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증 례

엑솜 시퀀싱으로 진단된 가족성 당원병 IXa 형 증례

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Familial Glycogen Storage Disease Type IXa Diagnosed by Targeted Exome Sequencing

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Glycogen storage disease type IX (GSD IX) is caused by deficiency of phosphorylase kinase which plays a role in breakdown of glycogen. Mutations in PHKA2 are the most common cause of GSD IX (GSD IXa). Clinical manifestations of GSD IXa include hepatomegaly, elevation of liver enzyme, growth retardation, fasting hypoglycemia, and fasting ketosis. However, the symptoms overlap with those of other types of GSDs. Here, we report Korean familial cases with GSD IXa whose diagnosis was confirmed by targeted exome sequencing. A 4-year old male patient was presented with hepatomegaly and persistently elevated liver enzyme. Liver biopsy revealed swollen hepatocyte filled with glycogen storage, suggesting GSDs. Targeted exome sequencing was performed for the differential molecular diagnosis of various types of GSDs. A hemizygous mutation in PHKA2 were detected by targeted exome sequencing and confirmed by Sanger sequencing: c.3632C>T (p.Thr121Met), which was previously reported. The familial genetic analysis revealed that his mother was heterozygous carrier of c.3632C>T mutation and his 28-month old brother had hemizygous mutation. His brother also had hepatomegaly and elevated liver enzyme. The hypoglycemia was prevented by frequent meals with complex carbohydrate, as well as cornstarch supplements. Their growth and development is in normal range. We suggest that targeted exome sequencing could be a useful diagnostic tool for the genetically heterogeneous and clinically indistinguishable GSDs. A precise molecular diagnosis of GSD can provide appropriate therapy and genetic counseling for the family.

Key words: Glycogen storage disease type IXa, Targeted exome sequencing, PHKA2, Hepatomegaly

Introduction

Glycogen storage disease (GSD) is a group of disorders caused by inborn errors of glycogen metabolism. To date, 23 types of GSD have been recognized showing various clinical spectrum involving liver, muscle, heart, and sometimes central nervous system[1)]. The types of GSD are classified by the specific enzyme deficiency involved in glycogen metabolism. However, the clinical differentiation among the various types of GSD is difficult because the clinical symptoms including hypoglycemia, hepatomegaly, and muscle weakness are overlapped among the various types of GSD. Moreover, the wide range of phenotypic variation is

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present, and the disease may have different clinical course even though the same enzyme is deficient[1]. Early onset forms of GSD presented with more severe typical symptoms, whereas other forms are relatively mild or even asymptomatic, and often cause confusions in making differential diagnosis. The molecular differential diagnosis of GSD has been largely depended on conventional gene by gene serial sequencing that is time- and cost-consuming. The targeted exome sequencing of candidate gene panel using massive parallel sequencing has been enabled the time- and cost-saving correct molecular differential diagnosis of various types of GSD having high genetic heterogeneity[1,2].

GSD type IX is one of common types of GSD caused by a deficiency of phosphorylase kinase (PhK, EC.2.7.1.38)[3]. Phosphorylase kinase is composed of four different subunits with four copies of each (αβγδ) and is responsible for catalyzing inactive phosphorylase into an active form, which initiates glycogen degradation[4,5]. Each of the subunits is encoded by different genes or alternative splicing of a single gene[4,5]. The PhK enzyme subunit deficiencies causing liver GSD IX include three types: GSD IXa (deficiency of α subunit, encoded by the PHKA2 gene, X-linked inheritance) (MIM # 306000); GSD IXb (deficiency of β subunit, encoded by the PHKB gene, autosomal recessive inheritance) (MIM #261750); and GSD IXc (deficiency of γ subunit, encoded by the PHKG2 gene, autosomal recessive inheritance) (MIM # 613027)[6]. GSD IXa (MIM 306000) is most common type of the GSD type IX cases, accounting for 75% of GSD IX cases, results from the deficient liver-α subunit[3] encoded by PHKA2 gene located at Xp22.2-22.17). Clinical symptoms of GSD IXa include growth retardation, hepatomegaly, elevated liver transaminase, hypercholesterolemia, hypertriglyceridemia, hypoglycemia, and occasional elevated levels of uric acid and lactic acid[8]. However, the phenotype ranges from mild hepatomegaly or elevated liver enzymes to severe hypoglycemia, lactic acidosis, and growth retardation[9].

Here, we report a Korean familial cases with GSD IXa whose molecular diagnosis were confirmed by targeted exome sequencing.

Case Report

A 4-year-old male patient was presented with hepatomegaly and persistently elevated serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The AST/ALT elevation was first noticed incidentally after influenza infection when he was 3 years old and persist for 1-year follow up. His parents were healthy non-consanguineous Koreans and had no familial history of liver diseases. He had one elder brother and one younger brother. He was born at 40 weeks of gestation and birth weight was 3.4 kg. The prenatal and postnatal period was uneventful and his developmental milestone was normal and hypoglycemic episodes during infancy was not definite. On physical examination, his height was 103.8 cm (10-25 percentile) and weight was 16.2 kg (10 percentile). He had distended abdomen and the liver was palpable at three finger breadths below the right costal margin. He had no jaundice or splenomegaly. Neurologic exam was normal. His hemoglobin, white blood cell counts, and platelet counts were 11.5 g/dL (normal range, 11.0-14.0 g/dL), 12.6×10^3/μL ($4-13\times10^3$/μL), and 285×10^3/μL ($134-387\times10^3$/μL), respectively. Serum aspartate aminotransferase (AST) was 106 IU/L (5-40 IU/L) and alanine aminotransferase (ALT) was 48 IU/L (8-41 IU/L). Serum

total bilirubin was 0.2 mg/dL (0.2–1.2 mg/dL). Total cholesterol was 129 mg/dL (120–180) and triglyceride was 192 mg/dL (44–180 mg/dL). Serum creatine kinase (CK) was 53 IU/L (58–348 IU/L). Lactic acid was 2.21 mmol/L (0.7–2 mmol/L) and uric acid was 6.4 mg/dL (2.0–5.5). Serological tests for viral hepatitis revealed no evidence of infection. On sonographic examination, his liver was enlarged with homogeneously increased parenchymal echogenicity suggesting fatty liver, but the intrahepatic and extrahepatic biliary ducts were normal. A liver biopsy was performed and histological examination revealed the enlarged hepatocyte with clear and wispy pink cytoplasm on H&E stain (Fig 1A). On PAS-stained tissue section showed diffuse glycogen deposits throughout the cytoplasm (Fig 1B). With a high suspicion of GSD, Sanger sequencing for *G6PC* was performed to rule out mild type of GSD Ia but no pathogenic variant was found. Therefore we decided to perform targeted exome sequencing for various types of GSD to appropriated molecular diagnosis. GSD panels included *GYS1, GYS2, G6PC, SLC37A4, GAA, AGL, GBE1, PYGM, PFKM, PYGL, PHKA1, PHKA2, PHKB, PRKAG2, PHKG2, PGAM2, LDHA, ALDOA, ENO3, PGM1, GYG1* and *LAMP2*. Briefly, genomic DNA was extracted from the peripheral blood. Library was prepared using the TruSight One Sequencing Panel (Illumina, Inc., San Diego, CA). Massively parallel sequencing was performed on the Illumina NextSeq platform. Average coverage of depth of the entire panel was 77x, and 97% of targeted bases were covered by 10x sequence reads. Sequence reads were aligned to hg19 with Burrow-Wheeler Aligner (version 0.7.12, MEM algorithm). Duplicate reads were removed by using Picard-tools1.96. Local realignment and base quality recalibration was done by The Genome Analysis Toolkit (GATK version 3.4-46). Variant calling was performed by GATK Haplotype Caller. Variants were annotated by Variant Effect Predictor (79) and dbNSFP (3.0). The targeted exome sequencing and confirmatory Sanger sequencing identified one hemizygous variant in *PHKA2*, c.3632C>T (p.Thr 121Met) (reference sequence: NM_000292.2), which was previously reported[3] (Fig 2).

After diagnosis of GSD IXa for the proband, familial mutation analysis found that his mother carried the heterozygous mutation and his 28 month-old younger brother also had same hemizygous mutation whereas his older brother did not carry the mutation (Fig 3). We evaluated the younger brother who was born at 40 weeks of

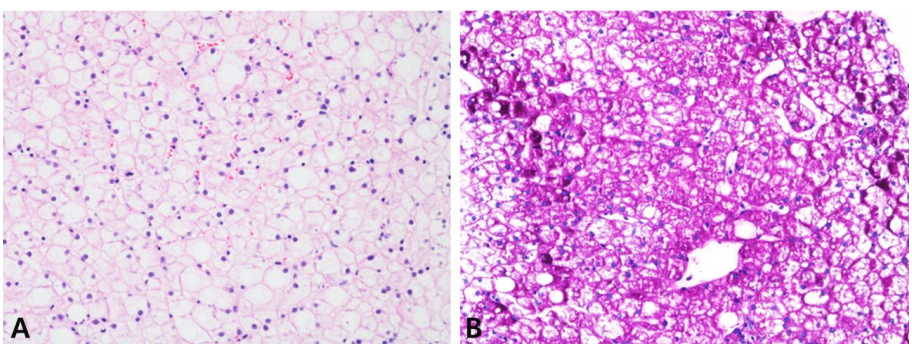

Fig. 1. Histologic examination (original magnification, ×200). (A) H&E stained tissue section shows enlarged hepatocytes with clear and wispy pink cytoplasm. (B) PAS-stained tissue section shows diffuse glycogen deposits throughout the cytoplasm.

gestation and birth weight was 3.4 kg and had normal developmental milestone to that moment. Height was 89.9 cm (50 percentile) and weight was 14.9 kg (75–90 percentile). His abdominal protrusion was more evident compared to that of the proband and liver was palpable by palm size below the right costal margin. Neurologic exam was normal. His hemoglobin, white blood cell counts, and platelet counts were 12.3 g/dL, 12.2 $\times 10^3/\mu L$, and $333 \times 10^3/\mu L$, respectively. Serum AST/ALT was 370/586 IU/L and total bilirubin was 0.3 mg/dL. Total cholesterol was 153 mg/dL and triglyceride was 363 mg/dL and CK was 53 IU/L. Lactic acid was 3.11 mmol/L and uric acid was 2.8 mg/dL. Sonographic examination also revealed diffusely increased liver echogenesity with hepatomegaly. Echocardiographic exam showed normal cardiac structure and function.

We educated his parents on preventing hypoglycemia via frequent meals high in complex carbohydrates and protein, as well as cornstarch supplements. At the latest evaluation of the proband at 6 years of age, his growth velocity was

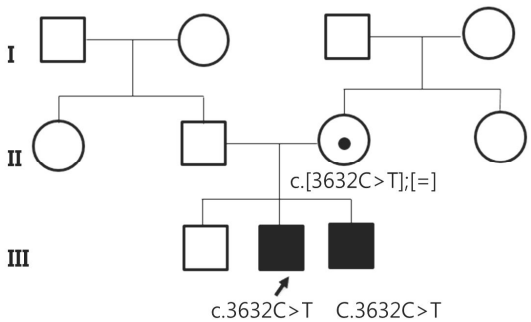

Fig. 3. Pedigree of the proband with results of familial mutation analysis. Familial mutation analysis found that his mother carried the heterozygous mutation and his younger brother also had same hemizygous mutation that made diagnosis of familial GSD IXa.

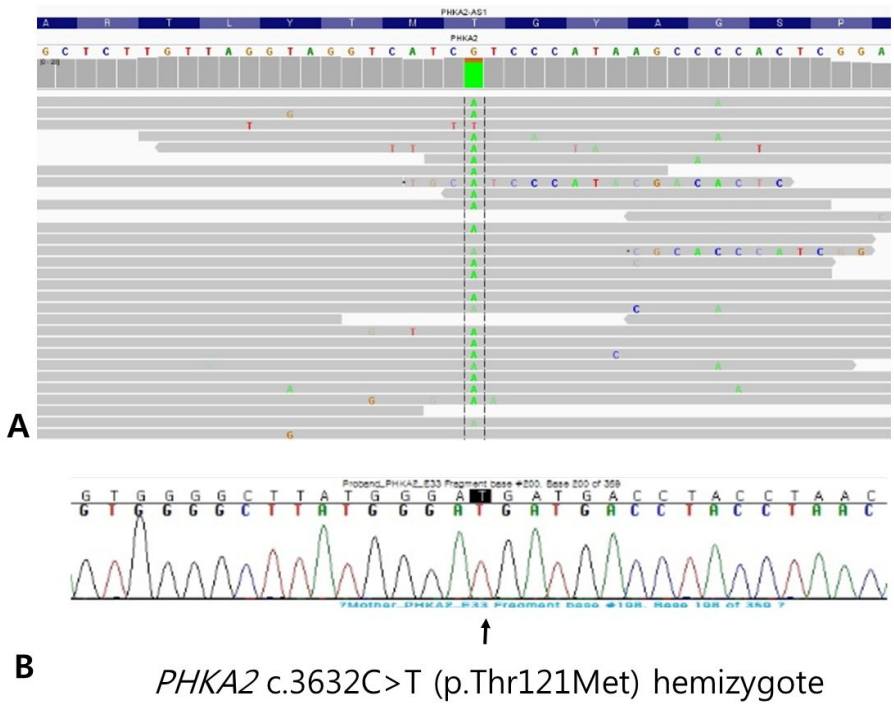

Fig. 2. *PHKA2* variant detected by targeted exome sequencing and Sanger sequencing. The targeted exome sequencing (A) and confirmatory Sanger sequencing (B) identified one hemizygous variant in *PHKA2*, c.3632C>T (p.Thr121Met) (reference sequence: NM_000292.2).

improved. His height was 113 cm (25 percentile) and his weight was 19.2 kg (25–50 percentile). He had not experienced any hypoglycemic episodes. Remarkably, elevated AST/ALT was normalized to 40/41 IU/L. His younger brother also showed normal growth velocity. At 38 month of age, height was 96 cm (50 percentile) and weight was 18 kg (90 percentile). He neither had any hypoglycemic event. Unlike the proband, his AST/ALT elevation was persisted (341/284 IU/L).

Discussion

GSD is clinically and genetically heterogeneous group of diseases and many clinical manifestations are overlapped among the different types. Clinical differentiation of various types of GSD could be difficult especially in the cases with mild or atypical presentation. Therefore, molecular differential diagnosis can provides the advantage of appropriate diagnosis, surveillance and therapeutic approaches[10,11]. Targeted exome sequencing of the candidate gene panel is a useful diagnostic tool and enables to shorten the diagnostic odyssey of the diseases with genetic heterogeneity including GSD. In this case, although GSD was highly suspected by histologic exam of liver biopsy, the clinical presentation of our patient was uncertain to differentiate the types of GSD. He had no evident history of neonatal hypoglycemia, profound growth retardation, developmental delay, or muscle weakness. The results of biochemical evaluation showed no hypoglycemia, mild elevation of triglyceride, lactic acid, and uric acid. Therefore, targeted exome sequencing of GSD gene panel provided a useful diagnostic option for differential diagnosis in this situation.

GSD IX is one of the most common type of GSDs, accounting for 25% of patients with all GSDs[3]. Until recently, GSD type I has been seemed to be the most prevalent type of hepatic GSDs in Korea. However, several studies reported that GSD IX is also common in Korea[11,12]. GSD IX could be underdiagnosed due to the mild clinical presentation and benign course. The next generation sequencing platform might be helpful for identifying undiagnosed patients.

The clinical manifestation of GSD IX ranges from mild (hepatomegaly and elevated liver enzymes) to severe (hypoglycemia, short stature, mild gross motor delays, progressive liver disease and liver cirrhosis)[6]. Our two sibling patients had mild clinical presentation with hepatomegaly and hepatic enzyme elevation, without any severe symptoms of liver–type GSD, such as hypoglycemic seizure, delayed development, or growth retardation. Our case could provide an additional clinical and genetic information of Korean patients with GSD IXa.

In terms of treatment and prognosis of GSD IX, liver fibrosis can occur and in rare instances progress to cirrhosis, but it is typically associated with mutation of *PHKG2* responsible for the GSD IXc[6]. Currently, there are no reports demonstrating liver failure in patients with GSD IXa[13]. Recently, Schippers et al.[14] proposed that GSD IXa is a mild disorder and it is questionable whether strict dietary therapy should be required for a normal growth pattern. Roscher et al.[9] also reported that the majority of the patients who were not treated showed improved, normalized or stable liver enzymes and liver adenoma or cirrhosis seemed rare. Moreover, a recent report of 13 chinese patients with GSD IXa also demonstrated a favorable prognosis of the patients during 0.5 to 25.7 year of follow–up[13]. The authors reported that the clinical and biochemical manifestations improved and even disappeared with age[13]. In

our cases, both of them were treated with uncooked corn starch supplement. The proband's hepatomegaly was improved and liver enzymes has been normalized at the age of six. However, the younger brother showed persistently elevated liver enzymes until age of 38 month. Further follow-up is needed for changes of younger brother's liver enzyme whether the elevated liver enzymes will be improved with age. In Korea, all 13 previously reported patients with GSD IX were GSD IXa, their clinical manifestations were variable from mild hepatomegaly to severe hypoglycemic episodes and growth retardation[11,12,15]. All of them were treated with uncooked corn starch and none of them showed poor outcome till this moment[11,12,15]. However, the data is still insufficient to draw a confirmative conclusion due to the short-term follow up period. More long-term and detailed delineation of clinical course and prognosis is needed for the natural history as well as developing optimal treatment guideline.

Although our proband's mother carrying the heterozygous mutation was asymptomatic, there is a report demonstrating female carriers who have mild hepatomegaly and increased liver enzyme which had been gradually improved. This manifesting female carrier could be explained by skewed inactivation of normal X chromosome[16]. Therefore, carrier female might need careful evaluation.

In conclusion, we suggest that targeted exome sequencing could be a useful diagnostic tool for the genetically heterogeneous and clinically indistinguishable GSDs. A precise molecular diagnosis of GSD can provide appropriate therapy and genetic counseling for the family.

요 약

당원병 IX형은 phosphorylase kinase 효소 결핍으로 분해되지 않은 당원이 간 또는 근육에 축적되는 유전성대사이상질환이다. 당원병 IXa형은 당원병 IX형 중 가장 흔한 형태로 *PHKA2* 유전자 변이로 발생한다. 당원병 IXa형의 임상증상은 간 비대, 간 효소 수치 상승, 성장 지연, 저혈당 등이 있다. 그러나, 이러한 임상 증상은 다른 타입의 당원병의 증상과 비슷하거나 겹쳐서 임상적으로는 구분하기가 어렵다.

저자들은 표적 엑솜 시퀀싱으로 진단된 가족성 당원병 IXa형 증례를 보고하고자 한다. 4세 남아가 간 비대와 간 효소 수치 상승을 주소로 내원하였다. 간 조직검사결과 간세포에 당원이 축적되어 있어 당원병을 의심하였으나 *G6PC* 유전자 검사는 음성이었다. 이에 당원병 타입을 감별진단 하기 위해 표적 엑솜시퀀싱을 시행하였으며, *PHKA2* 유전자에서 질환과의 연관성이 이미 보고된 바 있는 c.3632C>T (p.Thr121Met) 변이가 반접합체(hemizygote)로 발견되어 당원병 IXa로 진단하였다. 가족 유전자 검사를 통해 어머니가 이형접합체 보인자임을 확인하였으며, 남동생이 같은 변이를 가진 반접합체임을 확인하였다. 28개월 된 환자의 남동생 역시 신체 검진 상 간 비대가 있었으며, 혈액검사상 간 효소 수치가 상승되어 있어 같은 질환으로 확진하였다. 이환된 형제 모두 생 옥수수 전분 섭취와 복합 탄수화물을 섭취하도록 식이 조절을 하였으며 2년 추적관찰 동안 정상 성장 발달을 보이고 있다.

당원병과 같이 임상적으로 구분이 어려우며 유전학적으로 다양한 유전자 변이를 보이는 당원병과 같은 질환의 분자 유전학적 감별진단에 표적 엑솜 시퀀싱이 유용한 진단법이 될 수 있다. 신속하고 정확한 분자 유전학적 감별진단을 통해 환자와 보호자에게 질병의 적절한 치료법, 질병의 예후에 관한 정확한 정보를 제공할 수 있을 뿐 아니라, 적절한 유전상담을 제공할 수 있다.

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