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Pompe Disease: Literature Review and Case Series

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INTRODUCTION

Pompe disease (GSD II) is an autosomal recessive disorder caused by deficiency of the lysosomal enzyme acid-α-glucosidase (GAA, EC 3.2.1.20), leading to generalized accumulation of lysosomal glycogen especially in the heart, skeletal and smooth muscle, and the nervous system. Pompe disease was first described in a 7 month-old girl with severe muscle weakness who also had hypertrophic cardiomyopathy and generalized glycogen accumulation in various tissues throughout the body.[1] Bischoff and Putschar also independently described the disease in the same year.[2,3] Hers identified alpha-glucosidase deficiency and localized the GAA enzyme activity to the lysosomes of liver, heart and muscle tissues of five infants with classic Pompe disease and was the first to recognize impaired autophagy.[4] Pompe disease is generally classified based on the age of onset as infantile (IOPD) when it presents during the first year of life, and late onset (LOPD) when it presents afterwards. Childhood, juvenile and adult-onset Pompe disease are examples of the late onset form. IOPD associated with cardiomyopathy is referred to as classic Pompe disease and in the absence of cardiomyopathy as non-classic Pompe disease.[5,6,7] Similar to other lysosomal storage disorders, Pompe disease clinically presents as a continuum in its age of onset and multisystem involvement. The role of autophagy in the pathogenesis of Pompe disease, especially the late onset form, has increasingly become evident and may be clinically relevant. Autophagy (self-eating) is a highly complex, ubiquitously expressed, and evolutionarily conserved lysosomal degradative process, which is controlled by a multi-gene network (http://autophagy.lu/index.html). Its main function is to recycle obsolete cellular constituents and eliminate damaged organelles and protein aggregates. It involves dynamic membrane rearrangement for sequestration of cytoplasm and its delivery into the vacuole/lysosome. Basal autophagy plays a role in cellular development and differentiation,[8] innate and adaptive immunity[9] and is induced in response to various stress conditions, such as nutrient limitation, heat, and oxidative stress. Ammonia derived from the deamination of glutamine via glutaminolysis supports basal autophagy and protects cells from tumor necrosis factor alpha (TNFα)–induced cell death.[10] As a result basic metabolites are released into the cytoplasm for new synthesis or as sources for energy. Autophagy is also implicated in a wide range of disorders such as neurodegeneration, cancer and ageing and now various lysosomal storage diseases especially Pompe disease.[11-14]

Clinically, infants with classic Pompe disease typically present during the first few weeks of life with hypotonia, progressive weakness, macroglossia, hepatomegaly and hypertrophic cardiomyopathy. With this typical clinical presentation, diagnosis is usually straightforward. The natural history of IOPD is that most of these infants die by their first birthday. On the

other hand, the diagnosis of Pompe disease in older children and adults can be more challenging as these patients generally present with slowly progressive limb girdle type weakness and respiratory insufficiency without significant cardiomyopathy.[5-7,15] Cardiac involvement in late-onset Pompe disease manifests as Wolff-Parkinson-White syndrome, left ventricular hypertrophy and dilatation of the ascending aorta. Rigid spine syndrome (a progressive limitation of the neck and trunk), scoliosis, and low body weight had also been reported in a subset of patients with LOPD with onset in adolescence and resulting in postural anomalies.[16] The diagnosis of Pompe disease is usually made based on typical clinical presentation followed by the demonstration of deficiency of GAA enzyme activity in muscle, skin fibroblasts or more recently dried blood spots (DBS) as well as *GAA* mutation analysis.[5,6] Diagnosis of Pompe disease through newborn screening is also now possible. Pompe disease is still considered to be a rare inborn error of metabolism with an estimated frequency of about 1/40,000 and a higher incidence in certain populations such as African Americans (1/14,000), Northern Europeans of Dutch origin and South East Asians. However, early results of newborn screening pilot studies from Taiwan and USA indicated a higher incidence. Interest in Pompe disease has grown significantly since the FDA approval of the first specific enzyme replacement therapy (ERT) with recombinant human acid α-glucosidase (alglucosidase alfa) for this metabolic myopathy in 2006. Glycosylated alglucosidase alfa is targeted to the lysosomes through uptake via the mannose-6-phosphate receptors. Clinical experience with alglucosidase alfa showed more dramatic improvement of cardiac pathology compared to skeletal myopathy and especially in children more than adults. Abnormal autophagy in Pompe disease results in abnormal recycling of the cation independent mannose-6-phosphate receptors (CI-M6PR), which may explain the less satisfactory clinical response of skeletal muscles.[17,18] Therefore, correction of abnormal autophagy in individuals with Pompe disease may improve therapeutic response to ERT.

In this report, we describe our experience with 12 patients with classic infantile (1 child), non-classic infantile (2 siblings), juvenile (1) and adult onset (8) Pompe disease one of whom had a first trimester miscarriage while receiving ERT. We also report 4 potentially pathogenic, novel *GAA* gene variants in this group and review the recent advances in the pathogenesis, diagnosis and treatment of individuals with Pompe disease.

Genetic Etiology and Prevalence

Pompe disease is also considered a polyglucosan vacuolar myopathy which results from absence or partial deficiency of the lysosomal acid α-glucosidase (GAA) activity due to recessive mutations in the autosomal *GAA* gene. *GAA* (NM_000152.3) is approximately 18.3 kb long and contains 20 exons (Fig.1). Its cDNA has 2859 nucleotides of coding sequence which encode the immature 952 amino acid enzyme. GAA is synthesized as a membrane bound, catalytically inactive precursor which is sequestered in the endoplasmic reticulum. It undergoes sugar chain modification in the Golgi complex, followed by transport into the (minor) secretory pathway, or into lysosomes where it is trimmed in a stepwise process at both the amino- and carboxyl-termini.[19,20] Phosphorylation of mannose residues ensures efficient transport of the enzyme to the lysosomes via the mannose 6-phosphate receptor. GAA catalyzes the hydrolysis of α1→ 4 glucosidic linkages in glycogen at acid pH. Specificity for the natural substrate (glycogen) is gained during its maturation.

The activity of mature (76/70-kDa) GAA for its natural (glycogen) substrate is considerably more robust than its activity towards the artificial substrate (4-methylumbelliferyl-α-D-glucopyranoside4; 4-MU), which is frequently used in in-vitro assays.[20] However, 4-MU is also a substrate for several other enzymes including "leukocyte" neutral isoenzymes, glucosidase II (GANAB) and neutral α-glucosidase C (GANC), and maltase glucoamylase (MGAM). Muscle tissue and cultured fibroblasts do not contain MGAM allowing measurement of GAA (as the activity ratio of neutral to acid glucosidase, GANAB + GANC/GAA) without interference. Because MGAM is expressed in neutrophils, not in lymphocytes, the same activity ratio determined in purified lymphocytes has also been used for the diagnosis of GSD II which is not possible in dried blood spots (DBS). Using maltose or acarbose as an inhibitor of MGAM activity, the measurement of GAA activity in DBS samples with minimal interference by other α-glucosidases was accomplished which now serves as the basis for newborn screening and the non-invasive diagnosis for Pompe disease.[21-23] As a result, multiplex newborn screening for Pompe disease and other lysosomal storage disorders using fluorometric, digital microfluidic and tandem mass spectrometry based GAA enzyme activity assays had been developed.[24-27] In addition to qualitative and quantitative assessments of the disease burden, and clinical measures of the impact of Pompe disease on various affected systems, urinary glucose tetrasaccharide (Glc4), a biomarker of glycogen storage with 94% sensitivity and 84% specificity for Pompe disease, is frequently used in monitoring the response of patients to enzyme replacement therapy and as an adjunct to acid α-glucosidase activity measurements.[28] Also, in addition to the traditional 1-dimensional thin layer chromatography (TLC) for urine oligosaccharide analysis, a new MALDI–time-of-flight/time-of-flight (MALDI-TOF/TOF) mass spectrometry based assay of urinary free oligosaccharides useful for the diagnosis of Pompe disease and other lysosomal storage diseases is now available.[29]

Infants with Pompe disease are considered as cross reactive immunologic material (CRIM) positive if they have residual GAA enzyme activity and CRIM negative if no residual GAA activity is detected. Based on pooled clinical studies data, 28% of Pompe disease cases are infantile-onset, of which about 85% are classic infantile-onset and three quarters of those are CRIM+(http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/nominatecondition/reviews/pompereport2013.pdf). CRIM status is usually determined by Western blot analysis in cultured skin fibroblasts, a process that can take a few weeks, and more recently via a blood-based CRIM assay that can yield results within 48 to 72 hours.[30] Recombinant human acid α-glucosidase (rhGAA) was first produced in dihydrofolate reductase deficient Chinese hamster ovary (CHO) cells, was targeted to heart muscle and corrected glycogen accumulation in fibroblasts from patients with Pompe disease.[31] Prior to the initiation of enzyme replacement therapy, rapid determination of CRIM status in patients with infantile onset Pompe disease who at risk of developing neutralizing antibodies against rhGAA is extremely important.

Many normal allelic variants exist in *GAA* and are responsible for the three known alloenzymes (GAA1, GAA2, and GAA4). More than 450 mutations in *GAA* have been reported in individuals with Pompe disease. Nonsense mutations, large and small gene rearrangements, and splicing defects have been observed with any mutations being

potentially specific to families, geographic regions, or ethnicities (http://www.pompecenter.nl/). Combinations of mutations that result in complete absence of GAA enzyme activity are seen more commonly in individuals with infantile-onset disease, whereas those combinations that allow partial enzyme activity typically have a later-onset presentation.[32] *GAA* mutations result in mRNA instability and/or severely truncated acid alpha-glucosidase or an enzyme with markedly decreased activity. By means of homology modeling, and using the crystal structure of the N-terminal subunit of human intestinal maltase-glucoamylase as a template, analysis of the three-dimensional models of human GAA encompassing 27 relevant amino acid substitutions causing a processing or transport defect responsible for Pompe disease showed that they were widely spread over all of the five domains of GAA from the core to the surface of the enzyme and the predicted structural changes varied from large to very small.[33]

The c.1726 G>A (p.G576S) variant in cis with c.2065 G>A (p.E689K), also known as the c.[1726A; 2065A] pseudodeficiency allele, causes low GAA activity in normal individuals and is relatively common in Asian populations.[34,35] About 3.9% of apparently healthy Japanese were reported to be homozygous for this pseudodeficiency allele which may complicate newborn screening (NBS) and result in a high false positive rate in such populations.[36] In their newborn screening pilot program in Taiwan, Labrousse et al. identified 36 babies (0.027% screened) who had no pathogenic *GAA* mutation but were homozygous for the c.[1726A;2065A] pseudodeficiency allele.[37] In the United States, the prevalence of Pompe disease is approximately 1 in 28,000 with the prevalence of pseudodeficiency being less than 1% as confirmed by genetic analysis of healthy individuals with low GAA enzyme activity level.[88]

Clinical Presentation

Diagnosis of Pompe disease can be made clinically based on a typical clinical presentation as in infantile cases or suspected in a young child or adult with limb weakness, difficulty walking or limb girdle dystrophy. In combination with the clinical diagnosis, a histological diagnosis can be confirmed in a muscle biopsy which shows typical intra-myofibrillar cytoplasmic membrane bound glycogen, periodic acid–Schiff–positive vacuolar myopathic abnormalities and acid phosphatase positive vacuoles (Fig.2).[38-42] Low GAA activity in muscle, skin fibroblasts and more recently dried blood spots confirms the diagnosis even in the absence of diagnostic histological findings which are not regularly detected in patients with LOPD. Patients presenting with either a limb-girdle syndrome or dyspnea secondary to diaphragm weakness should undergo further testing. A blood-based GAA enzyme activity assay is the recommended tool to screen for GAA enzyme deficiency, confirmed by a second test: either a second GAA enzyme activity assay in another tissue (such as lymphocytes, fibroblasts or muscle) or *GAA* gene sequencing.[43] Deficient GAA enzyme activity leads to generalized tissue lysosomal glycogen accumulation especially in skeletal, cardiac, and smooth muscles.[44,45] Typically, affected newborns present with hypotonia, upper and low limb weakness, macroglossia, hepatomegaly, failure to thrive, progressive hypertrophic cardiomyopathy and cardio-respiratory insufficiency leading (if untreated) to early death. Generalized glycogen storage had been identified in autopsy material from fetal tissues and adults with IOPD and LOPD respectively.[46-54] On the other hand, older children

and adults usually present with slowly progressive limb girdle weakness, respiratory deterioration, rigid spine syndrome, scoliosis and low body mass.[53,56] A growing number of large series of patients with LOPD had been described in the literature and were reviewed recently.[57-64] As a result, the clinical presentation in LOPD has been expanded to include: ptosis, bulbar palsy and urinary incontinence. Taken together, the pathologic accumulation of glycogen in several tissues identified on autopsy examination and clinical experience with patients with Pompe disease revealed the following clinical correlations: diaphragm and intercostal muscles - respiratory failure, proximal skeletal muscle - progressive limb-girdle myopathy, genioglossus - tongue weakness, extraocular muscles - unilateral or bilateral ptosis, smooth muscle - abdominal pain/nausea/vomiting/diarrhea/urinary incontinence, and cerebral vasculature - cerebral aneurysm.[65,66] However, the following associations do not seem to have clinical correlates: broadened cerebral gyri, increased number of cerebral and cerebellar astrocytes, lipofuscin deposits in neurons and astrocytes of the spinal cord and cerebellum, fibrillary gliosis and anterior horn cell degeneration, and glycogen vacuoles in the Schwann cells surrounding myelinated and unmyelinated axons of peripheral nerves.

Pathogenesis of Pompe disease

In Pompe disease, it is well established that the initial insult is due to the accumulation of the intra-lysosomal glycogen. However, recent studies showed that multiple other cellular abnormalities occur and that the pathophysiology of Pompe disease is far more complex than appreciated previously. In particular, the central role of autophagy is becoming more important now.[67-78] Hers was the first to describe the histological features of autophagy which were then explicitly demonstrated using light and electron microscopic examination of skeletal muscle and confirmed by Engle and Dale.[4,77,78] Large pools of autophagic debris in skeletal muscle cells especially in type II fibers, were seen in both mouse *Gaa* knockout model and patients with Pompe disease.[70-73] MAP1LC3, often referred to as LC3, and its membrane bound isoform (LC3-II) is commonly used as a specific marker of autophagosomes.[76] Skin fibroblasts from patients with Pompe disease had been shown to have abnormal morphology with abnormal mannose-6-phosphate receptor trafficking which secondarily impaired rhGAA uptake in these cells. By electron microscopy, various features of enhanced autophagy such as the accumulation of multi-vesicular bodies, expansion of Golgi apparatus, abnormal intracellular distribution of CI-MPR and reduced availability of the receptor at the plasma membrane were identified. These abnormalities resulted in less efficient rhGAA uptake, processing and correction.[71] Accumulation of autophagosomes is a key pathological finding in skeletal muscle fibers and skin fibroblasts from patients with Pompe disease and is implicated in the poor response to ERT.[79] Mutant GAA initiates autophagy via the induction of endoplasmic reticulum (ER) stress as well as Akt inactivation (ER stress-independent) using mTOR suppression. Treatment with insulin which activates Akt signaling restored phosphorylation of both Akt and p70S6 kinase and suppressed autophagy in patient fibroblasts. Also, combination therapy using rhGAA and insulin enhanced correct co-localization of the enzyme with lysosomes.[80] On the other hand, suppression of autophagy in the whole organism by knocking out critical autophagic genes (Atgs), such as *Atg5* or *Atg7* is lethal.[74,75]

Metabolic abnormalities in tissues and body fluids of GAA2/2 mice and humans respectively had also been identified. Abnormal glycogen metabolism (suppressed phosphorylase activity, elevated glycogen synthase, glycogenin, hexokinase activities, and glucose-6-phosphate) in the heart, skeletal muscles and liver from GAA2/2 mice was demonstrated.[81,82] The effect of GAA deficiency in muscles of patients with Pompe disease extends to various vesicle systems linked to lysosomes including the early endosomes (rab5), recycling endosomes (transferrin receptor) and trans-Golgi network as they all showed increased immunoreactivity.[83] Expression of the insulin responsive glucose transporter 4 was also markedly increased and partially co-localised with all vesicular markers, a phenomenon which may contribute to its abnormal homeostasis. In addition, abnormal energy metabolism, diminished plasma methylation capacity, elevated IGFBP1 and IGFBP-3 levels were found in patients with LOPD.[84] Low carbohydrate and high protein-calorie diet was beneficial.

Newborn Screening for Pompe disease

Over several decades and since the inception of the universal newborn screening programs for inborn errors of metabolism, the number of disorders and the laboratory assays used to detect them continued to be limited. However, the introduction of tandem mass spectrometry in the late 1990's resulted in a significant and rapid expansion of the number of such disorders some of which may not fulfill the classical inclusion criteria of Wilson and Junger.[85] Once again, tandem mass spectrometry based GAA enzyme activity assays had been shown recently to be potentially useful in newborn screening for Pompe disease and other lysosomal storage diseases using DBS [Table 5]. Newborn screening efforts for Pompe disease started in Taiwan since 2005. Recently, the United States Secretary's Discretionary Advisory Committee for Heritable Disorders in Newborns and Children recommended universal newborn screening for Pompe disease, an effort which started in some states based newborn screening laboratories. Few other newborn screening pilots were conducted in other countries around the world [Table 5].[86-97] It is unclear how asymptomatic cases destined to have LOPD but detected on newborn screening should be followed and managed.[64] While newborn screening for lysosomal storage diseases including Pompe disease is gaining acceptance, some investigators and ethicists recommended that screening for these conditions should only be performed in the research context with institutional review board approval and parental permission.[98]

THE UNIVERSITY OF KANSAS MEDICAL CENTER CASE SERIES

We performed a retrospective review of all patients diagnosed with infantile (IOPD) and late onset Pompe disease (LOPD) at the University of Kansas Medical Center between 2000 and 2013 (Tables 1-4). Muscle biopsies, *GAA* mutation analysis and GAA enzyme activity of muscle, skin fibroblasts, amniocytes and dried blood spots (DBS) were performed at various medical centers and reference clinical laboratories using standard techniques. Medline database was searched for reports of large series of patients with IOPD and LOPD, autopsies performed on patients with IOPD and LOPD and abortuses/abortions as well as those reports which describe new diagnostic techniques related to Pompe disease.

We identified 3 patients with infantile (1 classical and 2 siblings with non-classical), and 9 patients with late onset (1 patient with juvenile and 8 with adult) Pompe disease. Male:female ratio was 1.4:1 and the average age of onset was 17.7 years (0-39). In this group, all 3 patients with infantile Pompe disease (cases 1, 2, 3) had varying degrees of hypertrophic cardiomyopathy which was most severe in patient 3. Unlike patients 1 and 2 (Fig.3A, B), patient 3 (Fig.3C) appears to have classical Pompe disease with severe hypotonia and minimal strength in the upper and lower limbs. Overall, the presenting symptom was limb girdle weakness in 43% while 25% presented with shortness of breath and 17% had myalgia. Delay from first symptom to diagnosis was 6 years (1-22). In LOPD cases, shortness of breath affected 3/7 cases, presenting within 1-2 years of the first symptoms. Besides limb weakness, scapular winging was evident on presentation in 8% and 4/7 (57%) had Trendelenburg gait. Low back pain was reported in 1 LOPD patient (12) in whom back surgery was done. MRI examination showed lower limb muscle fatty infiltration and edema (patient 4) and dilated cerebral -circle of Willis- vessels (patient 8). Echocardiography revealed septal hypertrophy in two patients, left ventricular hypertrophy (LVH) in one patient and hypertrophic cardiomyopathy (HCM) in the child with classical IOPD. Urinary tetrasaccharide (Hex4) level was elevated in two patients (3, 8) and normal in three other patients with LOPD (4,7,9). Creatine kinase level ranged from 59 to 1,684 IU/L (mean 878) and electromyography showed evidence of myotonia in one out of four studied patients, with fibrillation and myopathic motor unit action potential (MUAP) present in this and another patient (6 and 7). The other 2 cases were either normal (episodic severe myalgia) or revealed myopathic MUAPs. DBS-GAA enzyme activity was less than 40 % of the lower normal limit (10 pmol/punch/hour) in 7/9 patients and in the other 2 was in the borderline range of 40 to 50% of normal lower limit.

Most patients (8/12) had muscle biopsy as their first test followed by DBS-GAA activity level as a confirmatory test. Out of three IOPD cases, two underwent muscle biopsy; Case 2 showed vacuoles and microaggregates of glycogen on PAS confirmed to consist of abundant membrane bound glycogen on ultrastructural analysis (and markedly reduced muscle GAA enzyme activity) and Case 3 had vacuoles in 50% of muscle fibers and accumulation of glycogen in less vacuolated fibers that was also seen in smooth muscle fibers of erector pili muscles on skin biopsy. Altogether all 9 LOPD cases underwent muscle biopsy except for Case 5, 7/8 muscle biopsies were suspicious for Pompe disease and the eighth biopsy showed nonspecific myopathic changes. Vacuoles were seen in 5 LOPD cases along with abnormal glycogen deposition on Periodic Acid Schiff (PAS) stain while the other 2 cases showed abnormal glycogen deposition without vacuolation. Hematoxilin and eosin stained muscle tissue showed rimmed vacuoles in 2 cases and non-rimmed in an additional 2cases. In one of these cases, rimmed vacuoles could not be confirmed on modified Gomori trichrome stain, but in Case 4 (severe episodic myalgia), there were non-rimmed vacuoles only seen on trichrome. Acid phosphatase positive vacuolar aggregates were seen in 2/5vacuolated muscle biopsies. Abnormal glycogen deposition on PAS was present in 7 cases and was the only finding in 2/7 biopsies suggestive of Pompe.

Ten patients received ERT and reported subjective improvement although this was not measurable on clinical examination except in case 2 with non-classical IOPD (see below) and case 9 where objective improvement in proximal arm strength was noted 4 months after

the start of ERT. Anti-rhGAA antibody titers were elevated in 6 patients and negative in 2 patients (Table 4). In this cohort, all patients are still alive except patient 11 who was intolerant of ERT and died at 53 years of age due to progressive and severe respiratory insufficiency. *GAA* mutation analysis was done in 8/12 patients and a total of 12 mutations and 4 variants of unknown significance (VUS) were identified. All patients with identifiable mutations were compound heterozygotes except case 3 with classical IOPD who was "c. 1843G>A; p.Gly615Arg " homozygote. Two unrelated patients were heterozygous for the common "IVS1-13T>G/c.-32-13t>g" mutation. The novel (heterozygous) mutation (c.-1402A>T p.I468F) was identified in 2 unrelated patients while another novel splice site (c.546G>A) mutation was found in case 6. Both novel alleles are predicted in-solico to be pathogenic. In this series, family history was positive in 3 sib pairs (1 and 2; 5 and 6; 10 and 11). Next, we describe 4 cases in this cohort in more details.

Patients 1 and 2 (Fig. 2A, B) are African American sib pair. The older brother was diagnosed with hypertrophic cardiomyopathy at 2 months of age when he was also found to be carnitine deficient. Cardiomyopathy did not resolve despite carnitine supplementation. At 5 years of age, he presented with skeletal muscle weakness and respiratory failure triggered by influenza pneumonia for which a tracheostomy was placed. He then became ventilator dependent. Membrane bound glycogen was abundant on muscle biopsy and muscle and DBS GAA enzyme activity were markedly reduced. About 18 months following the initiation of ERT, daytime ventilation was discontinued and muscle strength improved significantly. His younger sister was diagnosed prenatally with infantile Pompe disease via amniocentesis. GAA activity in amniocytes was undetectable. However, postnatal GAA activity in DBS was detectable but reduced. She had mild macroglossia (Fig. 2B) and her echocardiogram showed septal hypertrophy only. She is doing quite well despite the delay in starting her enzyme replacement therapy at 10 months of age. Both siblings maintain negative anti-rhGAA antibody titer. CRIM status was not tested in either sib since they had significant residual GAA activity and by definition would be CRIM positive.

Patient 3 is 8.5 year old Vietnamese boy (Fig.1A) with severe, classical, infantile Pompe disease and severe hypertrophic cardiomyopathy. His diagnosis was made at 4 months of age and received ERT almost immediately. Cross Reactive Immunologic Material (CRIM) testing of his skin fibroblasts by Western blot analysis was positive (Duke University). He continues to be ventilator dependent with minimal muscle power in both upper and lower limbs. He maintained a negative anti-rhGAA antibody titer until 1 year ago when he developed a non-neutralizing low titer at 1:200.

Patient 4 had a muscle biopsy at 10 years of age when she presented with severe recurrent myalgia. A provisional diagnosis of "atypical" dermatomyositis was made for which she was treated with steroids and hydroxychloroquine. Numerous other laboratory studies were uninformative. A second muscle biopsy was done at 18 years of age and showed membrane bound glycogen. Low GAA enzyme activity was detected in muscle tissue and skin fibroblasts while DBS GAA activity was borderline reduced at 4.8 (normal 10-49). Only one predicted to be pathogenic novel *GAA* mutation (c.1402A>T; p.I468F) was identified. Deletion/duplication analysis using exon array (GeneDx, Gaithersburg, MD) was negative as well.

Case 7 presented as a young adult with progressive skeletal muscle weakness. The diagnosis of Pompe disease was made based on abnormal findings in muscle biopsy. GAA activity in DBS was low and *GAA* mutation analysis revealed compound heterozygosity for the common mutation (IVS1-13T>G) and a novel, likely deleterious, variant (p.R527F). She became pregnant for the first time while she was receiving alglucosidase alfa. She suffered a spontaneous miscarriage at 10 weeks gestation. Unfortunately, no pathological examination of the abortus was done.

Discussion, Current Management and Therapeutic Options

Our clinical experience with the 12 patients with Pompe disease which we report here is consistent with the literature. Findings in our case series suggest that a short latency between muscle symptoms and shortness of air should raise suspicion for LOPD. Muscle biopsy histopathology was done in 10/12 cases and was the first test to yield suspicion for Pompe disease in 8/12 cases and the second test in 2 out of the 4 remaining cases. DBS GAA enzymatic activity was done in 9/12 cases, being the first test in 2 cases, the second confirmatory test in 6 cases (in 5/6 after muscle biopsy) and the third test in Case 4 following skin fibroblasts and muscle GAA analysis. Our only patient with classic IOPD who was CRIM positive still has profound muscle weakness and hypotonia despite early and adequate ERT and a very low non-neutralizing anti-rhGAA antibody titer which suggests another mechanism for his suboptimal clinical response. Case 11, an adult with LOPD, died due to progressive respiratory failure which was associated with a high anti-rhGAA antibody titer, an experience consistent with what Patel et al reported recently.[99] Our 26-year old Caucasian female (case 7) who became pregnant for the first time while receiving alglucosidase alfa had a serious adverse event as she suffered a spontaneous miscarriage at 14 weeks gestation. Unfortunately, we did not have access to the product of conception and therefore we were unable to determine the genotype. Her fetus may or may not have been affected with Pompe disease. While recombinant enzyme replacement therapy is generally thought to be safe during pregnancy, a possible direct/indirect detrimental effect on that pregnancy could not be excluded. In addition, the maternal anti-rhGAA antibodies may have played a role as well. While high sustained antibody titers in infantile and more recently late-onset Pompe disease correlate with poor outcome, their potential effect in pregnancy had not be reported yet.[99] On the other hand, there are only two reports in the literature which describe a normal outcome in 2 babies whose mothers with Pompe disease were successfully and safely treated with alglucosidase alfa throughout their pregnancies.[100,101] More clinical experience with the prenatal use of alglucosidase alfa is needed to establish its safety.

Since the recognition that maltose and acarbose inhibit neutral α-glucosidase activity, reliable measurement of acid GAA activity in DBS became feasible and was quickly adopted as a non-invasive alternative to skin fibroblasts and muscle biopsies which are invasive, more expensive and take longer time to process. Preisler et al used this approach to identify three patients with LOPD among 38 patients with unclassified LGMD (8%).[15] Also, as newborn screening for metabolic disorders utilizes blood collected on dried filter papers (DBS), various platforms to measure GAA activity in DBS were developed. They include: tandem mass spectrometry, fluorometric and microfluidics based enzyme assays (Table 5).

Emerging evidence from these studies suggests a higher incidence of Pompe disease (1/28,000) compared to lower estimates reported previously. In addition, GAA pseudodeficiency (< 1% in Caucasians and up to 3.9% in Asians) was recognized which prompted an adjustment of the newborn screening algorithms.[37]

Based on a pooling of clinical studies, 28% of Pompe disease cases are infantile-onset, of which about 85% are classic infantile-onset and 75% of those are CRIM positive.[30,103,104] CRIM negative patients with classic IOPD very likely will develop neutralizing antibodies upon exposure to rhGAA. Such antibodies limit the efficacy of ERT. Induction of immune tolerance using various regimens such as rituximab with plasma exchange or alternatively the combination of rituximab and methotrexate with or without intravenous gamma-globulin is an important therapeutic intervention which should be accomplished prior to initiation of ERT in such naïve patients.[102-106] The recent development of a blood-based assay for determining CRIM status is expected to facilitate this process in a timely manner.

The growing literature on Pompe disease reveales significant clinical variability in the age of onset of symptoms among patients with late-onset Pompe disease which can be only partially predicted by their *GAA* genotype. Recently, expert opinion based guidelines about newborn screening, confirmatory and symptomatic diagnostic testing as well as management of patients with Pompe disease had been published.[7,107] International Pompe disease registry (https://www.registrynxt.com/Pompe/) as well as country based registries had been established.[87-92] Recent reports from these registries indicate that diagnostic delay for patients with Pompe disease is still significant, less than 2/3 of muscle biopsies done in French patients showed specific features of Pompe disease thus confirming the importance of GAA enzymatic assessment, and high prevalence of scoliosis (33%) especially among patients with IOPD.[109,110,113] Systematic analysis of data collected from the Pompe Registry will help improve recognition of the disease, enhance understanding of its variable course and the effect of direct interventions such as current ERT and other potential future therapies.

The literature contains 29 autopsy examination reports of Pompe patients including 9 in the non-English literature and 8 reports of autopsies of fetuses affected with Pompe disease. Collectively, these studies demonstrated the extensive and generalized accumulation of lysosmal glycogen in various organs including the brain, well beyond the liver, heart and skeletal muscles. In the brain, the cytoplasm of Schwann cells but not neurons was shown to accumulate glycogen. This observation suggests that progressive neurodegeneration of the brain is not expected. While glycogen deposition was also demonstrated in the spinal cord and peripheral nerves, there is no clinical correlate to this finding since peripheral neuropathy had not been demonstrated clinically, by electromyography or by histologic examination of muscle. These observations are consistent with the report of better than expected cognitive outcomes in a small group of 10 children with classic infantile Pompe disease treated with alglucosidase alfa. They were evaluated prospectively both developmentally and by neuroimaging.[114] Cognitive development at school age improved and ranged between normal and mildly delayed. Periventricular white matter abnormalities were found in 4 children. While treatment with alglucosidase alfa had been demonstrated to significantly increase survival in patients with IOPD, and since Pompe disease affects many

tissues, including the brain, rhGAA is not expected to have beneficial effects on the central nervous system since it does not cross the blood-brain barrier.

The new insights into the complex pathogenesis of Pompe disease explain, at least partially, the suboptimal clinical response in patients with LOPD and some patients with IOPD. As a result, novel therapies including modified ERT are being investigated. Novel experimental modified rhGAA therapies include glycosylation-independent lysosomal targeting of GAA (rhGAA-GILT), ICAM-1-targeted nanocarriers which aim to enhance delivery of α-glucosidase and muscle glycogen clearance, and neo-GAA.[115-117] The neo-rhGAA is a carbohydrate-remodeled enzyme with higher affinity for the cation-independent mannose 6-phosphate receptor and improved delivery to muscles of Pompe mice. Since CRIM negative IOPD patients are likely to develop neutralizing anti-rhGAA antibodies, early identification of their CRIM status and initiation of immunomodulation tolerance therapy using various approaches are very important for these patients. Co-administration of the pharmacologic chaperone, Duvoglustat hydrochloride (AT2220), with alglucosidase alfa in Pompe disease fibroblasts, blood cell lines and in-vivo appears to stabilize the enzyme and enhance its activity.[117-119] A bacterial glycosidase which enables mannose-6-phosphate modification also improves cellular uptake of yeast-produced recombinant human lysosomal enzymes.[120]

Additional potentially promising investigational therapeutic approaches include: autophagy suppression, gene therapy using modified single-stranded oligonucleotides, short hairpin ribonucleic acids (shRNA), transcription factors (TFEB), AAV1-CMV-hGAA, hematopoietic stem cell (HSC) transplantation and induced pluripotent stem cells (iPS).[120-134] GILT-tagged rhGAA (BMN 701), Duvoglustat hydrochloride (AT2220), neo-rhGAA and AAV1-CMV-hGAA are currently in clinical trials.

Conclusion and Future Directions

Pompe disease is the first metabolic myopathy for which corrective targeted enzyme replacement therapy was developed. Besides limb-girdle weakness and scoliosis in LOPD cases, shortness of air affected nearly half of our cases and led to early presentation within the first 2 years of symptom onset. The most common first diagnostic test to raise Pompe suspicion was muscle biopsy and confirmation was often with DBS assay. DBS GAA enzymatic activity levels in the borderline 40 to 50% range warrants further investigation. The efficacy of the current form of ERT is generally variable and unpredictable especially in patients with LOPD given the long diagnostic delay. There is a need for improved early recognition and therapy for this disorder which will be aided by improved understanding of its pathogenesis. Novel therapies based on improved understanding of the disease pathogenesis are already under study and some are in clinical trials. Early identification through newborn screening and more effective and specific therapies will likely significantly improve the outcome for all patients with Pompe disease.

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KEY POINTS

- Pompe disease, also known as type II glycogenosis, is a progressive autosomal recessive glycogen storage disease caused by deficiency of lysosomal acid alpha glucosidase (GAA) primarily in skeletal and cardiac muscle with age of onset ranging from infancy through adulthood. Extramuscular phenotypes are also recognized.
- Recognized clinical presentations of Pompe disease include infantile (with/without cardiomyopathy) and late onset (childhood, juvenile and adult) forms. In addition to cardiomyopathy in the classic infantile form, musculoskeletal signs and symptoms are the most frequent.
- Excessive lysosomal glycogen storage and defects in autophagy are the main determinants of pathogenesis of Pompe disease.
- Diagnosis of symptomatic individuals as well as screening in healthy newborns is now possible by demonstrating low GAA enzyme activity in dried blood samples complemented by DNA mutation analysis.
- Diagnostic gaps in Pompe disease patients across the disease spectrum continue.
- In our cohort of patients, 3 with infantile and 9 with late onset Pompe disease, we identified 4 novel, potentially pathogenic *GAA* mutations and one pregnancy which was complicated by prenatal exposure to recombinant human rhGAA and spontaneous miscarriage.
- In addition to supportive therapy, rhGAA enzyme replacement therapy (ERT) is now available. Oral chaperone therapy, modified rhGAA, autophagy suppression and gene transfer represent potentially promising novel therapies that are being tested in clinical research trials.

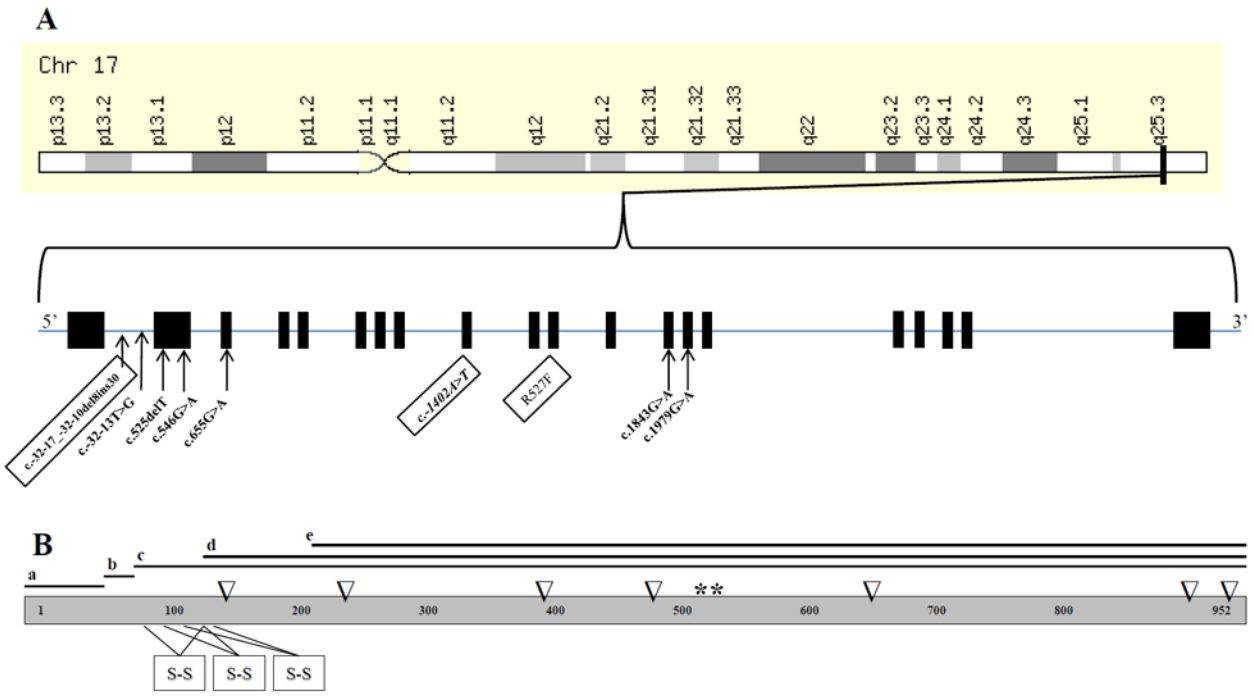

Figure 1.

Schematic representations of *GAA* genomic location and structure (A) and its protein structure (B). *GAA* maps to chr17q25.3 and consists of 20 (19 coding) exons which encode 952 amino acids. Mutations (DNA variants) identified in this study are shown according to their respective genomic position. Novel variants are boxed. GAA has 4 isoforms (a-d), 2 catalytically active sites (*), 3 disulfide bonds (s-s) and 7 N-linked glycosylation sites (∇).

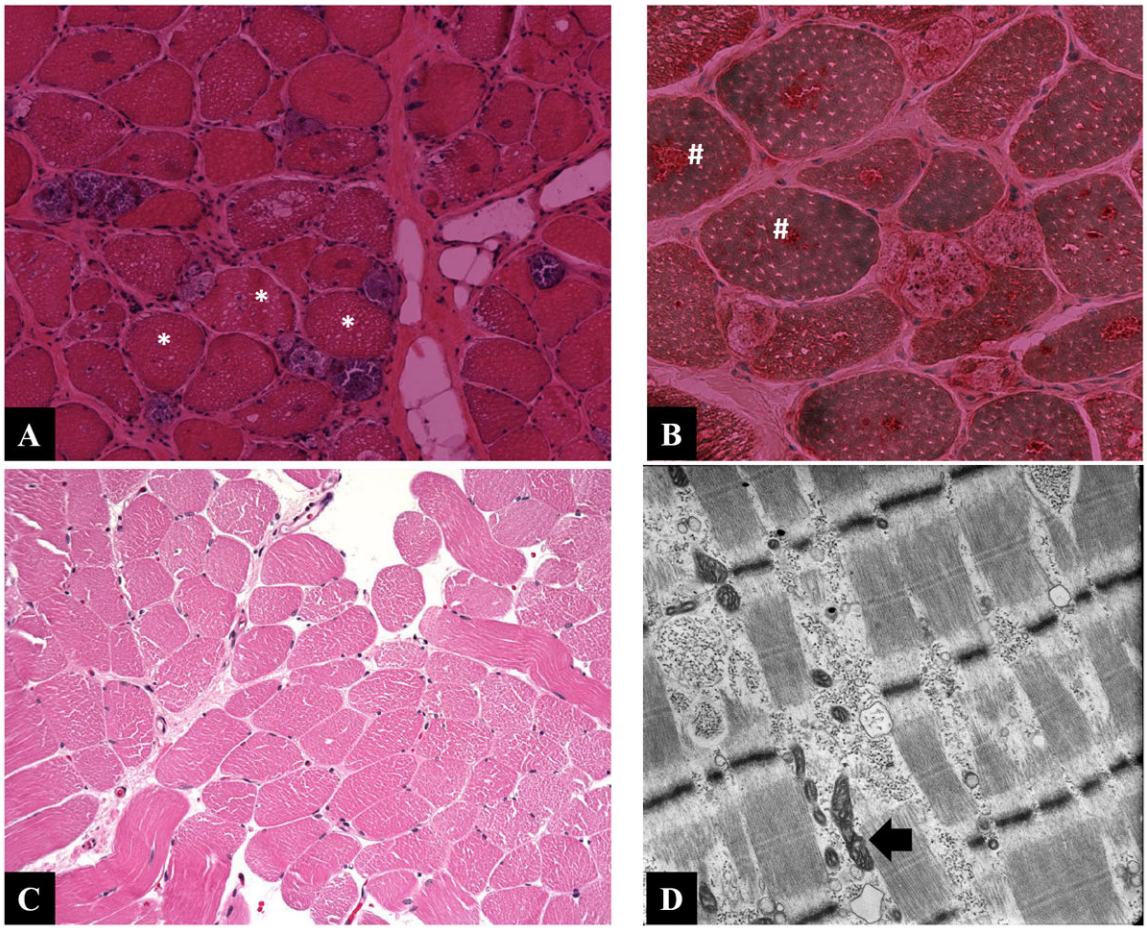

Figure 2.

Histological examination of muscle biopsies of patients 9 (A, B) and 4 (C, D). H&E stained muscle biopsy (A) from patient 9 shows extensive vacuolar changes (asterisk) and positive acid phosphatase aggregates (#) in panel B. The H&E stained muscle biopsy (C) from patient 4 is essentially unremarkable while the muscle electron micrograph (D) showed membrane bound glycogen deposits and mildly distorted mitochondrial morphology.

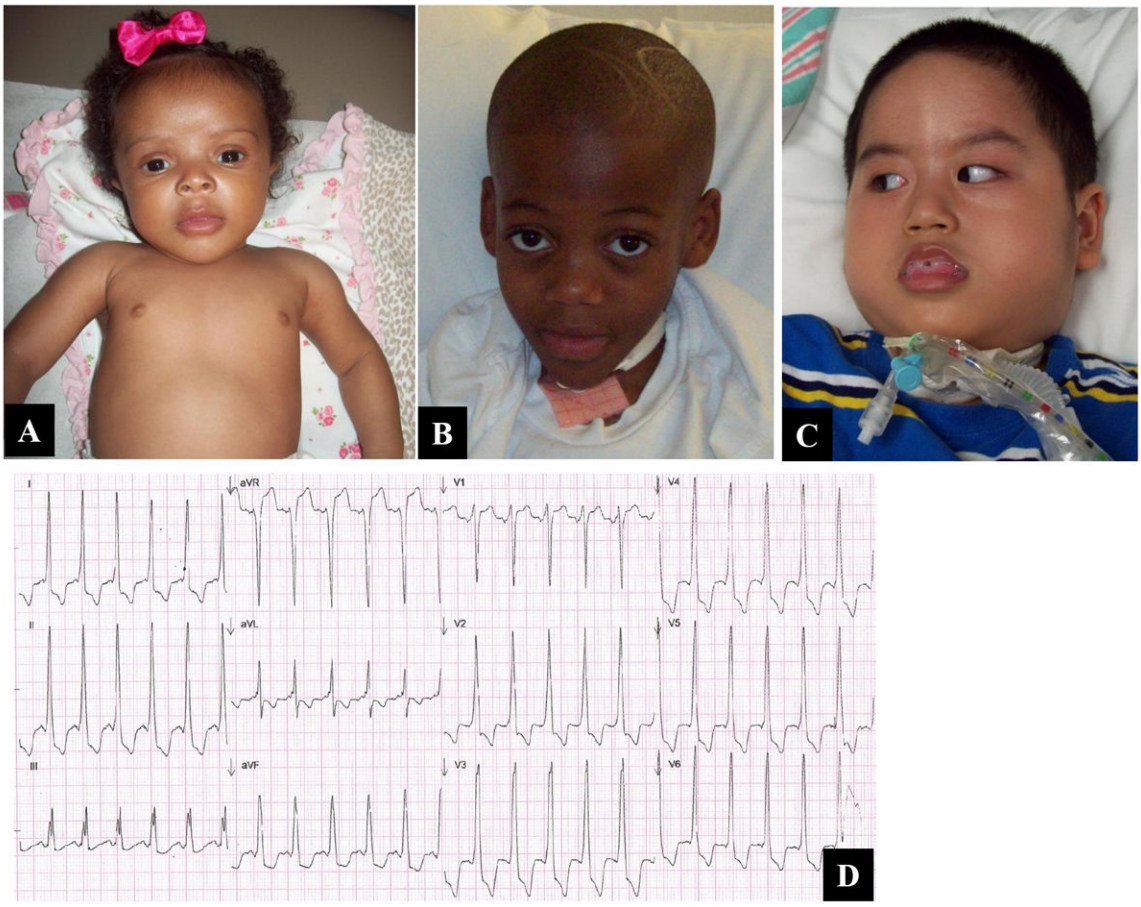

Figure 3.

Photographs of patients with infantile onset Pompe disease. The sib pair (A, B; patients 1 and 2 respectively) have non-classical IOPD while patient 3 (C) has classical IOPD with macroglossia, tracheostomy and severe hypotonia. Abnormal EKG of patient 3 shows sinus tachycardia, short PR interval, ST segment and T wave abnormalities as well as left ventricular hypertrophy.

Table 1

Symptoms characteristics in patients with infantile and late onset Pompe disease.

Patient	Family history	Sex	Manifestation sequence						Presentation		Age		Time from 1st symptom-Dx
			Age (yr)	1st	Age (yr)	2nd	Age (yr)	3rd	Sign / Symptom	Age (yr)	At diagnosis (yr)	At last visit (yr)	
1	yes (brother)	F	0	PD	0	RD	0	MG	PD	0	PD	2	0
2	yes (sister)	M	5/12	SOB	6/12	MY	2	DW	RF	5	7	12	2
3	no	M	2/12	GW	4/12	CM	4/12	MG	GW	4/12	4/12	8.5	2/12
4	no	F	10	My	12	LBP	16	LW	Myalgia	10	19	22	9
5	Yes (brother)	M	15	AW	17	Fatigue	-	-	AW	19	19	20	5
6	yes (brother)	M	15	AW	17	My	17	Fatigue	AW	19	19	25	4
7	no	F	9	LGW	16	My	21	Fatigue	LGW	23	21	26	12
8	no	M	2	LW	4	My	4	LGW	LGW	6	6	34	4
9	yes (sister)	M	38	Weight loss	39	SOB	40	LGW	LGW	40	40	41	2
10	yes (sister)	F	37	My	38	LGW	39	SOB	LGW	40	40	50	3
11	yes (sister)	F	36	LBP	38	LGW	38	SOB	LGW	40	38	53	2
12	Yes (sister)	M	10	LW	30	abn LFT	32	AMW*	AMW	32	32	52	22

Abbreviations: AMW axial muscle weakness, AW arm weakness, CM cardiomyopathy, DW delayed walking, F female, GW generalized weakness, LBP low back pain, LFT liver function test, LGW limb girdle weakness, LW leg weakness, M male, MG macroglossia, MY myalgia, PD prenatal diagnosis, RD respiratory distress, RF respiratory failure, SOB shortness of breath,

* with scoliosis.

Table 2

Summary of neurological motor examination in patients with infantile and late onset Pompe disease at last follow up.

Patient	CK level (IU/L)	Enlarged tongue	Tongue weakness	scapular winging	Trendelenberg gait	Leg Weakness	Arm Weakness	Ankle Weakness	Hand weakness
1	699	yes	no	no	na	+	+	+	+
2	1,173	no	no	no	yes	-	-	-	-
3	889	yes	yes	na	na	+	+	+	+
4	59	no	no	no	no	+	-	-	-
5	529	no	no	no	no	-	+	-	-
6	1,684	no	no	no	no	-	-	-	-
7	1,039	no	no	no	yes	+	+	+	+
8	396	no	no	no	yes	+	+	-	+
9	732	no	no	no	yes	+	+	+	-
10	329	no	no	no	yes	+	+	-	-
11	169	no	no	no	yes	+	+	+	+
12	107	no	no	yes	yes	+	+	-	-

Abbreviations: na not applicable.

Table 3

Respiratory status in patients with infantile and late onset Pompe disease

In this group of patients, 4/12 (33%) needed assistive ventilation including invasive ventilation in the 2 patients with infantile Pompe disease.

Patient	FVC (L)	Ventilatory support (onset in years)
1	-	-
2	0.96	Tracheostomy/5
3	-	Tracheostomy/0.5
4	4.6	-
5	-	-
6	5.08	-
7	3.8	-
8	4.06	-
9	1.7	BiPAP/39
10	3.15	-
11	2.00	BiPAP/48
12	4.14	-

Abbreviations: BiPAP (Bi-Level Positive Air Pressure), FVC forced vital capacity, L liter.

Table 4

***GAA* mutations and enzyme activities in various tissues in patients with Pompe disease**

Four novel (predicted to be pathogenic) were identified including "p.I468F" which was found in 2 unrelated individuals. Amniocytes in patient 1 showed undetectable GAA activity, which predicts severe classical IOPD, while postnatal DBS showed detectable but markedly reduced GAA activity.

Patient	GAA mutation		GAA enzyme activity			Order of diagnostic studies	Maximum anti-Lumizyme IgG antibody titer*
	Alelle 1	Allele 2	Muscle	Skin	DBS (normal)	Ist-2nd-3rd	
1	c.1979G>A (p.R660H)	VUS [c.-32-17_-32-10del8ins30]	nd	nd	<2.5 (>7.5)	Amniocytes GAA activity-DBS-DNA	negative
2	c.1979G>A (p.R660H)	VUS [c.32-17_32-10del8ins30]	0.023 (0.42 ± 0.2)	nd	1.9 (10-49)	MBx-DBS-DNA	negative
3	c.1843G>A (p.Gly615Arg)	c.1843G>A p.Gly615Arg	low	low	0#	DBS-MBx-DNA	1:1600
4	VUS [c.-1402A>T (p.I468F)]	unknown (negative GAA deletion/ duplication)	0.08 (0.42 ± 0.2)	88.77 (260 ± 82.2)	4.8 (10-49)	Skin fibroblast GAA MBx Muscle GAA -DBS-DNA	na
5	nd	nd	nd	nd	2.8 (10-49)	DBS	na
6	c.655G>A (p.G219R), exon 3	c.546G>A (p.T182T), exon 2 (splice site)	nd	nd	2.9 (10-49)	MBx-DBS-DNA	na
7	c.-32-13T>G ((IVS1-13T>G)	Multiple VUS (R527F)	nd	nd	4 (10-49)	MBx-DBS-DNA	1:1600
8	nd	nd	nd	nd	2.85 (10-49)	MBx-DBS	1:1600
9	c.-32-13T>G (IVS1-13T>G)	VUS [c.1402A>T (p.I468F)]	nd	nd	<2.5 (10-49)	MBx-DBS-DNA	nd
10	nd	nd	29.23 (260 ± 82.2)	nd	nd	MBx-Muscle GAA	1:12,800
11	nd	nd	38.54 (260 ± 82.2)	38.54 (177.8-342.2)	nd	MBx-Muscle GAA-Skin fibroblast	1:200
12	nd	nd	17.7 (63-123	nd	nd	MBx-Muscle GAA-DNA	1:1600

Abbreviations: DBS dried blood spot, GAA acid α glucosidase, MBx muscle biopsy, na not applicable, nd not done, VUS variant of unknown significance.

*Anti Lumizyme antibody titers were reported by Genzyme,

#GAA activity was measured in blood lymphocytes (mean 35 nmol/hr/mg protein). Units: GAA enzyme activity in muscle/skin fibroblasts (μmol/min/gm tissue), DBS (pmol/hr/punch).

Table 5

Newborn screening studies for Pompe disease.

Country	Newborns screened	Screening Method/Criteria	Outcome	Ref.
USA-Washington	111,544	MS/MS; cutoff of < 2.60 mmol/h/L (<15% of mean)	4 PD cases (1/27, 800), 3 carriers with an additional pseudodeficiency allele, 6 were heterozygotes for a pseudodeficiency allele only; PPV 0.24; FPR 1/8600	86
	5055	MS/MS; cutoff: < 20% daily mean activity	5 with low GAA activity	87
USA-Missouri	27,724	digital microfluidics	3 PD cases (1/8,657): 1 classic, 1 non-classic IOPD & 1 LOPD; 3 false positive results (carrier status unknown), 1 pseudodeficiency, 2 carriers, 2 pending cases	88
USA-Illinois	8,012	digital microfluidics	2 false positive	89
Taiwan	344,056 (2005-2009)	fluorescence assay, NAG/GAA > 60 & GAA inhibition by acarbose > 80%, 2nd tier: lymphocyte GAA activity < 5% of normal mean & GAA activity in skin fibroblasts, *GAA* sequencing	13 LOPD (1/26,466) & 6 IOPD cases	90
	473,738 (2005-2011)	fluorescence assay, NAG/GAA ratio ≥ 100	9 IOPD & 19 LOPD cases; NAG/GAA cutoff ratio≥ 60 (PPV) of 63.4%	91
Japan	496 healthy controls, 29 PD cases & 5 PD carriers (530 DBS)	GAA activity < 8% of normal mean & % GAA inhibition > 60% and NAG/GAA ratio > 30	5 healthy pseudodeficiency homozygots & 1 obligate carrier	92
Italy	3403	Fluorescent GAA activity; cutoff: < 35% of average control activities	3 cases with low GAA activity (final status not confirmed)	93
Hungary	40,024	MS/MS followed by molecular confirmation	9 PD cases	94
Germany	3251	MS/MS & fluorimetric assays; repeat testing in < 0.5% of DBS samples	No PD cases	95
	944 (symptomatic individuals)		14 PD cases and 8 GAA carriers	
Colombia	4700 (DBS samples from symptomatic, high risk individuals; 3 months – 73 years old))	Fluorometric microfluidic, molecular GAA analysis (some)	16 PD cases	96
Austria	34,736 (January - July, 2010)	ESI-MS/MS	4 confirmed by *GAA* mutation analysis (1/8684). Most *GAA* missense mutations were LOPD; PPV 80%; 1 false positive case (FPR 30 per million)	97

Abbreviations: acid glucosidase (GAA), false positive rate (FPR), infantile onset Pompe disease (IOPD), late onset Pompe disease (LOPD), MS/MS tandem mass spectrometry, neutral alpha-glucosidase (NAG), Pompe disease (PD), positive predictive value (PPV).