Glycogen-storage disease type II, also named Pompe disease, is caused by the deficiency of the enzyme acid alpha-glucosidase, which originates lysosomal glycogen accumulation leading to progressive neuromuscular damage. Early-onset Pompe disease shows a debilitating and frequently fulminating course. To date, more than 300 mutations have been described; the majority of them are unique to each affected individual. Most early-onset phenotypes are associated with frameshift mutations leading to a truncated alpha-glucosidase protein with loss of function. Founder effects are responsible for many cases from few high-prevalence world regions. Herein we described two apparently unrelated cases affected with classical early-onset Pompe disease, both pertaining to a small region from Central Mexico (the State of San Luis Potosí), with the same novel homozygous frameshift mutation at gene $\text{GAA}$ (c.1987delC), identified by complete gene sequencing.

Key words: Early-onset Pompe disease, Acid maltase deficiency, Founder effect

Glycogen storage disease type II (Online Mendelian Inheritance in Man, OMIM, accession number 232300), also called Pompe disease, was described by Johannes C. Pompe in 1932. The disorder is caused by a deficiency of the enzyme alpha-glucosidase (acid maltase, EC 3.2.1.20, Swiss) which originates lysosomal glycogen accumulation leading to lysosomal swelling, cellular damage and dysfunction (1-3). Affected individuals develop progressive neuromuscular damage, showing a debilitating and frequently fulminating course on the classical, early-onset type of the disease. Other main findings are hypertrophic cardiomyopathy, hypotonia, hepatomegaly, macroglossia, feeding problems and breath difficulty. Currently it is recognized that the late form of Pompe disease has a very variable phenotype that can be confused with a wide range of neuromuscular, pulmonary and cardiovascular diseases with mild, moderate or severe symptoms that present either alone or combined (4-6).

Pompe disease has an autosomal recessive inheritance and it is caused by more than 300 mutations that occur all over the gene coding for acid alpha-glucosidase (GAA) located at locus 17q25.2q25.3. The molecular phenomenon responsible of the different types of clinical expression occur by the presence of two either homozygous or compound heterozygous pathogenic mutations in early-onset Pompe cases, whereas late-onset Pompe have one variant and one pathogenic mutation (7). The majority of disease-causing mutations are unique; nonetheless, relatively frequent mutations have been described in certain populations with a possible founder effect traced from the original mutated carrier to the newly occurring cases. Affected cases have been described worldwide with a few high-prevalence regions like South-Africa, Taiwan and Holland (1, 8-10).

Herein, we described two unrelated cases affected with classical early-onset Pompe disease, both pertaining to the same small Mexican region, with the same novel homozygous frameshift mutation at gene GAA (c.1987delC), identified by complete gene sequencing.
**Report of cases**

**Case 1**

A 6 month-old boy was referred to our institution from his community hospital due to a febrile disease, productive cough and respiratory distress during a week without response to infection treatment. On physical examination he was found with heart failure, hepatomegaly and severe cardiomegaly. He was the first child born to young, healthy and presumably unrelated parents. The baby was obtained by uncomplicated vaginal delivery, with normal birth weight (3,400 g). Soon after birth the mother noticed perioral cyanosis during breast feeding. Two previous hospitalizations due to pneumonia were recorded. Motor development was delayed, head control or sitting position was not reached; however he was able to place objects in his mouth, smile at parents and follows adult gaze. At our center the patient received evaluation by the pediatric cardiologist, who found a systolic murmur grade II-III, reinforcement at tricuspid focus, and pulmonary auscultation with fine generalized crackles. Abdominal exam showed hepatomegaly. A radiogram showed enlarged heart and liver (Fig. 1A, B). EKG showed an inverted T wave from V4-V6 as well as AVF, suggesting left systolic overload. The echocardiogram showed a prominent biventricular hypertrophy, with an ejection fraction of 52%, and thus, severe hypertrophic cardiomyopathy was diagnosed. On the neurological exam he showed a weak cry, profound muscle weakness, during traction of the patient from a supine position the head control was completely absent, and both legs remain in a position of profound hypotonia. Three weeks later the child died due to heart failure. Postmortem histologic examinations showed glycogen accumulation in heart, liver and skeletal muscle (Fig. 1C-E).

**Case 2**

A 7-month-old boy with history of repeated respiratory infections since the age of 3 months was referred to our institution. He was the first child of healthy unrelated parents. He was initially admitted at his local clinic and days later referred to our institution due to fever, productive cough, respiratory distress and heart failure. On admission we found pale skin and teguments, severe generalized hypotonia, macroglossia, pulmonary crackles, right basal hypoventilation and hepatomegaly. EKG and echocardiogram showed signs of biventricular hypertrophy, severe systolic and diastolic dysfunction with ejection fraction of 40%. The patient worsened rapidly and died within few hours after admission.

**Biochemical and molecular studies**

**Case 1** and **Case 2** had mildly elevated serum creatine kinase (CK) to 386 and 650 IU/L, respectively. In **Case 1** alpha-glucosidase activity was decreased (1.08 nmol/L; normal range: 1.5-10 nmol/L) on dried blood spots (DBS) tests performed at the University Medical Center Hamburg-Eppendorf. In **Case 2** the alpha-glucosidase DBS assay performed at Duke University was 0.6 pmol/L, also below normal levels (normal range: 10.0-49 pmol/L).

Whole exon and exon-intron boundaries direct sequencing revealed a homozygous single base deletion c.1987delC in both cases, and the same heterozygous mutation in both parents of **Case 2** (Fig. 2). This frameshift muta-
Novel Pompe mutation in Mexicans

and fatal outcome before the age of 1 year. A number of conditions affecting this age group may have similar findings including metabolic and non-metabolic neuromuscular disorders. A systematic multistep approach is recommended to reach a definite diagnosis, starting with a complete general and neurological examination followed by the measure of CK serum activity. Immediately after this initial approach it is suggested to store blood samples for DBS and leucocytes to perform alpha-glucosidase enzymatic assay and DNA testing as necessary. The diagnostic approach must continue through careful electrophysiological or pathological investigations.

Sometimes clinical findings are enough to rule out other neuromuscular disorders of the infant. Cardiomyopathy discards Werdnig-Hoffman disease (OMIM #253300) and some congenital myopathies, while lactic acidosis support the diagnosis of cytochrome C oxidase deficiency (OMIM #220110) (2). Other glycogen-storage diseases such as phosphorylase B kinase deficiency (glycogenosis type VII, OMIM #232800) shows early and severe cardiomyopathy without liver or muscle involvement. Andersen disease or glycogenosis type IV (OMIM #232500) highly resembles the phenotype of early-onset Pompe disease and the distinction between both disorders is made by muscle biopsy or enzymatic assay (11). Danon disease (OMIM #300257) also has many of the Pompe manifestations; however, Danon disease is an X-linked disorder and the presence of mental retardation is the distinguishing feature between both conditions (12). The treatment is a true challenge, the heart failure and the pulmonary symptoms need to be aggressively treated until the diagnosis is confirmed. Then, the enzymatic replacement therapy must be immediately started (4).

Before the development of the enzymatic assay for alpha-glucosidase, the diagnosis was classically made by muscle biopsy, being the enormous amount of glycogen storage in all muscular fibers, heart muscle and hepatocytes the most remarkable finding. Nowadays, the measure of alpha-glucosidase activity in DBS followed by alpha-glucosi-
dase activity in lymphocytes or fibroblasts confirms the enzyme deficiency, and peripheral leukocytes DNA sequencing of the GAA gene is the preferred method for documenting the responsible mutation. Our cases started at a quite similar age of onset with a rapid worsening of the heart failure and respiratory distress, dying within the first months of life. The enzyme deficiency was present in both of our cases showing the very low enzymatic activity associated with classical Pompe disease. We also demonstrated a clearly pathogenic GAA mutation.

The July 1st, 2011 version of the Pompe disease mutation database at www.pompecenter.nl contains a list of 393 sequence variations in the GAA gene, 257 of which are confirmed to be pathogenic. They are spread all over the 19 coding exons. We found that our patients turn to have the same GAA genotype with a novel single base deletion that disrupts the reading frame and result in the introduction of a premature stop codon. The trinucleotide code is altered by the shift, and a different type and order of amino acids is assembled from the point of deletion. The highest percentage of pathologically severe amino acid substitutions is found in the catalytic barrel of the GAA protein (c.1039–c.2454). This mutation herein described provides further evidence of the previous observation that early-onset Pompe disease is caused by truncating mutations (13), while the late-onset Pompe disease usually retains some enzymatic function that fluctuates around 1% to 40% with at least one variant or missense mutation with relatively little effect over the protein function and structure (1, 4).

The worldwide Pompe disease frequency is estimated from 1 per 250,000, to as high as 1 per 14,000 newborns (8, 14-16). The two cases described here came from two small communities from the Center of Mexico (San Luis Potosí State) with less than 1000 inhabitants. The finding of these cases from the same region with the same novel mutation suggests a possible founder effect. Pompe patients usually have their own private mutation, but as with other single-gene diseases, the common mutations have been traced to common ancestors. The best documented example is the mutation, c.2560C>T, that also results in a truncated protein (p.Arg854X) (10). This sequence variation was traced back to a small village in North Africa and has spread through migration along the West-African coast to Namibia. Becker et al. (10), considered that this mutation was brought to the Americas by the slave trade. The c.1935C>A mutation which leads to a amino acid substitution (p.Aspa645Glu) is the second well-known example of a founder mutation. This mutation has a high frequency in Taiwan and along the coast of China (14, 9). The third founder mutation to be distinctly mentioned is c.2481+102_2646+31del (deletion of exon 18), which is common in some subsets of the Caucasian population (24-26). By far, the most frequent GAA mutation among Caucasian children and adults with Pompe disease is the well-known c.-32-13T>G (13, 17, 27).

Two sequence changes are even more frequent among Asian populations: c.1726G>A and c.2065G>A (17-21). These two non-pathogenic sequence variants are most often found together on the same allele, and an estimated 3.3%–3.9% of people in Asian populations are homozygous for both variants (18, 22). Individuals carrying this allele can have a very low alpha-glucosidase activity, as low as fifty percent and can be difficult to distinguish from individuals with Pompe disease in newborn screening programs, but they do not manifest a Pompe disease phenotype (16, 19, 22, 23). Few other pathogenic sequence variations occur in certain populations with higher frequency than expected, but the large majority of mutations in the GAA gene are either unique or very rare (7).

In conclusion, to the best of our knowledge, these are the first published Mexican patients with early Pompe disease who harbor a novel mutation (c.1987delC) with a possible founder effect.

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