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Targeted population screening of late onset Pompe disease in unspecified myopathy patients for Korean population

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Abstract

We performed targeted population screening of late onset Pompe disease (LOPD) in unspecified myopathy patients, because early diagnosis is difficult due to its heterogeneous clinical features. We prospectively enrolled 90 unrelated myopathic patients who had one or more signs out of five LOPD-like clinical findings (proximal weakness, axial weakness, lingual weakness, respiratory difficulty, idiopathic hyperCKemia). Acid alpha glucosidase activity was evaluated with dried blood spot and mixed leukocyte simultaneously. For a final diagnosis of LOPD, 16 patients with decreased enzyme activity were genotyped by *GAA* molecular analysis. We found two patients with LOPD (2.2%), and the remaining 14 patients had at least one G576S or E689K mutation, known as the pseudodeficiency allele. Acid alpha glucosidase activity of LOPD patients was significantly lower than that of patients with at least one pseudodeficiency allele ($p = 0.017$). This study is the first LOPD screening study for targeted Korean population, and more generally, an Asian population. Our findings suggest that for diagnosis of LOPD in Asian population, modified cutoff value of acid alpha glucosidase activity with dry blood spot considering that of patients having heterozygote pathogenic variants or pseudodeficiency alleles may reduce time and cost requirements and increase the comfort of patients.

Keywords: Late onset Pompe disease; Screening study; Unspecified myopathies; Pseudodeficiency

1. Introduction

Pompe disease (OMIM 232300, glycogen storage disease type II) is a rare autosomal recessive metabolic disease caused by deficiency of the lysosomal enzyme acid-α-1,4-glucosidase (GAA) [1]. Pompe disease can be classified as either infantile onset Pompe disease (IOPD) or late onset Pompe disease (LOPD), based on the age of onset and clinical phenotype. Patients with IOPD present with generalized hypotonia, early hypertrophic cardiomyopathy, and respiratory failure, due to massive glycogen storage in cardiac, skeletal, and smooth muscles, and die within the first year of life [2]. In contrast, patients with LOPD mostly present with progressive limb-girdle muscular weakness and respiratory insufficiency without cardiomyopathy [3].

In comparison to those with IOPD, patients with LOPD tend to present with a more heterogeneous age of onset and symptoms and signs that overlap with other neuromuscular disease [4]. Furthermore, diagnostic difficulties can be encountered in LOPD, because some of patients with LOPD may be associated with normal serum creatine kinase levels, unremarkable electromyographic findings, or even nonspecific histological features on muscle biopsy [5]. Consequently, diagnosis of LOPD tends to be 6–7 years after disease onset, resulting in a delayed treatment and poor outcome [6,7].

There were several reports demonstrating the consistent clinical benefits of ERT in both IOPD and LOPD [8,9], as well as reports confirming early diagnosis and treatment with newborn screening [10]. Additionally, the development of easy and rapid diagnostic test, such as GAA activity test with dried blood spot (DBS), has allowed a nationwide or multi-centered screening of Pompe disease in a few countries.

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So far, the estimated prevalence of Pompe disease is considered as different from country to country, and had been reported, as 1 in 33,333 (Taiwan) [11], 1 in 600,000 (Northern Portugal) [12], and 1 in 40,000 (Netherlands [13] and New York [14]). Meanwhile, in Korea, there have been only a few published reports on Pompe disease. Those reports include studies on the clinical manifestations and pathogenic variants of *GAA* gene [15–17], the treatment response of IOPD [17–19] and LOPD [20], and the usefulness of tandem mass spectrometry for diagnosis of Pompe disease and five other lysosomal storage diseases [21]. To date, more than about 37 Pompe disease patients (IOPD and LOPD) have been treated with recombinant human alglucosidase alfa (Myozyme®, Genzyme, Cambridge, MA). The previously reported and the most common pathogenic variant of LOPD and IOPD patients in Korea is c.1316T>A (p.439M>K) variant [15,16]. However, the study about the prevalence or screening of Pompe disease in large scale has not been performed in Korea. Furthermore, in Asia, there have been no reports about LOPD screening study in high risk group patients.

Therefore, we conducted a LOPD screening study in a targeted group of unspecified myopathy patients. The main purpose of this study was to identify the frequency of Pompe disease among the group of patients with unspecified myopathy in Korea. In addition, we also tried to conduct a clinical profiling of LOPD in Korea to determine the effects of pseudodeficiency alleles and to identify better methods of diagnosing LOPD and differentiating false positive patients.

2. Methods

2.1. Patient's enrollment and clinical data review

Ninety unrelated patients with unspecified myopathy were prospectively recruited from June 1, 2013 to August 31, 2014 in two neuromuscular centers in Korea (Gangnam Severance Hospital and Pusan National University Yangsan Hospital).

Inclusion criteria were: (1) age at onset ≥ 12 months old; (2) clinically diagnosed as myopathy, having one or more signs among these five LOPD-like clinical findings (a. proximal muscle weakness, b. axial muscle weakness, c. lingual weakness, d. respiratory difficulty, e. idiopathic persistent hyperCKemia); (3) patient or patient's legal representatives, including parents, could understand the oral or written informed consent. Exclusion criteria were: (1) diagnosis or suspected diagnosis of another neuromuscular disorder genetically or pathologically; (2) autosomal dominant or X-linked inheritance patterns. Informed consent was obtained from all patients, or from legal representatives, including parents, if patients were underage. Clinical history and laboratory data were collected in a Case Report Form (CRF).

2.2. Standard protocol approvals, registrations, and patient consents

Oral and written informed consent was obtained from each patient prior to blood sampling. This study was conducted according to the Declaration of Helsinki and was reviewed and approved by the Internal Review Board at Gangnam Severance Hospital of the Yonsei University College of Medicine (No. 3-2013-0085) and institutional review board at Pusan National University Hospital (No. 04-2013-014).

2.3. Acid alpha-1,4-glucosidase activity assay

GAA enzyme activity was analyzed by two different methods simultaneously: assays using dried blood spot (DBS) and mixed leukocyte (ML) using venous blood. When the patient showed a decreased GAA activity, genetic analysis was performed for the *GAA* mutations.

For GAA activity assay, we used 4-methyl-umbelliferyl-α-D-glucopyranoside (4MUG) as the main substrate for measuring enzyme activity. Acarbose is used to inhibit the interfering α-glucosidase activity of maltase-glucoamylase [22]. Enzyme activity was expressed as nanomoles 4-methylumbelliferone released per punch per hour (nmol/mg protein/hr) for DBS assay and nanomoles 4-methylumbelliferone released per milligram cellular protein per hour (nmol/mg protein/hr) for ML assay.

For DBS assay, a 3-mm sized punch of DBS filter paper was placed in a well of a 96-well white microtiter plate. 40 μL of prepared 4MUG (1.40 mmol/L, pH 4.00) was added with either 40 μL of acarbose (6 μmol/L) or 40 μL of distilled water. After shaking for 15 minutes, the plate was incubated at 37 °C for 20 hours. The reaction was quenched with 200 μL of 0.15 mol/L EDTA at pH 12 with vortex for 1 minute, and fluorescence intensity was measured with a Perkin Elmer LS 55 luminescence spectrometer at excitation and emission wavelengths of 360 and 450 nm, respectively.

GAA activity using the ML method with acarbose was performed as previously reported [23], with minor changes. Briefly, we prepared diluted leukocyte homogenates (2.0 mg/mL) using heat-inactivated 0.2% bovine serum albumin diluent buffer from patients' whole blood. 10 μL of the diluted leukocyte homogenates (2.0 g/L) was added to 60 μL of the substrate solution containing 1.47 mmol/L of 4MUG in 0.1 mol/L citrate and 0.2 mol/L sodium-phosphate buffer at pH 4.0 with and without 3.5 mmol/L acarbose (3.0 μmol/L acarbose in the final reaction mixture) in a 96-well white microtiter plate. The reaction mixture was then incubated at 37 °C for 2 hours, and the reaction was stopped with the addition of 200 μL of 0.4 mol/L glycine with NaOH buffer at pH 11.9. Fluorescence intensity was measured with a Perkin Elmer LS 55 luminescence spectrometer at excitation and emission wavelengths of 355 and 460 nm, respectively.

With the above method, GAA activity was measured with 4MUG without acarbose (total GAA, tGAA) and 4MUG with acarbose (true acid-α-1,4-glucosidase, GAA). The ratio of GAA activity was calculated as 'GAA/tGAA'.

In our study, we defined a 'decreased GAA activity' when either absolute level of GAA or GAA/tGAA ratio is low in both DBS and ML (Fig. 1A). Reference ranges were set as normal mean ± 2 standard deviation and were as follows (normal means were calculated with previous data of normal controls in Kim Sook Za's Children Hospital); DBS: tGAA: 4.50–27.15 nmol/punch/hr, GAA: 1.90–7.45 nmol/punch/hr, ratio: