

CASE REPORT / OLGU SUNUMU

Pompe disease: A case report

Pompe hastalığı: Olgu sunumu

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ABSTRACT

Pompe disease is inherited in an autosomal recessive manner, and is usually observed in the children of asymptomatic carriers. Pompe disease, known as Glycogen Storage Disorder type II, is caused by pathogenic mutations in the gene encoding lysosomal acid alpha-glucosidase (GAA). There are three types of Pompe disease: classical infantile form, non-classical infantile form and late-onset Pompe disease. Age of onset and severity of the disease determine the type of Pompe disease. We aimed to identify a mutation in GAA gene in parents who were first cousins and their baby girl was passed away due to the Pompe disease. The baby girl had reduced acid alpha-glucosidase activity, but genetic analysis had not been performed. Mutation analysis of parents was performed using high-throughput DNA sequencing method. Heterozygous mutation of c.896 T>C in exon 5 was found in parents, and prenatal diagnosis was performed for their next pregnancy. In conclusion, c.896 T>C substitution in GAA gene may lead to the severe type of Pompe disease. Using a relatively fast and reliable molecular genetic analysis method to confirm the early diagnosis of the Pompe disease is important for the management of the disease.

Key words: Pompe disease, GSD2, GAA deficiency, high-throughput DNA sequencing

INTRODUCTION

Glycogen storage disease type II or Pompe disease (MIM #232300), is an autosomal recessive disorder caused by mutations in the GAA gene encoding acid alpha-glucosidase (Glucosidase, Alpha; Acid) also known as acid maltase. The enzyme is required for

ÖZET

Pompe Hastalığı otozomal resesif olarak kalıtılır ve çoğunlukla asemptomatik taşıyıcıların çocuklarında gözlenir. Glikojen depo hastalığı tip 2 olarak bilinen Pompe hastalığına, lizozomal asit alfa-glukozidaz enzimini kodlayan gendeki (GAA) patojenik mutasyonlar yol açar. Pompe hastalığının; Klasik infantil tip, Klasik olmayan infantil tip ve geç başlangıçlı Pompe hastalığı olmak üzere üç tipi bulunur. Hastalığın başlangıç yaşı ve şiddeti, Pompe hastalığının tipini belirler. Burada, aralarında birinci dereceden kuzen evliliği bulunan ve bir kız bebeği Pompe hastalığı nedeniyle vefat eden anne-babada, GAA geninde bir mutasyon belirlemeyi amaçladık. Kız bebekte asit alfa-glukozidaz enzim aktivitesi azalmıştı, fakat mutasyon analizi yapılmamıştı. Anne-babada mutasyon analizi, yeni nesil DNA dizileme metodu ile yapıldı. Anne ve babanın GAA geninde, ekzon 5 'te c.896 T>C mutasyonu heterozigot olarak bulundu ve bir sonraki gebelik için prenatal tanı yapıldı. Sonuç olarak, GAA geninde bulunan c.896 T>C değişikliği Pompe hastalığının şiddetli tipine yol açabilir. Pompe hastalığının erken tanısını doğrulamak için nispeten hızlı ve güvenilir bir moleküler genetik analiz metodu kullanılması hastalığın yönetimi için önemlidir.

Anahtar kelimeler: Pompe hastalığı, GSD2, GAA eksikliği, yeni nesil DNA dizileme

glycogen degradation in lysosomes. Failure of this reaction leads to the accumulation of glycogen in tissues particularly in cardiac, skeletal and smooth muscles [1]. Pompe disease is usually classified into two forms, infantile- and late-onset. Infantile form is also divided into two subgroups. In the classical infantile form, the prominent findings are cardio-

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megaly, hepatomegaly, weakness and hypotonia which usually lead to cardiorespiratory failure and death in the first year of the life. In the non-classical infantile form of the Pompe disease, the cardiomyopathy is less severe and progression of the disease is slower than classical form [1].

In the late-onset (juvenile, childhood or adult onset) Pompe disease, the clinical symptoms are related to the involvement of skeletal muscle which leads to progressive muscle weakness and respiratory insufficiency [2].

The frequency of the Pompe disease may vary for different clinical form and ethnic groups. The prevalence of infantile onset Pompe disease is reported 1/57,000 in Taiwan [3].

In this case report, we described a couple with consanguineous marriage who applied to have pre-conception counselling about Pompe disease. Molecular genetic analysis has been performed for parents. Prenatal genetic test was carried out for the next pregnancy of the family.

CASE REPORT

Parents, who were first cousins (see pedigree, Figure 1), have been referred to get genetic counselling for Pompe disease. A baby boy and a baby girl had passed away when the parents applied to our outpatient department. Medical records including the echocardiography imaging and laboratory test results of the baby girl were evaluated. Concentric hypertrophy of the heart (particularly in the interventricular septum), dilatation of the left atrium and ventricle, moderate mitral valve regurgitation and small patent ductus arteriosus had been observed in her echocardiography. Blood sample of the second baby had been taken (by Genzyme, Turkey), and had sent to the Metabolic Laboratory, Hamburg University Medical Center, Department of Pediatrics and Institute of Clinical Chemistry for analysis. Alpha-glucosidase activity was 0.13 nmol/spot*21 hour at pH=3.8, and this was below the respective reference ranges (1.5-10 nmol/spot*21 hour). Therefore, the baby girl had been diagnosed with classical infantile onset Pompe Disease according to the decreased level of alpha-glucosidase enzyme activity, and an enzyme replacement therapy (Myozyme, 20 mg/kg, intravenously, once every 15 days) had been pre-

scribed at 7 months old due to the involvement of muscle and respiratory system. However, no genetic analysis had been performed for GAA gene, and the patient died at 10 months old. The confirmation of the Pompe disease by a second test could not be performed for the baby girl (The baby boy had passed away without a diagnosis at 17 days old. There was no data available for the baby).

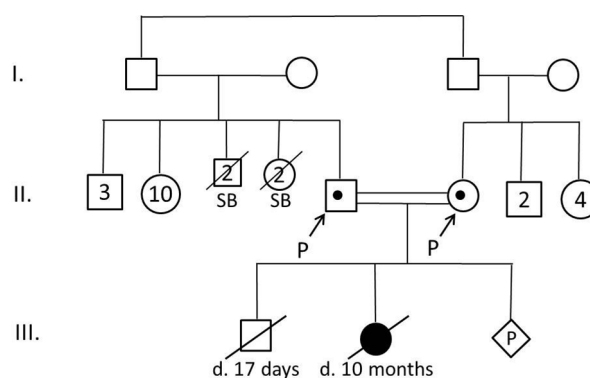


Figure 1. Pedigree of the family. GAA gene was first sequenced in parents. Unaffected couple were heterozygous for c.896 T>C mutation (p.L299P). The same mutation was also found in the prenatal test for the pregnancy. SB: Stillbirth

Mutation analysis for Pompe disease was carried out for parents. Peripheral blood samples of the parents were collected into tubes containing EDTA. DNA was obtained from blood samples by standard procedures. All exons and splice sites of the *GAA* gene were sequenced using next generation sequencing method on Illumina MiSeq platform. The results were evaluated using integrative genomics viewer (IGV). A heterozygous mutation leading to the substitution of Proline for Leucine at amino acid 299 of the acid α -glucosidase (p.L299P ; c.896 T>C) was detected in parents (Figure 2). Parents have been informed about assisted reproductive technology and pre-implantation genetic diagnosis. However, they preferred not to use this technology.

The couple applied for prenatal testing a few months later. Amniocentesis has been performed at 17th week of the pregnancy, and molecular genetic analysis has been carried out. Quantitative Fluorescence PCR (QF-PCR) has been performed to evaluate maternal contamination and the aneuploidy of chromosomes 13, 18, 21, X and Y. No maternal contamination has been detected in the analysis of short

tandem repeat markers (ChromoQuant® QF-PCR kit, CyberGene AB, Sweden). A heterozygous mutation of *GAA* gene (p.L299P) has been detected in

the amniotic fluid sample. Genetic counselling has been given to the family.

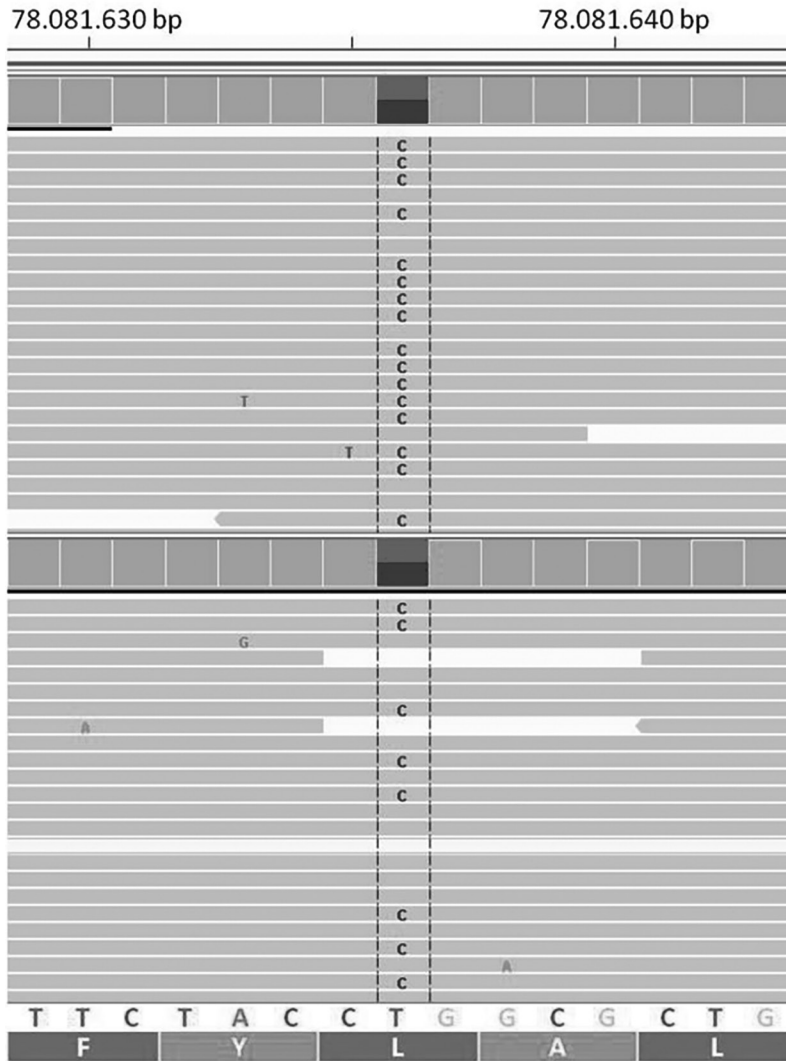


Figure 2. Result of the *GAA* gene sequencing encompassing the c.896 T>C. The pictures at the top and bottom are belong to mother and father respectively. The single nucleotide substitution in the *GAA* gene is observed in the middle column in both samples. At the top of the figure, the location of the sequenced region was indicated according to the genome reference consortium, GRCh37.p13 reference assembly. The original sequence, codons and corresponding aminoacids are shown at the bottom of the Figure.

DISCUSSION

Cardiomegaly, cardiomyopathy, hypotonia, muscle weakness, respiratory distress, respiratory infections, feeding difficulties and failure to thrive are the frequent signs and symptoms of the Pompe disease. In this case, hypertrophic cardiomyopathy had been probably developed secondary to Pompe disease. Spinal muscular atrophy type 1 (acute Werdnig-Hoffman Disease), hypothyroidism, endocardial fibroelastosis, myocarditis, congenital muscular dystrophy, other glycogen storage diseases, mitochondrial/respiratory chain disorders, Danon

disease, idiopathic hypertrophic cardiomyopathy and peroxisomal disorders should be considered for the differential diagnosis of Pompe disease [1]. In addition, classical infantile form of the Pompe disease may lead to symptoms similar to that of motor neuron diseases (spinal muscular atrophy type 2 or type 3) and congenital myasthenic syndromes [4]. If unexplained respiratory insufficiency and increased level of serum creatine kinase are observed, a health professional should suspect Pompe disease. To screen the Pompe disease in all age groups, usually *GAA* enzyme activity in a dried blood sample is

used. GAA enzyme activity in blood samples is performed under acidic conditions, using a synthetic substrate 4-methylumbelliferyl-alpha-D-glucoside or glycogen. However, the presence of isoenzyme maltase glucoamylase (another alpha glucosidase) can mask the GAA deficiency. For this reason, acarbose is used for competitive inhibition of isoenzyme maltase glucoamylase, and thus false-negative results are eliminated [5]. The diagnosis should be confirmed either by a second GAA enzyme activity assay or *GAA* gene sequencing [4]. Measuring the GAA activity in muscle biopsies or fibroblast culture is considered as invasive and slow. However, *GAA* gene sequencing is relatively fast and reliable method for the diagnosis of Pompe disease [2].

The location of the mutation in *GAA* may determine the onset or the severity of the Pompe disease. Recently, the effect of the *GAA* mutations on the clinical severity of the disease has been predicted by comparing the enzyme activity in the transfected COS-7 cells. When these cells transfected with a wild-type or a mutant form of the *GAA* gene [6], the pathogenic missense mutation of c.896 T>C in exon 5 has been reported to be classified under “potentially less severe” group [7]. In our case, reduced GAA enzyme activity, clinical findings of the baby (concentric hypertrophy of the heart, dilatation of the left atrium and ventricle) and early death at 10 months old suggest that the baby has homozygote pathogenic mutation in *GAA* gene. Although the c.896 T>C substitution classified under “potentially

less severe” group mutation, it may also cause to the severe classical infantile form of the Pompe disease.

In conclusion, Pompe disease is observed rarely and genetic counselling is required for families with the history of Pompe disease. The identification of Pompe disease-related mutations is useful for early diagnosis, early prescription of the enzyme replacement therapy and assisted reproductive technique with pre-implantation genetic diagnosis.

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