CASE REPORT

Alkaptonuria and pompe disease in one patient: metabolic and molecular analysis

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SUMMARY

Pompe disease is characterised by deficiency of acid α -glucosidase that results in abnormal glycogen deposition in the muscles. Alkaptonuria is caused by a defect in the enzyme homogentisate 1,2-dioxygenase with subsequent accumulation of homogentisic acid. We report the case of a 6-year-old boy diagnosed with Pompe disease and alkaptonuria. Urine organic acids and α -glucosidase were measured. Homogentisate 1,2-dioxygenase (HGO) and acid alpha-glucosidase (GAA) genes were sequenced by Sanger DNA sequencing. The level of α -glucosidase in white blood cells was markedly decreased (4 nm/mg) while the level of homogentisic acid was markedly increased (15 027 mmol/mol creatine). GAA sequencing detected two heterozygous GAA mutations (C.670C>T and C.1064T>C) while HGO sequencing revealed three polymorphisms in exons 4, 5 and 6, respectively. To the best of our knowledge, this is the first reported instance of Pompe disease and alkaptonuria occurring in the same individual.

BACKGROUND

Alkaptonuria is an autosomal recessive disorder of aromatic amino acid metabolism due to a deficiency in homogentisate 1,2-dioxygenase.¹ The incidence is 1 case in 250 000 to 1 million live-births and is increased in certain regions such as Slovakia and Dominican Republic.^{1 2} Homogentisic acid accumulate in the body and is oxidised to benzoquinone acetate, which polymerises to form melanin-like polymers, resulting in widespread deposition in fibrous tissue and cartilage leading to a wide range of symptoms.¹ There is no effective treatment for this condition but Nitisinone is currently used.¹

Pompe disease is an autosomal recessive disorder of glycogen metabolism due to deficiency in α -glucosidase with subsequent accumulation of glycogen in various body tissues.^{3 4} The incidence of Pompe disease is 1 in 40 000 birth.³ Early diagnosis and early institution of enzyme replacement therapy is crucial because it minimises morbidity and prolongs survival.⁵ In this article, we report a child who was initially diagnosed with Pompe disease and then with alkaptonuria. Data to support these diagnoses are presented.

CASE PRESENTATION

A 6-year-old boy was referred to us because of progressive motor weakness and failure to thrive. He was born by normal vaginal delivery; 10 days post the earliest due date with a birth weight of 2.3 kg.

Since early infancy, the child suffered recurrent respiratory tract infections with greenish mucoid stools, episodes of dark urine and gastro-oesophageal reflux disease. Few months ago, he started experiencing slowly progressing motor weakness predominantly over the lower extremities, feeding difficulties, shortness of breath on minimal exertion and inability to walk. The parents also noticed the appearance of cyanosis over both hands and lips. Investigations revealed an increased creatine phosphokinase (1679 IU/l) and a decreased α -glucosidase of 4.0 nm/mg P in white blood cells (control: 127 nm/mg P); and was diagnosed with 1,4- α -glucosidase deficiency or the Pompe disease. Two months prior to presentation, he developed pneumonia with cardiorespiratory distress. Cardiopulmonary resuscitation was initiated, put on mechanical ventilation and he improved thereafter. Heart ultrasound was normal and abdomen ultrasound revealed hepatomegaly (approximately 10.7 cm) with disturbed echotexture indicating liver parenchymal disease.

After physical examination, he was found to have normal vital signs, was pleasant and was able to communicate well without any sign of mental retardation. His height and weight were 85 cm and 13 kg, respectively. He was cachectic, had proximal muscle weakness of the lower limbs with preservation of the motor power in the upper extremities. The child had black spot in his right eye with normal cardiological and pulmonary examinations. There is no parental consanguinity. Both his parents and his sister are clinically well. In the family pedigree, one of his cousins died at the age of 14 years secondary to pneumonia and hepatosplenomegaly, another cousin have episodes of dark urine but they could not be reached (figure 1).

INVESTIGATIONS

Urine organic acids measurements were done by gas chromatographic-mass spectrometric determinations (GC-MS) using O-(2,3,4,5,6-pentafluorobenzyl) oxime-trimethylsilyl ester derivatisation and cation-exchange chromatography⁶ and modified in our lab. Measurements of α -glucosidase were done using 4-methylumbelliferyl- α -D-glucopyranoside in leucocytes⁷ which is based on the principle that α -glucosidases are first isoelectrically precipitated at pH 5.0 and the acid α -glucosidase is then quantitated by the hydrolysis of 4-methylumbelliferyl- α -D-glucopyranoside substrate with the concomitant release of 4-methylumbelliferone, highly fluorescent in alkaline medium.

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Figure 1 Family pedigree: the patient is indicated by an arrow. Two of his cousins died and a third one has episodes of dark urine.

The assay for GAA sequencing used PCR amplification followed by Sanger DNA sequencing to detect the mutations. Complete coding sequence coverage for exons through 20 flanking and intronic sequences (minimum of 20 base pairs), and coverage of the common adult-onset mutation (intron 1) was obtained using forward and reverse primers. These sequences were compared with the reference DNA sequence (GenBank Accession: NT_024871). Data were analysed by the ABI Data Collection software V3.0, Sequencing Analysis Software V5.4 and SeqScape Software V2.6.

Complete blood count and comprehensive metabolic panel were normal. Urine organic acids revealed a markedly increased homogentisic acid level (15 027 mmol/mol creatine) and was diagnosed with alkaptonuria. Molecular studies were carried out on both the parents and child. Sequencing of all exons of the HGO gene revealed three polymorphisms in exons 4, 5 and 6, respectively in both the parents and child. GAA sequencing for Pompe disease revealed two heterozygous GAA mutations in the child: Single nucleotide change (C.670C>T) in exon 3 and C.1064T>C in exon 6 were found. The first one was also detected in the mother while the latter was detected in the father. For C.670C>T: this change alters the arginine codon at position 224 to a tryptophan codon (p.Arg224Trp); and for C.1064T>C: this change alters the leucine codon at position 355 to a proline codon (p.Leu355Pro).

OUTCOME AND FOLLOW-UP

He was started on orfadin (nitisinone) 2 mg every other day and repeat urine organic acids carried out 3 months later revealed a major drop in homogentisic acid (136 mmol/mol creatine; the normal being less than 2) and an increase in both *p*-hydroxyphenylpyruvic acid of 731 mmol/mol creatine and *p*-hydroxyphenyllactic acid of 1 820 mmol/mol creatine (the normal being less than 2). Plasma amino acids revealed an increased tyrosine level 385 µmol/l (normal 24–115). The patient was not started on enzyme replacement therapy yet.

DISCUSSION

Alkaptonuria is caused by a defect in the homogentisate 1,2-dioxygenase gene which leads to a defective enzyme.^{1 2} As a result, excess tyrosine is converted into homogentisic acid (HGA). Elevated levels of HGA polymerise and form a pigment that is deposited in connective tissues (ochronosis).

Diagnosis is confirmed by measuring HGA level in the urine.² Currently, there is no cure for alkaptonuria. The only available treatment is symptomatic treatment aiming at relieving arthritis symptoms. A potential drug, nitisinone, has been shown to reduce up to 95% the level of homogentisic acid in the urine

but it is not yet approved nor has it been found to prevent joint deterioration.^{8 9} Dietary restrictions of tyrosine and phenylalanine show limited efficacies.¹⁰

Nitisinone (also known as NTBC) is a competitive inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase. By inhibiting this enzyme, it prevents the formation of maleylacetoacetic acid and fumarylacetoacetic acid, which can be converted into succinylacetone and succinylacetoacetate, toxins responsible for causing liver and kidney toxicity. It was initially approved for the treatment of hereditary tyrosinemia type 1, but currently, it is yet to be approved for use in alkaptonuria patients.^{8 9}

Pompe disease (or acid maltase deficiency or glycogenosis type II) is caused by the deficiency of lysosomal α -glucosidase which results in lysosomal and cytoplasmic accumulations of glycogen eventually leading to tissue destruction.^{3 4} There are two forms of Pompe disease: the infantile and late onset forms, but in general the latter has a better prognosis.⁴ Diagnosis may be established by muscle biopsy or by measuring acid maltase activity from leucocytes or from fibroblasts obtained by skin biopsy.¹¹ Treatment is based on the administration of an enzyme replacement therapy using biologically active recombinant human α -glucosidase α that have proved its efficacy and safety.⁵

In this article, we have described the unusual findings of both of these rare autosomal recessive diseases in the same patient. Based on incidences of 1:250 000 for alkaptonuria¹ and 1:40 000 for Pompe disease,³ the incidence of the two conditions combined in the general population is 1:10 000 000 000 and considering the current human population of approximately seven billion, it is unlikely that there are many more individuals with a combined diagnoses of these two diseases.

More than 200 mutations in the GAA gene have been identified in patients with Pompe disease. The sensitivity of DNA sequencing is high for the detection of nucleotide base changes, small deletions and insertions in the regions analysed. Only the coding regions of the GAA gene and immediate flanking intronic sequences were examined. In this article, parental studies showed each parent to be a heterozygous carrier of one of these mutations, indicating that these mutations are indeed present in different copies of the gene (in trans). And these sequence changes are consistent with a diagnosis of Pompe disease. In vitro studies have confirmed that C.670C>T in exon 3 decreases enzyme activity, but a low level of catalytically active lysosome α -glucosidase remains.¹² C.1064T>C has been previously reported in these patients and was shown through in vitro studies to result in decreased enzyme activity.¹³

More than 40 mutations have been identified in the HGO gene that leads to alkaptonuria. No mutation was detected in the HGO gene, but only three polymorphisms. The reason for

no identification of mutation in the HGO gene is that conventional primer-type PCR analysis was used. Therefore, a whole exome analysis has been underway.

Several previous cases of patients having two autosomal recessive disorders exist in the literature; Alkaptonuria have been reported in a patient with glucose 6-phosphatase deficiency and Huntington disease,¹⁴ Glutaric aciduria type I combined with isobutyrylglycinuria,¹⁵ Alcaptonuria and sucrase-isomaltase deficiency¹⁶ but this is the first time that this particular combination has been observed. This patient improved after the initiation of Nitisinone and urine organic acid, showed a markedly decreased HGA level and an increase in the intermediates (*p*-hydroxyphenylpyruvic and *p*-hydroxyphenyllactic acid) as a result of enzyme blockage which prove treatment efficacy.

Learning points

- ▶ Combined Pompe disease and alkaptonuria in a single patient has never been reported.
- ▶ Pompe disease is due to the deficiency of acid α -glucosidase with subsequent accumulation of glycogen.
- ▶ Alkaptonuria is caused by a defect in the enzyme homogentisate 1,2-dioxygenase with subsequent accumulation of homogentisic acid.
- ▶ Rare genetic disorders may occur even without parental consanguinity.

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Competing interests None.

Patient consent Obtained.

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