The Diagnostic Path to Pompe Disease

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Abstract

Pompe disease (PD) is a lysosomal disease that primarily affects skeletal and smooth muscles due to a deficiency of acid alpha glucosidase. The resulting clinical phenotype reflects a continuum ranging from severe infantile to later onset forms of PD. Early diagnosis is essential to prevent long-term complications and/or disease progression through initiation of enzyme-replacement therapy. The diagnostic path to PD starts with the ascertainment of medical and family history as well as an in-depth clinical and neurologic evaluation. The involvement of proximal muscle groups including hip and thigh muscles as well as the diaphragm is characteristic for later onset PD. Once PD is considered creatine kinase (CK) should be measured, realizing that levels may be within normal limits in end stage disease. Ultimately, diagnostic confirmation is readily achieved by enzyme analysis in dried blood spots and/or leukocytes followed by molecular analysis. Muscle biopsy is not sensitive enough for diagnostic testing of PD but may be an important diagnostic test for the differential diagnosis of myopathies. Future diagnostic approaches include whole exome and genome sequencing, which may contribute to our understanding of genotype–phenotype correlation and phenotype prediction.

Keywords

Pompe disease, alpha glucosidase, glycogen storage disease II, diagnosis, laboratory testing, dried blood spot

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Pompe disease (PD, glycogen storage disease type II, OMIM # 232300) is an autosomal recessive lysosomal storage disease caused by deficiency of acid alpha-glucosidase (GAA) (acid maltase, EC 3.2.1.20) due to mutations in the *GAA* gene.[1] Progressive storage of intra-lysosomal glycogen in skeletal, cardiac, and smooth muscle cells leads to impairment of cellular integrity and function with eventual apoptosis of affected muscle fibers, inflammatory processes, and subsequent replacement through connective tissue.[2] Additional systemic metabolic abnormalities may be observed although their clinical relevance is unknown.[3]

PD represents a continuous clinical spectrum with about one-third of patients presenting during the first months of life or prenatally with hypertrophic cardiomyopathy, muscle hypotonia, and secondary complications, such as respiratory insufficiency, feeding difficulties, and failure to thrive.[1,4,5] The remaining two-thirds of patients present as clinical continuum resembling the clinical picture of a limb girdle muscular dystrophy (LGMD) without cardiomyopathy and with variable age of onset ranging from late infancy to adulthood.[6] The proximal muscle groups including thigh, pelvic, and shoulder muscles are affected while the distal muscle groups are typically spared.[7] The diaphragm in contrast to other genetic causes of LGMD is typically affected in PD contributing to the respiratory insufficiency and CO_2 retention in later onset patients.[6,8] Importantly, asymptomatic patients with PD have been reported.[9] Residual GAA activity correlates with severity of PD, such that infantile onset patients show complete absence of enzyme activity whereas later onset patients retain up to 30 % residual enzyme activity.[8]

PD is a pan-ethnic disease with an estimated frequency of 1:40,000, although recent reports from newborn screening studies suggest that PD may be more frequent.[1,8,10,11] The advent of recombinant human enzyme replacement therapy (ERT) has improved quality of life and reduced morbidity and mortality in the majority of patients with both infantile-onset PD[11,12] and later-onset PD.[13] Recombinant human GAA (rhGAA) is harvested from Chinese hamster ovary (CHO) cells and exerts its activity after proteolytic cleavage in the lysosomes. The uptake of rhGAA is readily achieved via mannose receptors on macrophages of the liver and spleen.[8]

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Diagnostic Approaches

The diagnostic path to late-onset PD may be long and extensive for adult patients presenting with muscular symptoms due to the phenotypic overlap with LGMD. Although the presence of clinical clues have been reported in the medical literature, the predominant and first-presenting symptoms are typically muscular in nature.[14] Early involvement of the diaphragm causing respiratory insufficiency[15–17] as well as cardiac arrythmias[18] or vascular involvement[19] may increase the clinician's suspicion for PD. Since late-onset PD lacks a definite clinical phenotype that allows clinicians to clearly differentiate PD from other LGMDs, it is important for physicians to include PD in the differential diagnosis for LGMD considering the availability of ERT.

A diagnosis of infantile-onset PD is readily suspected in any infant with hypertrophic cardiomyopathy, muscular hypotonia, and moderate elevation of creatine kinase (CK) levels.[1,4,5] Any delay in diagnosis is not acceptable since it is now well established that an early diagnosis and timely initiation of ERT will have a drastic impact on the natural disease course.[12] The delayed start of ERT, even by only a few weeks increases the morbidity in affected infants significantly.[11]

Laboratory Abnormalities

CK levels are a sensitive marker for PD as CK is moderately elevated in all infants with PD and in more than 95 % of adults with PD.[7,8] The remaining 5 % of adults with PD have either very mild disease or, which is more likely, are in the terminal stage of PD with little or no residual skeletal muscle mass. Muscle injury in PD will not only lead to elevations of CK, but also to a release of other muscle enzymes including aldolase, lactate dehydrogenase (LDH), and amino transaminases (AST, ALT). The ratio of AST to ALT in PD is typically close to 1 in contrast to acute rhabdomyolysis where AST/ALT is greater than 3.[20] However, elevated transaminases should not be misinterpreted in the context of PD or other myopathies as being secondary to liver disease.[20,21]

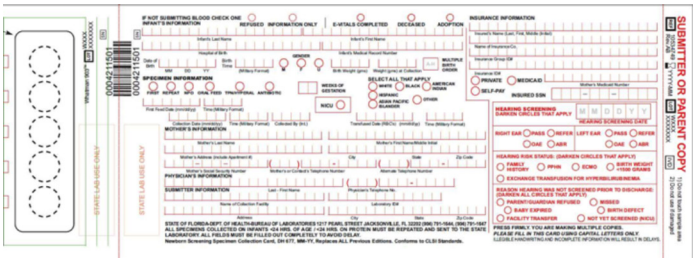
Muscle Biopsy

Muscle biopsies have an important place in the diagnostic workup of myopathies where the differential diagnosis may be extensive. However, if PD is considered in a patient based on the clinical phenotype, family history, and/or elevated CK levels, a muscle biopsy may not be used as the first-line diagnostic test. Instead, analysis of GAA activity is preferred for initial screening due to its short turnaround time and non-invasiveness.[22] In cases of uncertain or conflicting laboratory test results (enzyme and molecular), muscle biopsies as a second-tier test may guide the physician and provide additional valuable information for the differential diagnosis. Physicians need to be aware that up to 25 % of adult patients with PD may have normal muscle biopsies without pathologic storage of glycogen on periodic acid–Schiff (PAS) staining.[22,23]

Analysis of Acid Alpha-glucosidase Activity

GAA activity can be measured in a number of different biological matrices including dried blood spots (DBS), leukocytes, fibroblasts, and/or muscle using either a fluorometric assay[24] or tandem mass spectrometry (MS/MS).[25] The activity of another alpha-glucosidase, maltase-glucoamylase, may lead to false negative test results in cases of PD if not inhibited by acarbose.[26] Addition of acarbose is now standard for all blood-based GAA assays including the ones carried out in DBS.[26,27] DBS are generated ideally from transferring whole blood onto a filter paper (see *Figure 1*) avoiding blood with additives as these may interfere with enzyme analysis (see *Table 1*). Enzyme results are typically returned within 2–4 weeks depending on the individual circumstances, but can be expedited in suspected infantile onset PD.

Figure 1: Example of a Filter Card for Newborn Screening, State of Florida



Note the left hand side of the card including the five circles is made of filter paper to hold the dried blood. The remainder of the card is for identifying information and different parameters needed for newborn screening.

Table 1: Instructions on How to Obtain Dried Blood Filter Cards

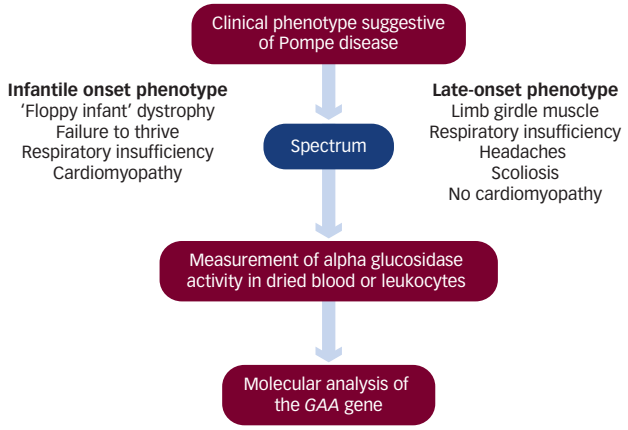
- Complete the identifying information on the filter card
- Transfer whole blood without additives drop by drop onto the filter paper
- Confirm that the blood has fully soaked the filter paper (from front to back)
- Air dry the blood completely (this may take up to 4 hours) without the filter paper touching any surfaces
- Put the filter card into a plastic bag and envelope and mail it overnight to the testing laboratory

Please note that whole blood without additives is the preferred sample type as additives may interfere with acid alpha-glucosidase analysis.

The fluorometric assay is based on the release of fluorescent 4-methylumbelliferone (4-MU) from a specific 4-MU substrate through enzyme cleavage. The measured fluorescence is directly proportional to the enzyme activity. 4-MU substrates are currently available for a number of different lysosomal enzymes including GAA.[24] The fluorometric assays for measuring GAA activity are as sensitive as the MS/MS assays although they lack multiplex capabilities and require an additional test run for analysis of a separate enzyme for quality assurance. Analysis of the latter enzyme is important to assess sample quality and integrity and a separate enzyme should always be included irrespectively of the analytical platform used.[26]

Measuring GAA activity in DBS using MS/MS is a fast and cost-effective way of diagnosing PD and allows multiplexing with additional lysosomal storage disorders for high throughput screening of newborn infants or at risk individuals.[10,25,27,28] The challenges of this approach are the higher initial investment cost for instrumentation and the longer overall analytical time from sample receipt to report of 48 hours compared with less than 24 hours for fluorometric assays.[24,26] The MS/MS method has so far to the best of our knowledge only be validated for enzyme analysis in DBS, but not in other biological matrices. In brief, DBS are extracted and incubated overnight with an assay cocktail including GAA substrate, GAA internal standard, buffer, and acarbose.

Figure 2: Diagnostic Algorithm



GAA substrate and internal standards are optimized for use in MS/MS to yield reproducible mass transitions.[25]

Glucose Tetrasaccharide (Glc4) in Urine

American College of Medical Genetics and Genomics (ACMG) guidelines recommend the measurement of glucose tetrasaccharide (Glc4) in urine for the diagnosis of infantile PD since elevations of Glc4 in urine in conjunction with decreased GAA activity exert close to 100 % sensitivity for infantile PD.[29] Various methods including measurement from urine on DBS have been established. Furthermore, Glc4 excretion in urine may be used for monitoring therapeutic response to ERT. However, Glc4 excretion in urine is not specific to PD but may be found in any condition affecting glycogen metabolism, including other glycogen storage disorders, Duchenne muscular dystrophy, and pregnancy.[30]

Cross-reacting Immunological Material

A subset of infants with the severe infantile form of PD is not able to synthesize any detectable endogenous alpha-glucosidase or cross reacting immunological material (CRIM).[31] These CRIM-negative infants will show increased antibody production in response to exogenous recombinant alpha-glucosidase causing ERT to be ineffective.[32,33] Determination of CRIM status will allow prompt initiation of immune tolerance therapy prior to ERT improving outcomes in CRIM-negative infants.[34,35] CRIM status is determined using Western Blot analysis in cultured fibroblasts, a process which may have take several weeks leading to delayed or ineffective therapies.[28] A blood-based CRIM assay with a turnaround time of less than 72 hours has been established and was published recently.[36] CRIM status may be predicted based on GAA genotypes in the majority of infantile onset PD cases.[31]

Molecular Testing

The *GAA* gene spans 28kb on chromosome 17q25.3 and contains 20 exons. The first exon is not translated.[1,8] The gene encodes a peptide of 952 amino acids with a molecular weight of 110 kD.[1,8] The peptide undergoes significant post-translational modification including glycosylation. To date more than 250 pathogenic mutations and variants in the *GAA* gene have been reported in the Pompe Disease Mutation Database (www.pompecenter.nl) including genotype–phenotype predictions.[37] Common pathogenic mutations include the c.-32-13T>G mutation in Caucasian patients, the p.R854X mutation in African American patients, and the p.D645E mutation in Chinese patients.[8] The p.G576S variant is associated with 'pseudodeficiency' of GAA leading to about 20 % of residual GAA activity.[38] Molecular test results are typically available within 4–6 weeks depending on the individual circumstances, but can be expedited for suspected infantile onset cases. Molecularly confirmed PD is an important pre-requisite for genetic counseling and prenatal testing.

Next-generation Sequencing

The advent of whole exome sequencing (WES) and whole genome sequencing (WGS) provides additional information on variants that may modify the phenotype, predict treatment response and potential side effects, while at the same time identifying pathogenic PD mutations.[39] Challenges for these new techniques include the cost, identification of variants of unknown significance in genes of interest, reduced sensitivity and specificity through variable coverage and read depth, management of large data sets, compilation and curation of results, and consenting and reporting of results to families.[39] To date, no reports have been published on the use of WES or WGS in PD although WES has been recently utilized to identify genetic modifier in Gaucher Disease, highlighting its potential.[40]

Newborn Screening

Taiwan was the first country to implement newborn screening for PD using a fluorometric assay in 2005.[11,41] Until the end of 2011 more than 470,000 infants were screened and nine cases of infantile PD and 19 cases of late-onset PD were diagnosed in addition to a number of individuals who carry the pseudodeficiency alleles.[5,41] Pilot studies in Washington State (US),[42] Austria[10,43] and Hungary[44] revealed comparable incidence rates and distribution between infantile and late-onset PD cases. PD has been recommended by the Newborn Screening Advisory Council for inclusion into the newborn screening panel (personal communication Rodney Howell, University of Miami).

Summary and Conclusions (see *Figure 2*)

- PD is due to intralysosomal storage of glycogen in muscle cells and shows a clinical continuum ranging from infantile onset to later (juvenile and adult) onset.
- Infantile-onset PD is characterized by hypertrophic cardiomyopathy, generalized muscle hypotonia, respiratory insufficiency, and others.
- Late-onset PD is characterized by limb girdle type muscle dystrophy with involvement of the diaphragm. Cardiomyopathy is not observed.
- Diagnosis of PD has to be timely to maximize the benefit of therapy.
- Laboratory abnormalities include moderate elevations of CK and transaminases in the vast majority of cases.
- The diagnostic test of choice is analysis of GAA activity in DBS or leukocytes.
- Molecular confirmation of PD is the prerequisite for prenatal testing and allows some prediction of the clinical phenotype.
- Muscle biopsy is an important test for the differential diagnosis of LGMDs including PD although physicians need to be aware of its limitations.
- Future testing for PD may include next-generation sequencing technology, which will further our understanding of genotype-phenotype correlations. ■

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