CASE REPORT

c.1437G>A intron 9 substitution on acid α -glucosidase gene associated with classic infantile-onset Pompe disease phenotype

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SUMMARY

Pompe disease, or glycogen storage disease type II (GSD2), an autosomal recessive disease first described by Joannes Cassianus Pompe (1901–1945), causes deficient activity of acid α -glucosidase (GAA) enzyme. GAA catalyses α 1,4 and α 1,6 glucosidic linkages in lysosomes; destruction of these linkages permits glycogen to be separated into glucose and later used for energy. Without proper function of this enzyme, glycogen accumulates in lysosome, causing muscle hypotonia. We report a previously undescribed association of c.1437G>A intron 9 substitution on the GAA gene with severe infantile-onset Pompe disease in a deceased proband and carrier status in four of five surviving family members. Previous authors have found late-onset or moderate severity infantile-onset Pompe disease associated with this allelic variation. Our proband's family's village was suspicious for locally endemic disease. While our proband developed all features of classic infantile onset GSD2, socioeconomic and geographic factors initially suggested an infectious aetiology.

BACKGROUND

We report a previously undescribed association of c.1437G>A intron 9 substitution with severe infantile-onset Pompe disease in a deceased proband (figure 1) and carrier status in four of five surviving family members (figure 2). Pompe disease, or glycogen storage disease type II (GSD2; MIM: #232300), first described by Joannes Cassianus Pompe (1901–1945),¹ is an autosomal recessive disease, with over 400 allelic variations described, causing deficient activity of acid α -glucosidase (GAA; MIM: 606800). GAA catalyses α 1,4 and α 1,6 glucosidic linkages in the lysosomes; destruction of these linkages permits glycogen to be separated into glucose and later used for energy. Without the proper function of the enzyme, glycogen accumulates within the lysosome, causing muscle hypotonia. GSD2 has two subtypes: (1) infantile or classic-onset, characterised by failure-to-thrive, motor delay and cardiac problems; and (2) late-onset, characterised by proximal muscle weakness and respiratory failure without cardiac failure. While GSD2 is lethal, enzyme replacement therapy with alglucosidase alfa (Myozyme, Genzyme Corporation, Cambridge, Massachusetts, USA), produced from recombinant human acid α -glucosidase, has been shown to improve ventilator-free survival in some patients.²

Figure 1 Proband. Notice severe hypotonia.

Diagnosis of GSD2 is based on positive history and examination; elevated creatine kinase (CK) values, especially in the infantile forms;³ elevated urinary oligosaccharides; and decreased GAA enzyme activity.

CASE PRESENTATION

The proband was a 6-month-old male boy (figure 1) with complicated pneumonia, transferred to a tertiary referral centre in Guatemala City from a rural hospital located in Northwest Guatemala. The proband was the sixth child and born at 37 weeks' gestation via out-of-hospital normal vaginal delivery to a non-consanguineous 29-year-old mother, who received no prenatal care, and a 32-year-old father. At birth, the proband weighed 3.63 kg and cried immediately. Approximately 12 years before the proband's birth, the parents' first two children died at ages 8 months and 12 days, respectively, of complicated pneumonia. The proband's history included delayed motor milestones, progressive hypotonia and dyspnoea from 2 weeks of age.

Figure 2 Family pedigree. Notice deceased siblings and carriers conforming to typical Mendelian autosomal recessive inheritance.

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The proband was breast-fed until referral and exhibited social smile at 1 month, held his head at 3 months, made guttural sounds at 5 months and rolled over at 6 months. His Guatemalan immunisation schedule was up to date. The mother first noticed his symptoms of fever and dyspnoea at 2 weeks of age; neighbours, shamans and, eventually, a local health clinic, were consulted without result. At age 3 months, the proband developed cough. Medical consultation at that time resulted in diagnosis of pneumonia; antibiotic therapy was started. He continued to show no progress and was admitted to the Regional Hospital, which later transferred him. His family came from a poor socioeconomic status; the father was a farmer and the mother, a housewife.

INVESTIGATIONS

On admission to the tertiary referral centre, physical examination revealed a severely underdeveloped, active and hydrated male infant (weight: 6 kg and length: 63 cm, both <2 SD normal range; body mass index: 15.1 kg/m²). The proband exhibited subcostal retractions with symmetric thoracic excursion, and diminished aeration over the left lung field, disseminated rhonchi, occasional right basal wheezing and a systolic II/VI cardiac murmur. Plain chest radiography suggested cardiomegaly. Admission laboratory tests revealed elevated white cell count, neutrophilia, hyperglycaemia, elevated liver enzymes and CK levels. On hospital day 9, echocardiogram showed hypertrophic cardiomyopathy and patent foramen ovale. On hospital day 16, fluorimetry GAA enzyme analysis revealed lysosomal values of 71.8 µmol/L/h (normal range <30 µmol/L/h) and 95.4% (normal range <87%) GAA enzyme inhibition, consistent with severe Pompe disease phenotype. GAA gene screening detected no heterozygous single nucleotide polymorphisms but did reveal a homozygous substitution (c.1437G>A) at the consensus splice donor site of intron 9 (GenBank RefSeq: NM_000152.3; ClinVar accession number: SCV000211952) in the proband. Sense and antisense oligonucleotide primers were synthesised, based on the sequences flanking the exons of the GAA gene.

DIFFERENTIAL DIAGNOSIS

Prior to genetics consultation on hospital day 9, working clinical impressions were complicated pneumonia, possible atrial septal defect and malnourishment; empiric antibiotic therapy, bronchodilators, oxygen supplementation and intravenous hydration were initiated. By hospital day 2, the proband's cardiopulmonary status deteriorated, requiring mechanical ventilation. The remainder of the hospital course was complicated by concomitant acute renal failure and clinically significant bacterial isolates from endotracheal tube and central venous catheter. Difficulty stabilising the proband delayed some investigations and eventual treatment. Findings suggesting GSD2 included: history of progressive hypotonia beginning at age 1 month, holosystolic cardiac murmur and hypertrophic cardiomyopathy on echocardiography. This diagnosis was confirmed and classified based on elevated CK enzyme levels.

TREATMENT

By hospital day 26, the proband had relatively stable vital signs and renal function, and continued to improve sufficiently, over the next 3 days, to be awakened and in a better condition for alglucosidase alfa infusion. Alglucosidase alfa was given according to the manufacturer's protocol of 20 mg/kg over 4 h, along with antihistamine.² The proband exhibited no hypersensitivity for 72 h postadministration. Five days later, his cardiopulmonary

status had improved, mechanical ventilation parameters were reduced and hepatic enzyme levels stabilised. He remained drowsy, with absent deep tendon reflexes in four extremities, and diminished muscular tone and strength, and was intubated.

OUTCOME AND FOLLOW-UP

Despite clinical improvement after alglucosidase alfa, the proband's parents signed discharge papers against medical advice, for personal and economic reasons. The child died approximately 1 h postdischarge. Three months after his death, GAA screening showed the same c.1437G>A intron 9 substitution in four of five surviving family members (figure 2). Despite exhaustive efforts to contact them again, the family was lost to further follow-up. Efforts to investigate the prevalence of c.1437G>A allelic variation in the proband's village were similarly infeasible.

DISCUSSION

Molecular genetic testing by targeted allelic variation analysis in families with a known defined allelic variation, sequence analysis, or deletion/duplication analysis, is important for genetic counselling, and for estimation of clinical severity and outcome. Deletion/duplication is the preferred test for the Dutch population because of a deletion in exon 18 of GAA gene on chromosome 17, locus 17q25.2-q25.3, which has not been found in populations outside Europe.⁴ The proband described had sequence analysis, as no previously described GAA allelic variation was identified.⁵ His single nucleotide substitution (c.1437G>A on intron 9) was thought to cause aberrant splicing that could alter and inhibit the protein formation. When screening the proband, there were five allelic variations reported on intron 9,^{6–9} with three being very severe and two, non-pathogenic. Zampieri *et al*¹⁰ reported a c.1437+2T>C intron 9 allelic variation, studied by an in silico approach, that could cause the alteration in splicing. Allelic variation severity using the molecular weight of the GAA enzyme⁹ could not be assessed in our proband; however, splicing and missense allelic variations may cause different phenotypes in different families. Hoefsloot *et al*¹¹ demonstrated diversified rates of enzyme function in a kindred corresponding to variable expression in their patients' clinical status.

Previous reports associated c.1437G>A intron 9 with late-onset or moderate severity infantile-onset Pompe disease.^{12 13} Considering (1) our proband's aggressive clinical progression, (2) markedly reduced GAA enzyme activity, (3) predicted enzyme formation inhibitory effect of the allelic variation through aberrant splicing, and (4) premature death of his previous siblings, we conclude c.1437G>A intron 9 may be associated with a very severe GSD2 phenotype in at least some cases.

Improper reliance by the proband's mother on local peers and shamans to solve the health problems experienced by her son initially delayed time to first presentation to medical services, until the proband had progressed considerably and become gravely ill, and though our proband developed all features of classic infantile onset GSD2, socioeconomic and geographic factors initially suggested an infectious disease aetiology, further delaying diagnosis and treatment. We suggest that, in resource depleted areas, Pompe disease should be a differential diagnosis for cases of complicated pneumonia in infants. Interestingly, the two previously reported patients with c.1437G>A intron 9 infantile-onset Pompe phenotype were Hispanic,¹³ possibly suggesting ethnogeographic allelic variation clustering, when considered with the present kindred. Finally,

we received anecdotal reports, from the family's village, of infants dying with complicated pneumonia. While our proband's parents' consanguinity was unproven, they shared a second last name, and many persons in this community shared our proband's parents' second last name, indicating probable consanguinity possibly perpetuating this severe GSD2 allelic variation, ostensibly making it locally endemic.

Learning points

- ▶ Pompe disease should be generally considered more as a differential diagnosis for infants presenting with complicated pneumonia from rural and resource-depleted areas, especially where consanguinity may exist among community members.
- ▶ The single nucleotide substitution c.1437G>A on intron 9 should be included in molecular screening for phenotypically severe as well as moderate infantile-onset and late-onset or glycogen storage disease type II (GSD2).
- ▶ As all three probands reported with infantile-onset GSD2 phenotypes associated with a c.1437G>A intron 9 substitution were Hispanic, this may illustrate an ethnogeographic allelic variation clustering.
- ▶ Expedient diagnosis and stabilisation of critical patients allows for enzyme replacement therapy with alglucosidase alfa, which may measurably improve ventilator-free survival.
- ▶ In rural areas, reliance by persons of low socioeconomic status on local peers and traditional healers for medical care may critically delay appropriate provision of life-saving interventions, as was probably the case with our patient. Physicians and others should make efforts to educate persons who may be at risk about the dangers of making decisions to seek non-medical care.

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Competing interests None declared.

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