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Fabry Disease: from Diagnosis to Therapy

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Abstract:*Fabry disease (FD) is an accelerating, X-linked hereditary disorder of glycosphingolipid metabolism which occurs due to improper lysosomal α-galactosidase A activity. Its pan-ethnic and the annually reported occurrence may underestimate the true frequency of the disease. Classically affected hemizygous males, with no residual α-galactosidase A activity may display all the characteristics like cardiovascular (cardiomyopathy), cutaneous (angiokeratoma), renal (kidney failure), neurological (pain), cerebrovascular and cochleo-vestibular signs of the disease while heterozygous females have similar symptoms ranging from very mild to severe. Incomplete action of lysosomal α-galactosidase A results in gradual increase of globotriaosylceramide within lysosomes, supposed to set off a flow of cellular events. Enzyme analysis may occasionally help to detect heterozygote but in females results are often questionable because of random X-chromosomal inactivation that obligates the molecular testing (genotyping). Due to ethical reasons determination of enzyme activity through prenatal diagnosis is carried out only in male's fetus. The subsistence of atypical variants and the unavailability of a specific therapy complicate the genetic counseling. Enzyme replacement therapy (ERT) is the only specific therapeutic option that introduced recombinant human α-galactosidase A. End stage renal disease and critical cardiovascular or cerebrovascular complications limit the life-expectancy of untreated males and females. Oral therapy drives the research forward into active site specific chaperones. Adjunctive therapy of ERT with chemical chaperone therapy can arrest disease succession.*

Keywords: *α-Galactosidase A (AGAL), chaperon therapy, fabry disease, glycoshingolipids, lysosomal storage disease*

I. Introduction

Fabry disease (FD) (OMIM: 301500) comparatively less prevalent in females is characterized by angiokeratoma corporis diffusum (skin abnormality), cornea verticillata, hypohidrosis (excessive sweating), gastroenteritis, obstructed airflow, improper kidney functioning and premature myocardial infractions [1]. The occurrence of clinical symptoms may vary in intensity and frequency in different patients. It is an X-linked inborn error of α-galactosidase A deficiency (AGAL). The multisymptomatic FD with its complex manifestations has slow progression of symptoms with age. The main cause of death is heart or renal failure. The Fabry Outcome Survey (FOS) data showed that patients experience neuropathic pain with burning sensation in the soles and palms. Pain attacks in joints and abdomen was also reported. The intensity of pain increases with fever, physical exercise and alcohol. Specific facial appearance with prominent lips thickening, frontal bossing and supraorbital ridges in hemizygous males of 12 to 14 years of age were also observed [1]. Angiokeratoma that is characterized by red, small, raised spots appear slowly in the areas of buttocks, and inner thighs. Some of the male patients become intolerant to exercise with reduced production of saliva and tears. Rare cases report hyperhidrosis at early age [2, 3]. Sensory organs specifically eyes and ear are affected by FD. Cornea verticillata (opacity) and hearing loss is most obvious [4]. Other severe symptoms include air flow obstruction, asthma dementia, learning difficulty, and depression [1, 5].

Atypical variants of FD manifest cardiac and renal involvement having AGAL activity between 2-20% than normal [6]. Cardiac involvements in hemizygous males include the defects of heart valve and conduction disturbance [7]. Low AGAL activity also found in FD patients of renal variants having mutation in galactosidase gene [8].

1.1 α- Galactosidase A

Fabry disease is actually caused by excessive accumulation of a class of lipids called glycoshingolipids, especially ceramidetrihexoside, or globotriasylceramide (Gb3) [9]. Sphingolipids have structural moiety of long chain aminoalcohol sphingosine Fig. 1. Second carbon atom two of long chain fatty acid sphingosine is bound to nitrogen atom and the structure formed is called ceramide. First carbon atom of ceramide portion of sphingosine is linked to varying numbers of sugars [10]. The structure formed is called glycosphingolipids. Glycosphingolipids (GSLs) are components of the plasma membrane. These components are degraded in the lysosome through endocytic pathway [11]. The catabolic pathway of GSLs needs different cofactors and enzymes. The AGAL enzyme in its active form requires saposin B to start its activity on substrate that is Gb3 [12]. The insufficient activity of AGAL to catalyze the hydrolytic cleavage of the terminal galactose from Gb3

causes the excessive accumulation of globotriaosylsphingosine (lyso-Gb3) in kidney, lungs and erythrocytes [13, 14]. Lyso-Gb3 and Gb3 are cytotoxic and the toxicity of lyso-Gb3 is greater than Gb3 [15].

Sphingosine

$$CH_3\text{-}(CH_2)_{12}\text{-}CH{=}CH\text{-}CH\text{-}CH\text{-}CHOH$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad OH\ NH_2$$

Ceramidetrihexoside/ globotriasylceramide (Gb3)

Sphingosine-Glucose-Galactose-Galactose

Fatty acid

Globotriaosylsphingosine (lyso-Gb3)

Sphingosine-Glucose-Galactose-Galactose

Fig. 1 The core structural moiety is long chain sphingosine [10].

1.1.1 The Structure of Human α-galactosidase A

The X-ray crystallography showed the homodimeric structure of AGAL, made up of two domains. The N- terminal domain contains the active site that is present at the C- terminal end of β-strands and second is β-sandwich of 8 anti-parallel β-strands at the C-terminal Fig. 2 [15]. The distance between two active sites is 50 Å that remained same in ligand and free enzyme showing the independent functioning of both domains. Structurally α-galactosidase A and α-galactosidase B (α-N-acetylgalactosaminidase) have same structure but the absence of an 'N-acetyl recognition loop' in α-galactosidase differs the substrate specificity of both enzymes [16]. The genes encoding α-galactosidase A and α-galactosidase B are localized on chromosomes Xq22.1 and 22q13, respectively. The genetically distinct locations further confirm the difference between two enzymes [17]. The reaction mechanism of enzyme carried out by double displacement reaction as the two nucleophiles attack on carbon of substrate (Gb3). D170 is the catalytic nucleophile and D231 is the catalytic base/acid in human AGAL [18].

1.2 The Genetic Basis

The homodimeric precursor α- galactosidase A (101kD) is encoded by 12kb gene present at X chromosome (Xq22.1 region) have 429 amino acids, 7 exons ranging from 92 to 291 base pairs Fig. 3 [14, 19]. Patients with FD have not only the structural changes in AGAL enzyme but also slower heat inactivation as compared to normal enzyme. Over 599 mutations were recorded in defective galactosidase gene that is making the enzyme non functional [20, 21, 22]. Mutations causing FD include base substitutions in exons, splice site mutations, small insertions, large deletions and partial gene rearrangements [23]. Dissimilar mutations at different positions showed lack of mutational hot spot. Galactosidase gene rearrangements with five partial gene deletions of size 0.4 to 5.5kb were detected by southern blot analysis. Four among the 5 deletions were in exon 2 containing Alu repeats [24]. Repetitive Alu elements are about 30% of the 12kb gene but only 1 deletion in case of FD is resulted from Alu repeats recombination [25]. Eng and Desnick reviewed 31 point mutations, 15 rearrangements, and 3 mRNA processing defects [26]. Rare intronic splice site mutations and other twenty novel mutations were reported by Topaloglu while testing 30 unrelated families of FD [27]. At low pH only one exonic variant D313Y found in 0.45% of normal Caucasian which had reduced and lowered the plasma activity of enzyme [28]. Upstream region of exon 6 showed single point mutation in two Japanese patients with cardiac phenotype of FD [29]. Missense mutation in exon 3, new splice site mutation in consensus sequence of exon 4 resulted in loss of catalytic activity of related enzyme [30]. New mutations including 20 missense, 2 nonsense, 1 splice site, 8 insertions/ deletions in exon 7 and complex rearrangements in exon 1 and 2 were also reported by Christine and his colleagues [31]. Missense mutations were also reported in the renal variants of FD patients [8, 32].

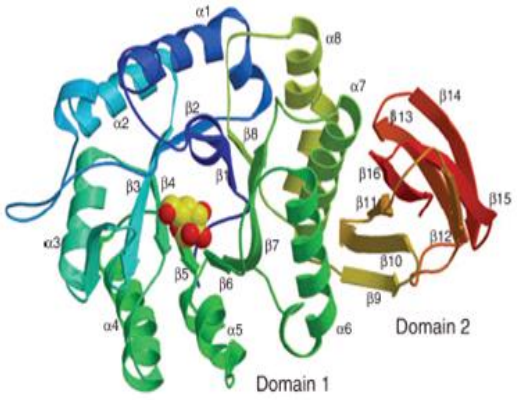

Fig. 2 Structure of AGAL monomer Domain 1 contains active-site; domain 2 contains antiparallel β strands [15]

1.2.1 Diagnosis at Molecular Level

The activity of AGAL in blood/ leukocytes can determine the affected patient. But mostly affected females show normal enzyme activity. So, the enzyme activity test is not the reliable testing for disease patient identification [33]. Another method for biochemical diagnosis is to check plasma Gb3 level that is comparatively lower in males even in the normal range [34]. Urinary Gb3 level is also unreliable option because Gb3 level will not increase in patients of late onset variants [35]. Sequence analysis of galactosidase gene identifies all the affected patients. Targeted mutational analysis is better option for the areas where the disease occurrence is high. Deletion testing detects the whole gene deletions in carrier/ heterozygous females. After the confirmation of disease family screening and pedigree analysis is effective to identify previous unrecognized affected individuals. Similarly genetic counseling before prenatal diagnosis can easily identify the risk level of associated symptoms but there are always unanswered ethical issues that limit the boundaries [36].

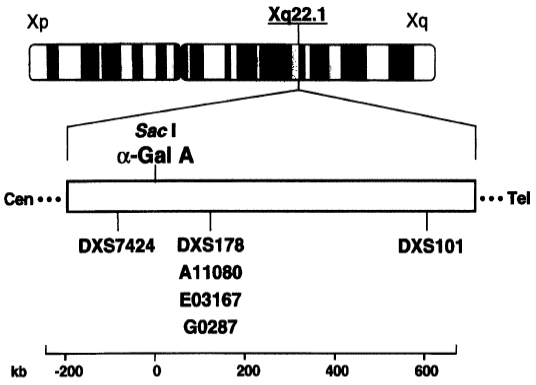

Fig. 3 Idiogram of the human G-banded X-chromosome (Xq22.1) with mapping of the genomic region flanking α-Gal A indicating the relative positions of the markers used for haplotype analysis [19].

Looking at the penetrance and expressivity pattern of FD in males and females Dobyns and his coworker suggested using the term of X-linked trait instead of recessive or dominant one. Females are mosaic for some X-linked genes due to random transcriptional silencing of X-chromosome known as X-chromosome inactivation (XCI). Therefore carrier females range from no symptoms to the atypical ones. The patterns of X-inactivation in different organ systems in females justify the variability pattern of FD symptoms to some extent [37]. A study was carried out to determine the relation between mutant α-galactosidase A allele and skewed X-inactivation. Highly skewed samples were tested for nonsense mutations in AGLA. Results showed no correlation between clinical signs, XCI ratios, age, and enzymatic activity of α-galactosidase A [38].

1.3 Therapy and Management of Fabry Disease

Treatment of FD commonly starts with the symptoms management because of the involvement of almost all the organs. Loss of renal function delayed by antihypertensive and ACE (acetyl cholinesterase) inhibitors. Heart functioning is normalized by coronary bypass grafting, artificial pacemaker, and antiarrhythmic drugs [39, 40]. But these are generalized treatments for dipping symptoms. Some specific treatments are discussed below

1.3.1 Enzyme Replacement Therapy (ERT)

The recombinant human AGLA introduced the disease specific enzyme replacement therapy (ERT). This therapy varies in different patients especially when it comes to children and females. In Europe agalsidase alfa and agalsidase beta are two commercially available enzyme preparations for FD treatment. Alfa galsidase alfa produced by human skin fibroblast culture and agalsidase beta produced by recombinant of human cDNA AGAL expression in Chinese Hamster Ovary (CHO) cells [41, 42]. The safety and efficacy of ERT is generalized by the reduction of disease severity of FD patients after one year of therapy with agalsidase alfa [43, 44]. In children the plasma Gb3 level along with GI symptoms were reduced after agalsidase beta therapy [45]. The enzyme replacement therapy has been implemented in clinical trials but it has limitations for long term trials [46].

1.3.2 Small Molecular Drugs

Small molecular drugs are 80-90% of marketed drugs and showed response quickly even after oral administration. Ability to cross blood-brain barrier, lack of autoimmune reaction, and lowest manufacturing cost favor these drugs therapeutically and economically [47]. Such type of drug therapy involves activation of

residual enzyme through substrate reduction therapy (SRT), chemical chaperone therapy (CCT) and activation of AGAL promoter. Small AGAL promoter molecules enhances the transcription of mutated gene and results in increase of AGAL protein in lysosomes [48]. Similarly in case of residual enzyme activation, it is practically more complicated to design specific activator molecules of protein [49]. SRT and CCT are relatively better option as compare to other therapies.

1.3.2.1 Substrate Reduction Therapy (SRT)

Glucosylceramide synthase inhibitors (N-butyldeoxynojirimycin) lowered down the rate of Gb3 synthesis thus reduces the accumulation of globotriaosylceramide, glycosphingolipid in lysosomes [50]. Contradictions of N-butyldeoxynojirimycin are due to its non specificity for glucosylceramide synthetase resulted in gastrointestinal problems [51, 52]. Combination of ERT and SRT might improve treatment efficacy.

1.3.2.2 Chemical Chaperon therapy (CCT)

Missense mutation in FD results in the synthesis of catalytically active but unstable lysosomal protein. These mutated enzymes are retained and then degraded in endoplasmic reticulum because of their misfolded conformation [53]. By using active site specific chaperones, the stability and misfolding can be enhanced that will prevent the premature degradation by endoplasmic reticulum [54]. Basically chemical chaperons are small molecules that assist in correct folding and trafficking of mutated enzyme [55]. Clinical trial is carried out for deoxygalactonojirimycin (DGJ, marketed as Amigal TM by Amicus Therapeutics, Inc.) as a chemical chaperone therapy [56]. Co-administration of small pharmacological chaperone AT1001 improvers the stability and pharmacological properties of recombinant human AGAL by preventing denaturation at neutral pH and normal body temperature. In this way CCT enhances the efficiency of ERT [57]. Oral administration of these pharmacological chaperones support this therapy but unfortunately only 42% of genotype respond these so called small molecules [58].

1.3.3 Gene Therapy

The alternative method is gene therapy with a number of options for gene delivery system. Dissemination of AGAL transduced haematopoietic cells from Fabry mice have enzymatic correction of recipient cells that led to reduction in lipid storage [46]. Gene therapy usually target liver and skeletal muscles for the production of therapeutic proteins followed by systemic action. Recombinant AGAL administered into the blood through the lower respiratory tract via adenoviral vector. The resulted enzyme was expressed in the epithelium of respiratory tract and after diffusion enters into the circulatory system. In this way lungs are reported to be the viable organ for gene therapy [59]. Unavailability of reliable data for clinical trials of gene therapy is due to involvement of viral vectors [47].

II. Conclusion

The complexity of Fabry disease at genetic, molecular and diagnostic level makes it the most sophisticated disease not only at diagnostic level but also at therapeutic level. Discovery of new exonic and intronic mutations and lack of mutational hot spot results in reluctance of clinical trials of gene therapy. Therefore target of the physiologist is basically to lessen the associated symptoms to expand the life span of patient. Combination of ERT, SRT and CCT promises to increase the efficacy of FD at different stages of clinical trials. Development of cost effective and long term stable therapy would definitely be a breakthrough in the world of human genetics.

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