

Case report

First clinical and genetic description of a family diagnosed with late-onset Pompe disease from Costa Rica

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Abstract

Glycogen storage disease type II, also known as Pompe disease, is an autosomal recessive disorder caused by deficiency of enzymatic activity of acid alpha-glucosidase. The wide phenotypical variation of this disease relates to the amount of residual enzymatic activity depending on the combination of mutations on each allele. We confirmed Pompe disease in a patient that presented with progressive weakness, recurrent episodes of respiratory failure associated with pneumonia, a predominantly demyelinating mixed sensorimotor polyneuropathy and paraspinal complex repetitive discharges. Genetic analysis of the *GAA* gene from this patient revealed two pathogenic compound heterozygous mutations: c.-32-13T>G (rs386834236, intronic), c.2560C>T (rs121907943, p.Arg854Ter); and one variant of unknown significance: c.1551+42G>A (rs115427918, intronic). We found expected mutations in two siblings and two nieces. Genetic variants reported in this family reflect on the European and African ancestry that we carry in our Costa Rican population.

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1. Introduction

Glycogen storage disease type II (GSD II), also known as Pompe disease, is an autosomal recessive disorder caused by deficiency of enzymatic activity of acid alpha-glucosidase (GAA). The reduced activity of this enzyme leads to the intralysosomal accumulation of glycogen in most tissues, including liver, skeletal and cardiac muscle [1]. It can present as an infantile form (IOPD) with failure to thrive, severe axial weakness, hypertrophic cardiomyopathy and rapid death [2]. Moreover, there is also a late-onset form of Pompe disease (LOPD) that presents after 1 year of age, usually beyond the first decade of life, it progresses in a gradual manner affecting mainly the skeletal muscles, and

most often involves the diaphragm and leads to respiratory failure with the need for ventilator support [3].

The wide phenotypical variation of this disease only relates in part to the amount of residual GAA activity and to the combination of mutations on each allele. Thus, for LOPD the genotype–phenotype correlation becomes less evident and patients with the same set of mutations can present with a different phenotype and at varying ages [4]. Moreover, mutations associated with a large reduction of GAA activity will manifest earlier in life with a more severe onset of disease, while mutations that leave a higher residual GAA activity will present later with a less severe course of disease [5]. The *GAA* gene comprises 20 exons and is localized in chromosome 17q25.2–q25.3; it encodes a polypeptide of 952 amino acids and a 27 amino acid signal peptide [6,7]. This is a highly polymorphic gene, and to date more than 550 variations have been described; however, only close to 375 are considered pathogenic (www.pompecenter.nl – updated April 2017). This is the first description of a clinical

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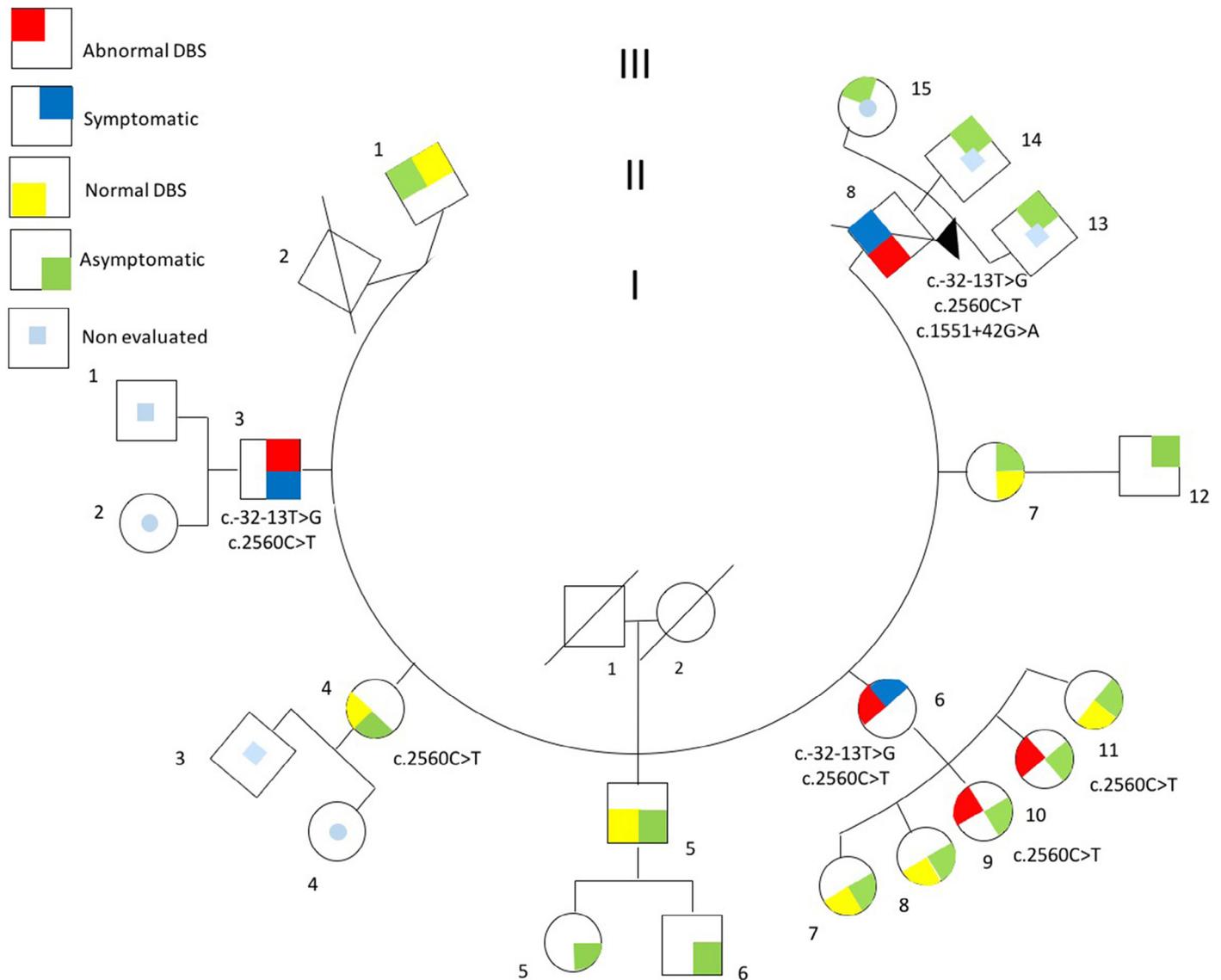


Fig. 1. Genealogy for LOPD patients in a family from Costa Rica. (Pedigrees were constructed and drawn using Progeny Clinical Version N/Progeny Lab Version N (modified from Progeny Genetics LLC, Delray Beach, FL, www.progenygenetics.com).

and genetic characterization of a family with Pompe disease from Costa Rica. We obtained the informed consent from all of the family members evaluated and the Ethics Committee from the Institutional Review Board of our hospital approved this report. Additionally, genetic variants found in our patients as well as their phenotypes were registered in a public open access genetic variation database (http://chromium.lovd.nl/LOVD2/home.php?select_db=GAA), following recommendations established in the 2006 Human Variome Project meeting [8].

2. Case report

A 50-year-old man (subject II-8, see Fig. 1) was admitted to a main hospital of Costa Rica (Caja Costarricense de Seguro Social) due to a two-year history of dyspnea, dysphagia and progressive muscle weakness. He had been hospitalized before, on three occasions, in the Critical Care unit because of respiratory failure associated with recurrent episodes of pneumonia. In his

last hospital admission, he received a tracheostomy and was fully dependent on a ventilator. His medical history was remarkable for hypertension, type II diabetes mellitus and dyslipidemia. When this patient was initially referred to us, he was receiving intravenous gamma-globulin therapy because a chronic inflammatory demyelinating polyneuropathy (CIDP) was considered as the most probable diagnosis. Physical examination revealed a thin man, unable to speak due to a tracheostomy but fully conscious, alert and able to follow orders. On neurological examination, cranial nerves were normal while motor examination showed a symmetrical 2/5 proximal and 4/5 distal strength, with muscle atrophy in his four limbs. Bilateral biceps, triceps, patellar and ankle jerk reflexes were all absent, as well as Babinski and Hoffmann signs. On exploration, there was no myotonic phenomenon and coordination was normal. The patient referred allodynia in the soles and distal part of both legs, and was not able to stand or walk. Cardiac examination was unremarkable, while pulmonary

evaluation revealed bilateral reduced breath sounds. Laboratory studies documented a creatine kinase (CK) of 132 IU/L (normal values, 25–90 IU/L) and an aspartate aminotransferase (AST) of 46 IU/L (normal values, 8–40 IU/L). Electrocardiography (ECG) did not show any alteration; echocardiography was not performed on this patient. Needle electromyogram reported denervation and spontaneous activity with complex repetitive discharges on muscles associated with cranial nerves and within cervical, thoracic and lumbar areas. Nerve conduction studies revealed a predominantly demyelinating, mixed sensorimotor polyneuropathy, with absence of F waves. We did not perform a muscle biopsy on the patient; however, as part of our routine diagnostic approach, we tested for Pompe disease on dried blood spot (DBS) analysis that confirmed an alpha-glucosidase reduced activity (Neutral/acid: 73.0, reference: <30). Genetic analysis of the *GAA* gene revealed two pathogenic compound heterozygous mutations: c.-32-13T>G (rs386834236, intronic), c.2560C>T (rs121907943, p.Arg854Ter); and one variant of unknown significance: c.1551+42G>A (rs115427918, intronic). After a long hospitalization this patient was discharged home with a tracheostomy and continuous ventilator support; however, six months after, he was admitted again and died from a respiratory failure due to a pulmonary infection.

Soon after confirming Pompe disease in this patient, we started studying his family. Both of his parents passed away years before and he had an older brother (*II-2*) that died in an accident. Nonetheless, we were still able to assess the rest of his six siblings with DBS and *GAA* genotyping. We found an altered DBS on two of his older siblings (*II-3* and *II-6*) and *GAA* mutations in three of them (*II-3*, *II-4* and *II-6*). Subject *II-6* was, at the time of the assessments, 56 years old and her past medical history was relevant only for hypertension treated with beta-blockers. She referred a one-year history of proximal weakness, with occasional cramping in both of her lower limbs. Physical examination revealed no alteration in cranial nerves; however, her voice sounded particularly nasal despite she did not complain of dysphagia or dysphonia. She presented a mild scapular winging and there was slight muscular atrophy on temporal, deltoids and thigh muscle extensors. Muscle strength was 5/5 proximal and distal for upper limbs, while 4/5 on hip extension but 5/5 on distal strength on both lower limbs. Abdominal muscle weakness was clinically evident and she did not have a positive Gower's sign. Biceps, triceps, patellar and ankle jerk reflexes were all diminished, with no Babinski or Hoffmann signs present. Pain, temperature, vibration and proprioception were preserved as well as her coordination. She had a mild lordosis, and despite her weakness was not severe, she showed a rather notorious waddling gait. CK levels were 86 IU/L (normal values, 25–90 IU/L) and AST was 33 IU/L (normal values, 8–40 IU/L). We performed electromyography that confirmed a myopathic pattern with reduced duration and amplitude of motor unit action potentials (MUAPs) specifically on thigh extensors; in spite of that we did not find paraspinal complex repetitive discharges, as we were able to observe on her brother. We confirmed on her a reduction in alpha-glucosidase activity (Neutral/acid: 61.0, reference: <30) and her *GAA* gene sequencing showed the two same pathologic heterozygous

compound mutations found on our first subject (*II-8*) (c.-32-13T>G (rs386834236, intronic) and c.2560C>T (rs121907943, p.Arg854Ter)). However, she did not have the third variant found on subject *II-8* (c.1551+42G>A (rs115427918, intronic)). We were also able to take a muscle biopsy from her thigh extensors but we did not find any alteration on the tissue specimen analysis using hematoxylin and eosin (H&E), Gomori trichrome and Periodic Acid–Schiff (PAS) method staining. As previously mentioned we identified one older and symptomatic brother (*II-3*), whom we considered to have a patient status, where we found reduced alpha-glucosidase activity on DBS assessment and the same pattern of mutations seen on the sister described above (*II-6*): (c.-32-13T>G (rs386834236, intronic) and c.2560C>T (rs121907943, p.Arg854Ter)).

Further analysis on the rest of the siblings revealed one sister (*II-4*) to have a carrier status, considering that she was clinically asymptomatic and that she also had normal values for alpha-glucosidase activity on DBS analysis but she was heterozygous only for the c.2560C>T (rs121907943, p.Arg854Ter) pathogenic mutation on *GAA* genotyping. Moreover, after the death of subject *II-8*, both subjects *II-3* and *II-4*, as well as their children, refused on participating on any other test or evaluation. We ascertained that the children of subject *II-8* were asymptomatic during one first clinical examination, yet afterward they also refused to be tested with DBS and *GAA* genotyping.

Only the five daughters of our subject *II-6* agreed on having DBS as well as *GAA* sequencing evaluations. Here we found that even though all of the daughters were asymptomatic, only two of them (*III-9* and *III-10*) had marginally altered DBS reports (Neutral/acid: 36.0 and 38.1, reference: <30) and both of them also carried one of the mutations (c.2560C>T (rs121907943, p.Arg854Ter)) already identified on their mother. Despite both of them having borderline results on one single DBS assessment, we have still considered them to have a carrier status in view of that they have remained asymptomatic until now.

Currently, none of the already diagnosed patients have treatment with enzyme replacement therapy (ERT) because it is still not provided by our public health care system.

3. Discussion

This is the first clinical and genetic description of Pompe disease in a family from Costa Rica. We found two pathogenic mutations and one genetic variant of unknown significance.

We decided to test for Pompe disease in our index patient (*II-8*) considering the repeated episodes of respiratory failure associated with pneumonia that he had presented; added to the progressive weakness and paraspinal complex repetitive discharges found on neurophysiologic evaluations. These three features that he showed are classically described in association with LOPD and should raise awareness for this diagnosis whenever present in a patient [9]. Unlike other neuromuscular diseases, respiratory insufficiency seen in LOPD is mainly due to diaphragmatic weakness and may precede loss of ambulation. This condition leads to sleep disorders, ineffective clearance of airway secretions, recurrent pulmonary infections and eventually

may contribute as a main cause for early death [10]. Nonetheless the absence of respiratory complaints should not guide the clinician away from the diagnosis, as these symptoms may not always be present [9]. The findings of complex repetitive discharges predominantly over paraspinal muscles have been described before [11,12]. In our first patient we found paraspinal complex repetitive discharges in cervical, thoracic and lumbar levels; however, it is possible not to find this phenomenon on every spinal level [9].

An interesting feature found in our first patient was the concomitant presence of a predominantly demyelinating sensorimotor polyneuropathy. Polyneuropathy has been described before [13,14] to be associated with LOPD and it has been explained to be due to glycogen accumulation in neurons and Schwann cells leading to a damage to the myelin sheath with an eventual axonal injury. Neuropathic symptoms can be seen to begin at the same time when the weakness becomes evident or they can even precede weakness and be present as an initial sign of the disease. Main manifestations described, as a result of peripheral nerve involvement include, sensory disturbances such as allodynia, burning pain and loss of pinprick sensation and autonomic derangements. All of them suggest essentially a small fiber neuropathy compromise [14]. In our case, because we rather identified a large fiber impairment instead of the small fiber neuropathy (before mentioned to be in association with LOPD), we considered the observed polyneuropathy to be likely related to the underlying diabetes mellitus as well as to the repeated and prolonged hospital admissions the patient had before we first assessed him, and not precisely to the underlying Pompe disease.

As mentioned before, Pompe disease manifestations and their severity relate to the residual GAA activity observed. Furthermore, this residual activity is primarily determined by the severity of the pathogenic mutations in both *GAA* alleles and is likely controlled by unknown secondary genetic and non-genetic factors that eventually contribute to the Pompe disease phenotype [15,16].

One of the mutations that we found in our first subject (*II-8*) and later in two other symptomatic siblings (*II-3* and *II-6*), c.-32-13T>G, also known as IVS1-13T>G, was first described in 1994 by Hirschhorn et al. and it is the most common mutation reported in Caucasian LOPD population. It is considered to have been mainly spread out through a founder effect, with a reported frequency of 34–47% among LOPD patients [16–18]. This variant corresponds to a “leaky” splice site mutation in intron 1 of *GAA* that leads to an impaired splicing of exon 2; this reduces the GAA synthesis but allows for the production of a 10% to 20% of normally processed mRNA rendering for some residual enzyme activity [17–19]. Even though there is no clear genotype–phenotype correlation established on patients that harbor this mutation, the course of the disease is seen to be slow and progressive in general [18,20]. However, regardless if it has been considered as a potentially mild mutation and therefore it would be expected to be associated with a milder course of disease, it has also been shown to have a broad spectrum of variability in the decline of locomotive and respiratory function, even in patients with the same genotype,

as enzyme activity varies per patient and per tissue [16,17,21]. Even though initially it was thought that patients that had this mutation, in conjunction with another severe mutation in their second *GAA* allele, would present with manifestations of Pompe disease in their childhood or early adulthood [18], recently Van Capele et al. evidenced that a second mutation (defined as severe, less severe or potentially less severe) did not seem to have an effect on the age of onset of the disease [22]. A homozygous status for this mutation has been shown to have a low frequency (maybe explained by a reduced or incomplete penetrance); however, patients homozygous for this mutation do not seem to differ, in their clinical phenotype variation, from heterozygotes [19]. All three subjects that harbor this mutation in the present family showed the mutation in a heterozygous status and yet, they presented with clinical manifestations and reduced GAA activity. This comes as a result of a compound heterozygosity where all of them held another and different heterozygous pathogenic mutations that when combined on the same gene brought a symptomatic phenotype. In addition, although the three subjects shared the same mutations they all presented with a different age of onset and severity of symptoms despite having a similar genotype. There is a description of 22 Dutch families carrying the c.-32-13T>G mutation, where the course and severity of the disease varied substantially among most of the siblings despite having identical genotypes. This suggests that other components, such as lifestyle, nutrition, environmental influences and other genetic or epigenetic factors, may contribute to explain such clinical inter and intra-familial variability [4].

Lastly, Rémiche et al. outlined five Italian Pompe patients with c.-32-13T>G that presented with pseudomyotonia and with a muscular and respiratory phenotype, and an age of onset of symptoms similar to our first patient described (*II-8*) [23]. As mentioned before, paraspinal complex repetitive discharges (pseudomyotonia) and respiratory compromise are both somewhat characteristic of this disease, and thus should raise awareness for LOPD diagnosis whenever seen together in a patient.

We found a second mutation on the *GAA* gene, c.2560C>T, that was present on all of the subjects that harbored *GAA* mutations in this family. This mutation was first described in 1993 by Hermans et al. and is considered pathogenic. It has been reported predominantly in association with various African populations and probably was brought to African American patients through slave trading and it can be found both in compound heterozygous and in homozygous states [24]. This variant corresponds to a nonsense mutation in exon 18, which results in a premature termination codon (p.Arg854Ter or R854X) that leads to the translation of a nonfunctional protein [16,18].

The third mutation that we found was present only on our index patient (*II-8*), c.1551+42G>A and it is an intronic mutation, first reported in 2008 in one patient from the United Kingdom, but there is no pathogenic effect identified for it [25]. Niño et al. reported this mutation as a frequent variation present in four of thirteen Colombian patients, with the same haplotype of the African mutation (c.2560C>T) previously mentioned [26].

In the population history of Costa Rica, inhabitants are mainly the result of interbreeding between the three basic roots:

native Spanish, African and indigenous populations [27]. Considering this, it sounds consistent that we found in our patients the most frequent mutations described on Caucasian and African populations. Likewise, this report matches the mutation description for Colombian LOPD patients (probably them having a similar ancestry like ours), where the mutation frequency estimation was 23.5% and 17.6% for the c.-32-13T>G and c.2560>T mutations, respectively [26].

Our biggest limitation with this report relates to the fact that large part of this family has not been tested yet, mainly because they have not agreed on being evaluated. Nonetheless we have provided for genetic counseling to the family as needed and we hope that in the future they will consent on being assessed. Also, we have not been able to perform any muscle imaging studies on our already diagnosed patients although we expect to have this evaluation done in the future.

4. Conclusions and future recommendations

We were able to characterize clinical and genetic aspects of the first diagnosed family with LOPD in Costa Rica and the mutations that we found showed the European and African ancestry that we carry in our population. Furthermore, clinical manifestations seen in our first patient illustrated features that suggest LOPD (i.e. paraspinal complex repetitive discharges (pseudomyotonia), diaphragmatic weakness) and should raise awareness for this diagnosis whenever present. Finally, DBS can be used as an easy, quick screening test, but requires for confirmation testing due to false positives.

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