증 례

Pseudodeficiency 및 potential late onset Pompe disease 보인자로 확인된 *cis*형 dual variant 돌연변이 두 개를 가진 여아 1례

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A Case of Pseudodeficiency in a Potential Late Onset Pompe Disease Carrier, with Double Dual Variant, Each in *cis* Formation

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Pompe disease (PD) is an autosomal recessive genetic disorder caused by a deficiency of the lysosomal enzyme acid α-glucosidase (GAA). It is easy to hastily diagnose as patients if they have two pathogenic variants. Clinical pathologists misdiagnosed our infant and her mother as PD. Here, we report a case of pseudodeficiency in a potential late-onset Pompe disease (LOPD) carrier with a double dual variant, each in cis formation in a 3-month infant. The person who has two pathogenic variants was diagnosed as a carrier, not a patient. It was first reported in Korea. The patient had: two likely pathogenic heterozygous mutations on exon #4: c.752C>T (p.Ser251Leu), c.761C>T (p.Ser254Leu), and a heterozygous mutation on exon #12: c.1726G>A (p.Gly576Ser), also with a heterozygous mutation on exon #15: c.2065G>A (p.Glu689Lys). By presenting this case we emphasize the possibility of *cis* formation of genes which may cause pseudodeficiency, and potential LOPD carrier form. Hereby we suggest that thorough evaluation of *GAA* gene is essential among whom initially diagnosed as PD.

Key words: Pompe disease, Dual variant in *cis* formation, Pseudodeficiency, Potential late onset Pompe disease carrier

Introduction

Pompe disease (PD) also known as glycogen storage disease type II (GSD2), acid α-glucosidase (GAA) deficiency, and acid maltase deficiency, is a rare autosomal recessive genetic disorder classified in the lysosomal storage disorder (LSD)[1]. Incidence of PD differs by ethnicity and geographic region. It affects between 1 in 14,000 and 1 in 300,000 individuals, as per reports. However, it is estimated to affect approximately 1 in 40,000 births[1]. The highly polymorphic *GAA* gene contains 20 exons and has many neutral variations[2]. The *GAA* gene contains the genetic information required for the production and function of GAA. Defect in function or shortage of GAA hampers the degradation process of glycogen to glucose. In PD, glycogen is accumulated in various tissues and the lysosomes of

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different types of cells, resulting in cellular malfunction and damage, leading to organ dysfunction[3]. Predominantly found to affect cardiac, skeletal, and smooth muscle cells, PD manifests by muscle weakness and wasting[3]. PD shows a variable rate of disease progression and diverse age of onset leading to the subdivision of PD according to clinical spectrum as Infantile-onset (IOPD) and late-onset PD (LOPD)[4]. IOPD was first described in 1932 by the Dutch pathologist Dr. J. C. Pompe[1]. Generalized muscle weakness and cardiomegaly were the initially evaluated characteristics of IOPD. Other terms such as childhood-, juvenile-, and adult-form refers to relatively less severe LOPD; with delayed onset or slow progression[1]. LOPD is now typically used to refer to PD without manifestation of hypertrophic cardiomyopathy, or to patients not diagnosed until the age of 1 year, and in which the age of first onset of symptoms is above the age of 1 year[1]. In LOPD, symptoms usually develop after the second decade and can present as slowly progressing skeletal muscle dysfunction. Proximal lower limb and paraspinal trunk muscles are known to be affected first. Diaphragm and respiratory accessory muscles are affected later[1]. Within the first three months of life, IOPD patients with near complete deficiency of GAA show signs of rapidly progressing muscle weakness. Decreased muscle tone, respiratory deficiency, and diminished cardiac function with hypertrophic cardiomyopathy, when left untreated, usually cause IOPD patients to expire owing to cardio-respiratory failure within the first two years of life. While diagnosing the disease itself is critical, it is also essential for clinicians to appropriately distinguish certain false positive cases such as genetic heterozygosity and pseudodeficiency in PD.

This report presents the case of a Pompe disease carrier with a double dual variant, each in cis formation, presenting as a potential late-onset Pompe disease with pseudodeficiency.

Case Report

The case concerns a preterm female infant born at 31 weeks and two days of gestational age. The patient's birth weight was 1610 grams (APGAR 8/9). Showing initial signs of desaturation and chest retraction, the patient was admitted to Dankook University Hospital, Department of Pediatrics, neonatal intensive care unit (NICU). The patient was diagnosed with respiratory distress syndrome of newborn and treated with exogenous surfactant. Her respiratory condition had improved after the use of continuous positive airway pressure. Enlarged tongue, abnormal posture and muscle weaknesses were not present. The patient's chest X-ray, electrocardiogram, and echocardiography showed no signs of cardiomegaly.

Newborn screening for LSD showed a low GAA level of 0.58 (>0.76 umol/h/L). GAA enzyme assay showed decreased total GAA level of 16.2 nmol/2hr/mg protein (30-90 nmol/2hr/mg protein), GAA with acarbose level of 2.0 nmol/2hr/mg protein (≥10 nmol/2hr/mg protein), and a ratio of acarbose/total level of 12.0% (≥25%). These results were indicative of PD. At 2 months old, her creatine kinase (CK) level had increased by 2,827 IU/L (2-146 IU/L). However, CK level normalized without treatment by 3 months of age to 130 IU/L. The results of the sensory nerve and motor nerve conduction study were normal. No signs of respiratory distress or decreased muscle tone were to be found at the age of 3 months. A *GAA* gene mutation test was performed to assess the patient's condition further. The gene analysis showed two likely pathogenic heterozygous mutations on exon #4: c.752C>T (p.Ser 251Leu), and c.761C>T (p.Ser254Leu). A heterozygous mutation on exon #12: c.1726G>A (p.Gly576Ser), and a heterozygous mutation on exon #15: c.2065G>A (p.Glu689Lys) were also found in the patient's *GAA* gene. *GAA* gene mutation tests for the patient's family members were additionally performed. The patient's

7-year-old brother's *GAA* gene analysis was normal. The patient's father had the same heterozygous mutation: c.1726G>A (p.Gly576Ser), c.2065G>A (p.Glu689Lys). The patient's mother also had two likely pathogenic heterozygous mutations: c.752C>T (p.Ser251Leu), c.761C>T (p.Ser254Leu) (Fig. 1). However, none of the family members had muscle weaknesses or cardiac complication.

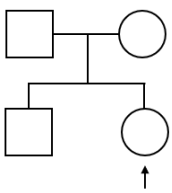
After consideration and further investigation, these mutations were concluded to be a dual variant in *cis* formation, resulting in pseudodeficiency and potential LOPD without signs or symptoms. Thus, the patient can be classified as a PD carrier and a potential LOPD patient with pseudodeficiency. The patient's mother and father can be classified as, potential LOPD patient and pseudodeficiency, respectively.

This case was approved by the Institutional Review Board of the Dankook Hospital (IRB No. 2022-08-010).

Discussion

PD can be divided into two major forms, IOPD and LOPD, depending on the age of onset[5]. IOPD, also known as the classic infantile form, occurs before the age of 1 year. Common features of IOPD are cardiomegaly, cardiomyopathy, hypotonia, muscle weakness, respiratory distress, respiratory infections, feeding difficulties, and failure to thrive[1]. LOPD usually develops symptoms after the second decade and can present as slowly progressing skeletal muscle dysfunction. Proximal lower limb and paraspinal trunk muscles are known to be affected first. The diaphragm and respiratory accessory muscles are affected later[1]. Early detection and treatment of PD is possible via the newborn screening test (NBS)[5].

However, due to diverse mutations currently found on *GAA*, careful considerations should be made before diagnosing the patient. Currently known *GAA* variations and clinical forms of PD are provided in the Pompe-variant database; www.pompevariantdatabase.nl. The c.1726G>A (p.Gly576Ser) variant in cis with c.2065G>A (p.Gly689Lys) is known to be a c.1726G>A; 2065G>A) pseudodeficiency allele. It is common in Asian populations, causing low GAA activity in normal individuals[2,8]. Whereas c.752C>T and c.761C>T are disease-associated variants, they always occur in *cis* formation as a dual mutation (c.752C>T; 761C>T))[6,7]. Kroos et al. experimentally demonstrated that the c.752C>T (p.Ser251Leu), c.761C>T (p.Ser254Leu) variant decreases GAA activity to approximately 1.5-3% of the normal levels[3]. According to Sawada et al., individuals with this mutation did not develop any signs or symptoms related to PD and thus were classified as



Family member	Gene	Exon	Nucleotide change	Amino acid change		Zygosity
Case	GAA	4 4	c.752C>T c.761C>T	p.Ser251Leu p.Ser254Leu	*cis*	Hetero Hetero
		12 15	c.1726G>A c.2065G>A	p.Gly576Ser p.Glu689Lys	*cis*	Hetero Hetero
Father	GAA	12 15	c.1726G>A c.2065G>A	p.Gly576Ser p.Glu689Lys	*cis*	Hetero Hetero
Mother	GAA	4 4	c.752C>T c.761C>T	p.Ser251Leu p.Ser254Leu	*cis*	Hetero Hetero
Brother	GAA	-	-	-		-

Fig. 1. Family pedigree of the case child and the *GAA* gene mutation test results of each family member.

potential LOPD carrier[6]. The c.752C>T (p.Ser251Leu), c.761C>T (p.Ser254Leu) variant is currently known to be associated almost exclusively with LOPD. All affected individuals were either homozygous or carried this variant in addition to another pathogenic variant on the other allele in *trans*, consistent with the autosomal recessive inheritance of PD[2,6], According to Genome Aggregation Database, these variants are not common in the general population. c.752C>T (p.Ser251Leu) and c.761C>T (p.Ser254Leu) are each observed in 100 of 282,488 alleles and 55 of 282,470 alleles respectively[9].

NBS programs for PD can contribute to early detection and early intervention in patients with IOPD and LOPD. Early detection and ERT is optimal for treating PD[10]. However, the frequency of LOPD and pseudodeficiency alleles in the population are high[5]. Since patients with potential LOPD may not ever develop PD symptoms, problems such as psychological stress for the family, the cost and time of visiting hospitals and receiving medical examinations, and the potential of overtreatment are likely to occur. Thus, clinicians should interpret each case with significant caution. It is easy to hastily diagnose those who have two pathogenic variants with PD.

However, as shown in our case, the currently available NBS programs that evaluate GAA activity cannot discriminate pseudodeficiency from PD or potential PD cases. Making an evaluation of pseudodeficiency or potential LOPD carrier status using the *GAA* gene is essential. Herby, we report the first case in South Korea of pseudodeficiency in a potential late-onset Pompe disease carrier with a double dual variant, each in cis formation.

요 약

폼페병(PD, Pompe disease), 2형 당원축적병(Glycogen storage disease type II)은 보통염색체 열성 질환으로 용해소체 효소인 acid maltase (acid α-glucosidase, GAA) 결핍으로 인한 대사근육병이다. 한 종류의 효소 결핍에 의한 질환이지만, GAA의 결핍정도와 유전자형에 따라 임상양상이 다르게 나타난다. 발병 시기에 따라 크게 영아형 폼페병(infantile onset Pompe disease, IOPD), 성인형 폼페병(late onset Pompe disease, LOPD)으로 나눌 수 있다. 저자들은 신생아기 때 호흡곤란증후군을 진단받고 치료받은 후 잠시 혈중 CK 증가가 확인되었으나 다른 임상증상 없이 경과 관찰 후 호전되었고, LSD 스크리닝 검사 결과 상 GAA 수치가 0.58 umol/h/L로 감소되어 있음을 확인한 1례에 대해 보고하고자 한다. 해당 환아를 PD 의증으로 고려하여 시행한 GAA enzyme essay 상 total GAA level은 16.2 nmol/2hr/mg protein, GAA with acarbose level은 2.0 nmol/2hr/mg protein, acarbose/total level의 비율은 12.0%로 낮은 수치를 확인하였다. 환아의 유전자 검사 상 exon #4에서 두개의 likely pathogenic heterozygous mutation인 c.752C>T (p.Ser251Leu), c.761C>T (p.Ser254Leu), exon #12에서 heterozygous mutation인 c.1726G>A (p.Gly576Ser), exon #15에서 heterozygous mutation인 c.2065G>A (p.Glu689Lys)이 확인되었다. 환아의 7세 오빠는 유전자 검사에서 정상으로 확인되었고, 아버지는 환아에서 동일하게 확인된 exon #12에서 heterozygous mutation인 c.1726G>A (p.Gly576Ser), exon #15에서 heterozygous mutation인 c.2065G>A (p.Glu689Lys)이, 어머니에서는 exon #4에서 두 개의 likely pathogenic heterozygous mutation인 c.752C>T (p.Ser251Leu), c.761C>T (p.Ser254Leu)가 확인되었다. Pathogenic한 유전자 두개가 있으면 보통 환자로 인식될 수 있으나 이 환아에서처럼 pathogenic한 유전자 두 개가 있더라도 *cis* 형태로 하나처럼 움직인 경우 PD 환자가 아니라 carrier 일 수 있다는 것을 경험한 증례였다. 이에 PD 환아의 유전검사 결과를 해석할 시 pathogenic variant 유전자가 두 개일지라도 혹시 *cis* 형태로 하나의 유전자인지를 확인하여, pseudodeficiency나 potential LOPD carrier일 수 있는 점을 고려하며 여러 임상 양상을 취합하여 진료를 시행하는 것이 필요하다.

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