ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Review Article

MOLECULAR ANALYSIS OF POMPE DISEASE AND TREATMENT

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ABSTRACT

Pompe disease (OMIM 232300) is also known as Glycogen storage disease or acid maltase deficiency. It is a rare autosomal recessive lysosomal disorder of glycogen metabolism with an estimated prevalence of 1 in 40000 in Caucasians. It is caused by the deficient activity of acid α-glucosidase enzyme (E.C 3.2.1.20) due to mutation in the *GAA* gene. The enzymatic deficiency leads to the accumulation of glycogen within the lysosomes in multiple tissues, including cardiac, skeletal and smooth muscle cells. Clinically, the disease has been classically classified in infantile and childhood/adult forms. The clinical features are cardiomyopathy and generalized muscle weakness that rapidly progress to death from cardiorespiratory failure in the first year of life. The *GAA* gene has been localized to chromosomes 17q25.2-q25.3 and to date, 582 mutations distributed throughout the whole gene in HGMD. All types of mutation have been described and among that missense mutation are the most frequent followed by deletions. Indeed, there are missense, small deletions, in frame, splicing variants, nonsense, small insertions/duplications, gross insertions/deletions, small indels and complex rearrangements. The mutations known to cause severe disease were coding mutation, c.2560C>T (p. Arg854X), and the splice acceptor site, c.1327-2 A>G. The splice site mutation c.1327-2A>G is very common in the patients of Caucasian origin with the frequency ranging from 40% to 70%. This disease can be treated by enzyme replacement therapy (ERT) and currently there is one approved drug available for its treatment: ERT with intravenous infusion of *rhGAA* (Myozyme, Lumizyme). Early diagnosis and ERT can benefit infants with this disease. This diagnostic test can facilitate prenatal diagnosis and help in identifying carriers in families with the identified mutations.

Keywords: Pompe, Diagnosis, Molecular, ERT (Enzyme Replacement Therapy), *GAA*

1. INTRODUCTION

Pompe disease or Glycogen storage disease type II is an autosomal recessive disorder caused by the deficient activity of acid α-glucosidase enzyme (*GAA*, EC 3.2.1.20) and it is also known as acid maltase. It is the key enzyme in hydrolysis of lysosomal glycogen to glucose. Deficiency of this enzyme leads to accumulation of glycogen in tissues particularly in cardiac, skeletal and smooth muscles. It is mostly classified into two forms, infantile and late-onset. Infantile form is also divided into two subgroups, classical infantile form and non- classical infantile form [1]. The infantile form has symptoms of rapidly progressive proximal myopathy and cardiomyopathy. The main cause of death within first 2 years of life is respiratory and cardiac failures. The late-onset is also known as juvenile, childhood or adult onset PD (Pompe disease) [2]. It is characterized by slowly progressive disease of proximal myopathy and also leads to increased creatine kinase and later on involvement of respiratory muscles and lead to respiratory failure. The altered function of this enzyme hampers the lysosomal degradation of glycogen that progressively accumulates inside the lysosomes, causing swelling and rupture of them with loss of glycogen in the cytoplasm and subsequently cell damage. The cellular pathology mainly affects muscle fibers displacing their myobrils and ultimately leading to skeletal muscle weakness, but also other tissues such as cardiac and smooth muscle cell [3]. The deficiency of α-1,4-glucosidase results from the mutation in the *GAA* gene.

As shown in figure-I the *GAA* enzyme is located in the lysosome. Its function is to breakdown glycogen into glucose. As we are aware that glucose is simple sugar and is main source of energy for the body. Glycogen is mainly stored in the muscles and in the liver. Some glycogen is transported to the lysosomes, where *GAA* breaks it down into glucose. When there is deficiency of *GAA* or its not functioning properly, glycogen build up in the lysosomes. This excess glycogen causes the lysosomes to swell and damage the cellular structure around them. The

lysosomes eventually swell so much that they burst, further damaging cells and the body. This build-up of glycogen and the accompanying damage causes the symptoms of PD [4].

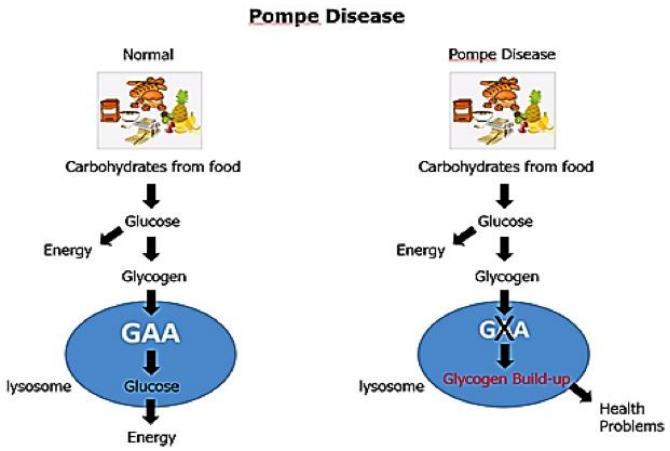

Fig. 1: Glycogen degradation in lysosomes [4]

2. PREVALENCE

The prevalance of Pompe disease worldwide is 5000 to 10,000 [5]. It is considered to be a rare inborn error of metabolism with an estimated frequency of about 1/40,000 and higher prevalance is 1/14,000 people in some countries like China and Taiwan, and among African-Americans, Northern Europeans of Dutch origin and South East Asians [6]. The new born screening pilot studies from Taiwan and USA also indicated a higher incidence. It is much less common in Australia than Holland, with an incidence of 1/145,000 people. It is even rare in Portugal, with 1/600,000 newborns diagnosed with it, based on acid alpha-glucosidase enzyme activity tests and genetic tests [5].

The early new born screening program data shows the incidence of IOPD is ~1/57,000 and for LOPD is 1/26,500. The incidence of infantile-onset Pompe disease varies between regions and ethnic groups.

3. PATHOGENESIS

The severe infantile-onset form of the disease is due to the complete deficiency of the GAA enzyme (activity <1% of normal controls). GAA enzyme activity of 2% to 40% is associated with some residual enzyme function and a later disease onset [7]. However, progrssion of Pompe disease is at all ages. This disrupts cellular function that occurs due to the excessive accumulation of lysosomal glycogen, causes cell destruction and lead to clinical manifestation such as proximal myopathy and respiratory muscle weakness. Significant cardiac abnormalities- massive cardiomegaly and hypertrophic cardiomyopathy can be commonly seen only in the infantile onset form of the disease. It is inherited in an autosomal recessive disorder, that means the affected individual has two abnormal copies of the *GAA* gene (one on each chromosome located on chromosome 17 at 17q25.2-25.3). There are 25% chances of two carrier parent to have an affected child with each pregnancy. If a person with this disease has children whose spouse is unaffected, all of his or her children will be asymptomatic carriers. At present, more than 300 different pathogenic sequence variations, and most of them belong to only one family. However, some patients are homozygous for a pseudo deficiency allele [c.1726G>A (p.Gly576ser)] can be seen in common in the Asian population, , which can complicate diagnostic confirmation; yet, more sensitive assays using dried blood spots have been developed to distinguish the pseudodeficiency alleles from disease-causing mutations [8].

4. CLINICAL SYMPTOMS

The symptoms of Pompe disease vary from person to person. Symptoms can start at different ages. Symptoms may start in infancy or not until late adulthood. Historically, it has been classified into infant onset with the symptoms seen in first year of life, juvenile onset with symptoms from second year of life through adolescence and adult onset seen later in life. Without treatment, the symptoms of this disease are often fatal. It is important to remember that each child is different and may experience symptoms differently [5]. In general, earlier onset of symptoms is associated with more severe and more rapidly progressive disease.

4.1. IOPD (Infantile Onset Of Pompe Disease)

The symptoms of early onset of this disease might be available at the time of birth or appear within the first few months. The general symptoms include: cardiomyopathy, hypotonia, rapidly progressive muscular weakness, in conjunction with delayed motor milestones, cardio-respiratory failure, by age 1year, impairment of respiratory muscles (including abdominal and intercostal muscles and the diaphragm) and rapid-onset respiratory insufficiency, difficulty in Swallowing, malnutrition, aspiration pneumonia, and respiratory tract infections, other common findings include macroglossia, hepatomegaly, hearing loss, osteopenia, and scoliosis. Patients with infantile onset of this disease create secondary cardiomyopathy because of the gigantic gathering of glycogen inside the heart muscles [9, 10].

4.2. LOPD (Late Onset Of Pompe Disease)

The symptoms of LOPD is milder than the infantile form, and heart is usually is not affected. The deficiency of acid α-glucosidase enzyme is mild and the general symptoms may occur after the primary year of life up to the 6th decade of life. Initially, the paraspinal muscles and lower limb proximal muscles are affected that prompts to motor impairment and difficulty performing daily activities and scoliosis, kyphosis. Diaphragm impairment and auxiliary respiratory muscles causes chronic respiratory failure with fatigue, carbon dioxide retention, respiratory insufficiency and sleep apnea. Repeated aspiration pneumonia is common. Other findings include; macroglossia, palpebral ptosis, cerebral aneurism, hyporeflexia, osteopenia and muscular weakness is the most prominent and detectable in cases of the disease. Respiratory problems also appear as the first symptom in few cases [11, 12].

5. *GAA* GENE

As shown in figure 2, *GAA* gene is localized to chromosome 17q25.2-q25.3 as shown in Fig. 2 [13]. The *GAA* gene is approximately 20 kbp long and encompasses 20 exons. The first exon contains the 5' untranslated sequences and is separated from the second one by a large intron of approximately 2.7 kb. The first ATG codon is located in exon-2; 32 nucleotide downstream from the beginning of the exon. The second and the last exons are quite big (578 and 607 bp, respectively), while the remaining exons length ranges from 85 to 187 bp [14]. In *GAA* gene, the promoter sequence is located upstream of the first exon. The cDNA of *GAA* is 3.6 kb long and encodes a precursor peptide of 952 amino acids with a predicted molecular weight of 105 kD [15]. Many normal allelic variants exist *GAA* and are responsible for the three known alloenzymes (*GAA1, GAA2* and *GAA4*) [16].

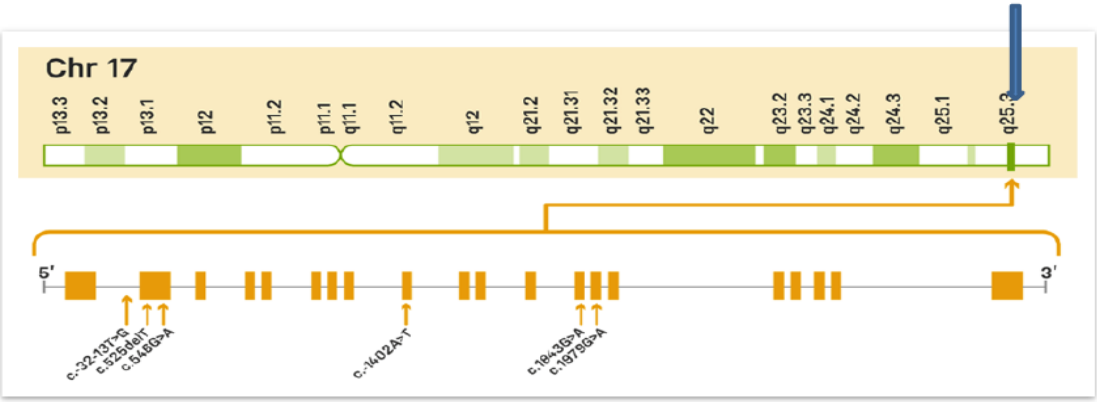

Fig. 2: *GAA* gene Source: HGMD: http://www.hgmd.cf.ac.uk/ac/[13]

6. *GAA* MUTATIONS

The mutation of *GAA* gene is very heterogeneous. All types of mutations have been described. To date, more than 582 mutations in the *GAA* gene have been reported in the human gene mutation database (HGMD: http://www.hgmd.cf.ac.uk/ac/) [13]. All types of mutations has been described. Missense mutations are most common followed by small deletions. As shown in Figure-3, 297 (51.0%) of reported mutations are missense, 87 (14.9%) are small deletions, 16 of which are in frame, 74 (12.7%) are splicing variants, 51 (8.8%) are nonsense, 35 (6.0%) are small insertions/duplications, 19 (3.3%) are gross insertions/deletions, 13 (2.2%) are small indels, 5 (0.9%) are complex rearrangements as shown in Fig.3 [14]. It is considered to be as pan ethnic disease.

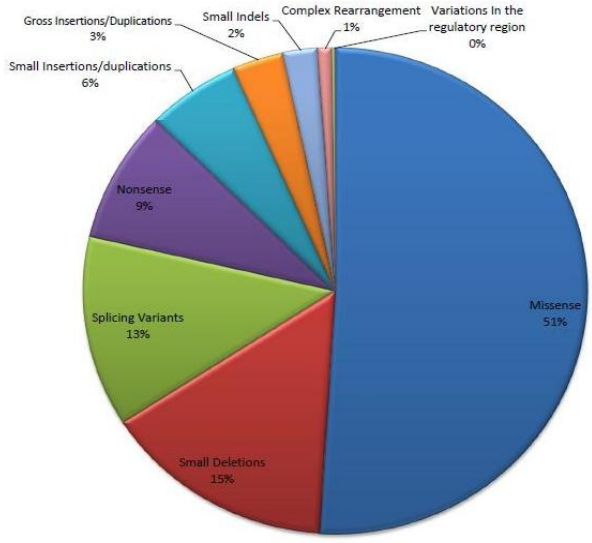

Fig. 3: Frequency of *GAA* mutant alleles reported in the HGMD [17]

7. BIOCHEMICAL ANALYSIS

7.1. Newborn Screening

In certain states, newborn screening for these diseases is done. In this test, blood spot from the baby's heel is used to screen for many different conditions. Newborn screening detects this disease by identifying for *GAA* enzyme activity. These enzymes are active in every healthy newborn's blood. Since babies with this disease have *GAA* enzymes that are absent or not working appropriately, they will have diminished activity of *GAA* enzyme. If a baby has a positive result on the initial Pompe screening, it doesn't yet confirm that the person has Pompe disease. Low GAA enzyme activity level can sometimes be found in people that never develop Pompe disease. This is called pseudodeficiency. It is observed in about 3.9% of East Asian populations. Therefore, a positive screening result implies that further testing must be done to assure or rule out Pompe disease. Rarely, there can also be false positives with additional testing [18].

In case of one or both parents are known to be carriers of Pompe disease, newborn screening is not enough there should be more sensitive diagnostic tests should be done in additional to NBS, even if the result is negative of NBS [18].

7.2. Serum Creatinine Kinase (CK) Concentration

This blood test measures the amount of an enzyme called creatinine kinase (CK) in the blood. People with Pompe disease will often have more CK in their blood than expected. Many other conditions also cause elevated CK levels in the blood, so this test cannot be used to make a definite diagnosis of Pompe disease. Also, late-onset Pompe disease that presents itself in late childhood may be missed by this test [18].

7.3. Urinary Glucose Tetra saccharides

This urine test looks at certain carbohydrates in the urine. Individuals with Pompe disease will have more of a particular carbohydrate than expected in their urine. However, people with other glycogen storage diseases will have the same results, so this test cannot by itself diagnose Pompe disease [18].

7.4. GAA Enzyme Diagnosis Test

The gold standard method for the diagnosis of Pompe disease is the determination of partial or complete deficiency of GAA enzyme activity in blood or fibroblasts. These assays are currently more reliable and sensitive. The traditional way of clinical diagnosis is confirmed by the virtual absence (infantile-onset) or markedly reduced (late-onset) GAA activity in tissues such as cultured fibroblasts from skin biopsy, muscle biopsy, purified lymphocytes, mononuclear cells and lymphoid cell lines However, in refined fibroblasts or muscle the GAA chemical estimation is mostly dependable upon the probability of interchange isoenzyme activities making disease in culture cell examines. The optimum activity of GAA is at pH 3.7 to 4.5 and hydrolyses alpha 1-4 and alpha 1-6 bonds in glucose polymers. This activity is measured by comparing acidic 3.7 to 4.5 with activity of neutral glucosidase at pH 7.0 by using maltose and glycogen or the maltose fluorescent synthetic analogue, 4- methyl umbelliferyl-α-D-glucosidase(4-MUG) as substrates [19].

The skin biopsy is obtained by cultured fibroblast that is then grown to confluency prior to the enzyme assay. This can take up to about a month and a half and can essentially postpone the diagnosis [19].

Muscle biopsy in disease indicates the presence of vacuoles that stain positively for glycogen. In advanced stages of the disease, glycogen accumulation is seen both in the lysosomes and dispersed in the cytosol [19].

Quantitatively, the amount of glycogen in muscle rose up to ten times normal than average in early onset patients and to a lesser degree in late onset patients [19].

Acid α-glucosidase activity can also be assayed selectively in mixed leukocytes by inhibiting neutrophil maltase glucoamylase by acarbose. The addition of acarbose to assays using purified lymphocytes prevents false-negative results. With the competitive inhibition acid α-glucosidase assays using blood samples can now be considered the method of choice for the enzymatic diagnosis of Pompe disease because they are reliable, less invasive, more convenient, and faster [20].

8. MOLECULAR ANALYSIS

Molecular analysis is particularly useful in the identification of carriers when the familial mutation is known. Analysis of *GAA* gene may be required to confirm the diagnosis. The PCR and RFLP tests are fast confirmatory tests for diagnosis and screening [21].

Mutation testing plays a vital role in PD. Due to the potential overlap of residual *GAA* enzyme activity in late onset Pompe disease with heterogenous, molecular analysis of the *GAA* gene may be required to confirm the diagnosis. More than 100 mutation and numerous

variants in the *GAA* gene have identified. The mutations in exon 14 may be over represented [22, 23].

Among the recurrent mutations in the infantile-onset cases is a single base pair deletion, _525T that is seen in 9% of U.S. cases. This same mutation accounts for 34% of Dutch cases. The exon 18 deletion mutation is seen in infantile-onset cases and accounts for about 25% of Dutch and Canadian cases but only about 5% of U.S. cases. The leaky IVS1(-13T-_G) splice-site mutation accounts for about 50% of late onset cases. There are some populations in which particular mutations are more common due to founder effects while allelic heterogeneity can be significant in admixed populations as exist in the U.S. The R854X mutation is found in many African American and African cases; D645E is seen in many Chinese infantile cases; the 2741AG-_CAGG insertion is seen in Turkish cases; and the G925A mutation is seen in many European cases [24]. Currently, mutation in India for this disease is still unknown as it is rare disease.

9. CURRENT MANAGEMENT

The management of this disease needs comprehensive multidisciplinary proceeds towards encompassing strategies that include proper and timely interventions that are disease-specific to target the underlying disease process and symptom-specific manifestations. The most important is to very have clinical experience and managing the disease. Care is a joint effort across multiple specialties and can include specialists in inherited metabolic diseases, developmental paediatrics, cardiology, pulmonology, neurology, anaesthesiology, urology, immunology, and nutrition. In addition to this, physical, occupation and speech therapy by the patient at early intervention, and should be estimated early for these needs. Genetic counseling is required for new families. Overall coordination of care across disciplines and continued oversight of the care and management by a clinician that is experienced in patients and knowledgeable about the disease itself, potential complications, and the nuances of treatment are essential. The geographic locations having limited resources and facilities with experience in the care of patients with Pompe disease and in this patients telemedicine also can play a part in monitoring and care coordination of patients. Most importantly, the treatment of any patient with Pompe disease, as with other inherited metabolic diseases, needs to be tailored to the individual patient. The management guidelines for patients across the disease spectrum have been published for various parts of the world. Clinicians should also refer to these guidelines when treating patients with Pompe disease [7, 25-27].

Most NBS programs do short-term follow-up of babies with a positive newborn screen until the diagnosis is confirmed or excluded. Decisions on treatments and long-term follow-up of patients are the responsibility of the clinical specialist. In the United States, the clinical specialist is usually the medical geneticist. In Pompe disease, monitoring of patients to determine need to initiate ERT is critical. The follow-up of Pompe disease is lifelong and is closely related to disease severity [7, 27].

10. THERAPEUTIC OPINION

10.1. Enzyme Replacement Therapy (ERT)

Enzyme replacement therapy (ERT) is the only effective treatment for Pompe disease. In this therapy, acid alpha-glucosidase, the enzyme that is deficient in Pompe disease, is given via an injection. This allows patients to break down the glycogen stored up in their tissues into the more usable glucose [5].

10.1.1. Mechanism of ERT

ERT is used to treat genetic diseases in the patients that are having an insufficient amount of enzyme produced or the enzyme produced but not working properly. For the treatment, the functional enzyme usually is produced in the laboratory by cells that have been genetically modified. The cells are then harvested and the enzyme is purified before being given to the patient [5].

10.1.2. Procedure

ERT should be started as early as possible in infants with Pompe disease, and as soon as symptoms appear in adults. Once started, patients need to be on the therapy for their entire lives in order to prevent the glycogen from building up again. The treatment usually is not stopped or interrupted even if negative side effects occur. Lumizyme is an ERT for Pompe disease produced by Sanofi Genzyme, this is the only ERT currently available in the U.S. The company also produces a similar ERT, marketed under the brand name Myozyme, in Europe [5].

As a result ERT reduced the risk of death by 99% and of invasive ventilation by 92% compared with the untreated patients. A relevant number of treated infants acquired motor and functional skills and some patients remained ambulatory in their teens [5].

10.1.3. Side effects of ERT

There are several side effects of ERT. Many of these are due to the body reacting to the needle and fluids involved in the intravenous delivery of the enzyme. The influx of fluid can cause electrolyte problems and the body may respond with swelling and irritation around the injection site or a more general fever. Other side effects involve an allergic immune reaction to the enzyme itself. Their immune system marks the enzyme as a foreign substance and attacks it and that can lead to problems such as severe anaphylaxis, a life-threatening allergic reaction that requires immediate treatment [5].

10.2. Gene therapy

This is an experimental therapy which aims to restore the body's ability to produce functional acid alpha-glucosidase enzyme on its own by providing cells with a functioning copy of the *GAA* gene [5].

10.2.1. Procedure

A working duplicate of a gene is embedded into the body utilizing a modified virus called an adeno-related infection (AAV) as a vector. The AAV vector has had its disease causing genes evacuated with the goal that it is no longer dangerous, and the gene of interest added. In this case, *GAA* along with a virus, it can still integrate the gene into the genome of the patient, providing cells with a new healthy copy of *GAA* that can be used to produce the functional enzyme. This can be targeted to specific tissues in the body, including affected organs such as the diaphragm, for example, to improve breathing capacity [5].

There are several research groups investigating gene therapy in Pompe disease. Some of these potential therapies have already been tested in clinical trials while others are in the preclinical stage and are being tested in animals [5].

10.3. Chaperone Therapy

Chaperone therapy for Pompe disease is currently in the clinical trial stage and aims to restore function to the defective *GAA* enzyme. This therapy is also being investigated in other lysosomal storage disorders, such as Gaucher disease and Fabry disease [5].

10.3.1. Procedure

In Pompe disease, chaperone therapy uses small molecules that bind to the dysfunctional *GAA* enzyme, helping it to fold correctly and ensuring that normal enzyme activity is restored. Chaperone therapy may help overcome the limitations of enzyme replacement therapy (ERT), the current standard of care for Pompe disease.

The limitation of chaperone therapy is all patients with Pompe disease have a defective *GAA* gene, there are many different variants of the *GAA* gene mutation that have been documented. Not all the mutations may respond to chaperone therapy. Chaperone therapy alone may not be enough to reverse enzyme defects [5].

11. FUTURE DIRECTION

Pompe disease is the first myopathy for which corrective target enzyme replacement therapy is developed [6]. Because ERT is currently the only approved treatment available research is focused on making the therapy more effective with fewer side effects. There are several research groups investigating gene therapy in this disease. Some of these potential therapies have already been tested in clinical trials while others are in preclinical stage and are being tested in animals [13]. Also the timescale for carrying out the PCR and RFLP test on a routine basis is about 3-8 hours and is therefore fast compared to either available method. Early identification through newborn screening and more effective and specific therapies will likely significantly improve the outcomes for all the patients with Pompe disease [6].

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