brought on by a number of things, including exhaustion, stress, physical activity, fever, and abrupt temperature changes.

Gastrointestinal symptoms, which begin in childhood and persist until adulthood, are typical after pain. They include diarrhoea, nausea, vomiting, and stomach pain, particularly after eating. Gb3 buildup in the mesenteric blood vessels may be the cause of these symptoms, which could result in anorexia.

Skin lesions, such as angiokeratoma and clusters of red-purple capillary vascular lesions, are another very distinctive characteristic that can be seen from childhood. They are seen on the buttocks, upper thighs, inguinal region, umbilical zone, and even mucosal areas like the mouth. These are tiny superficial angiomas, and the injury to the vascular endothelial cells causes the skin's vessels to dilate. They range in size from a pinpoint to several millimetres, and as they become older, both their size and quantity increase.

6. DIAGNOSIS:

The clinical management of FD patients depends on the monitoring of disease symptoms and the effectiveness of FD treatment. Clinical, radiographic, and laboratory analysis can be used to determine the beginning and course of a disease. However, because patient variability is so significant, it might be difficult to evaluate a therapeutic treatment's effectiveness. Furthermore, some severe effects of FD, like advanced renal failure, cannot be reversed. However, biomarkers are crucial for monitoring illness and treatment.

Identification of GLA gene mutations causing a missing or clearly

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defective α-GalA protein is a simple way to diagnose classic FD in men. Using artificial water-soluble substrates like 4-methylumbelliferyl-α-galactoside, it is easy to demonstrate extremely low α-GalA activity in leukocytes, fibroblasts, and dried blood spots. To further confirm the diagnosis, increased levels of Gb3 and lysoGb3 in plasma and urine can be found. For FD females, especially those with negatively skewed X-inactivation, enzyme activity tests are not usually useful. The diagnosis of FD in females can then be confirmed with the detection of increased lysoGb3. The diagnosis of atypical FD patients who arrive with an uncommon symptom (such as albuminuria, left ventricular enlargement, or white matter abnormalities) in addition to a GLA gene mutation with unclear effect is challenging. This is frequently accompanied by a comparatively high residual enzyme activity in cells and no obvious anomaly in the amounts of Gb3 and lysoGb3 in the plasma or urine. In difficult situations, biopsy analysis and Gb3 deposit demonstration are thought to be useful in supporting diagnosis.

Biopsies of various FD patients' tissues may also indicate the illness. The presence of cytoplasmic vacuoles containing the lipids can be observed using optical microscopy. Electronic microscopy reveals lysosomal inclusions with a lamellar structure. Immunoelectron microscopy can be used to look for anti-Gb3 antibodies when these results are contradictory.

7. Current Treatment:

Approved treatments such as enzyme replacement therapy (ERT) and chaperone therapy work to reduce intracellular Gb3 accumulation by replacing the deficient α-galactosidase A enzyme or stabilizing misfolded enzyme forms, thereby enhancing their transport and

enzymatic activity within lysosomes.

7.1. Enzyme Replacement Therapy (ERT): Purified human placental AGAL was successfully given to two FD patients by Brady and others in 1973, marking the first attempts at ERT. The infusion was well tolerated by both patients, and AGAL activity rose by roughly 68% over pre-infusion values. Over time, two brothers with FD were given human plasma and spleen-derived AGAL. Up to 70% of Gb3 was cleared after plasma-derived AGAL was administered. Clinical research advancement was constrained by the difficult purification and restricted supply of human AGAL for infusion. Agalsidase-alfa which is produced in a human cell line (human fibrosarcoma cells HT-1080) with an approved dosage of 0.2 mg/kg body weight and an infusion duration of about 40 minutes, and agalsidase-beta (Fabrazyme, Sanofi Genzyme), which is produced in Chinese hamster ovary (CHO) cells with a recommended dose of 1.0 mg/kg body weight and an infusion duration of about 240 minutes. The following therapeutic effects can be achieved with ERT, depending on the patients and their manifestations: stabilisation of kidney function or delay of progression to terminal kidney failure, stabilisation of the thickness of the heart wall and function or reduction of left ventricular hypertrophy, and improvement in sweating.

7.2. Chaperone Therapy: Reduced intracellular AGAL activities are frequently caused by missense mutations in the GLA gene, which produce an unstable and misfolded protein. Misfolded proteins will prematurely degrade before they reach the lysosomes because they cannot pass the endoplasmic reticulum's (ER) protein quality-control process. Pharmacological chaperones, which bind reversibly to the protein's active core, can be employed to restore the protein's folding and stability. It is initially discovered as a competitive inhibitor of the

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AGAL enzyme, the small molecule 1-deoxygalactonojirimycin (DGJ) is an iminosugar. The binding to the catalytic center of the enzyme (wild-type and susceptible mutations) enhances protein folding in the ER and accelerates maturation and trafficking to the lysosome at sub-inhibitory concentrations (extracellular: 20–100 μM). This causes an increase in enzymatic AGAL activity in both healthy control cells.

8. Emerging Therapeutic Approaches:

8.1. Next Generation ERTs: Pegunigalsidase alfa is a new version of AGAL that is covalently cross-linked and PEGylated (PEG, polyethylene glycol). It was created as an ERT for FD and made in tobacco cells with longer in vitro stability and a tenfold longer half-life in male Fabry mice (581 min) than licensed medications, pegunigalsidase appears to behave similarly to the ERTs currently on the market. Increased efficacy was found in a 1-year phase I/II clinical trial (dose-finding study) as a result of decreased immunogenicity and a longer plasma half-life (80 h). Because PEGylation stabilises the AGAL homodimer and improves its half-life (80 h), it may be possible to lengthen the infusion interval (monthly IV administration).

8.2. Substrate Reduction Therapy: While migalastat increases endogenous enzyme activities of AGAL due to amenable mutations and ERT seeks to replace missing or defective AGAL through the infusion of a genetically engineered enzyme, substrate reduction therapy (SRT) aims to reduce the substrate and, consequently, inhibit Gb3 accumulation in the cells. Lucerastat (Idorsia) is a low molecular weight iminosugar that inhibits the manufacture of glycosphingolipids, including downstream Gb3, by blocking the upstream-located glucosylceramide synthase (GCS). This suggests a

possible new oral therapy option. The initial in vitro research showed that fibroblasts generated from FD patients successfully reduced Gb3 and improved abnormal cellular membranes.

8.3. Gene Therapy: DNA containing the genetic code for the AGAL protein is introduced into patients' cells as part of gene therapy. The safety of gene therapy for FD is now being assessed in a number of clinical trials using varying therapeutic modalities. The lentiviral ex vivo transduction of haematopoietic stem cells is the foundation of the first interventional, multicenter, global, open-label trial. The goal of this strategy is to produce functional AGAL using transfected haematopoietic stem cell-derived cells, which will then be released into the plasma and taken up by AGAL-deficient cells. Adeno-associated virus (AAV) in vivo transduction of hepatocytes, utilising these cells as an AGAL-secreting platform rather than cells produced from haematopoietic stem cells, is the basis for two more clinical investigations.

9. Conclusion

Fabry disease is a rare X-linked lysosomal storage illness caused by mutations in the GLA gene that result in a shortage of the enzyme α-galactosidase A. Globotriaosylceramide (Gb3) gradually builds up in many tissues as a result of this enzymatic deficiency, causing multisystem involvement that affects the kidneys, heart, nervous system, and vascular endothelium. From early symptoms like neuropathic pain, gastrointestinal issues, and angiokeratomas to serious consequences including renal failure, cardiomyopathy, and cerebrovascular accidents, Fabry disease's clinical presentation is quite varied.

Recent advancements in gene therapy, substrate reduction therapy,

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and next-generation enzyme replacement medicines present encouraging opportunities for enhancing long-term illness management. These new methods seek to improve therapeutic efficacy, lessen the burden of treatment, and possibly offer longer-lasting repair of the underlying enzyme deficiency. To enhance clinical results and quality of life for Fabry disease patients, early diagnosis, prompt therapeutic intervention, and ongoing research into innovative treatment approaches are crucial.

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