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IDENTIFICATION OF p.HIS119LEU MUTATION IN THE *G6PC* GENE OF A VIETNAMESE PATIENT WITH GLYCOGEN STORAGE DISEASE TYPE Ia

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ABSTRACT

Glycogen storage disease type Ia (GSD Ia), a rare autosomal inherited disorder, is characterized by accumulation of excessive glycogen and fat in the liver. Primary symptoms of GSD Ia include hypoglycemia; metabolic acidosis; elevated levels of lactate, uric acid and lipids; hepatomagaly and growth retardation. Glycogen storage disease type Ia was caused by mutations in the *G6PC* gene. In this study, mutations in a Vietnamese patient with glycogen storage disease type Ia were analyzed using the whole exome sequencing method. A missense mutation c.356A>T (p.His119Leu) in the *G6PC* gene of the patient was identified in exon 3. Genetic analysis confirmed that this mutation was present under homozygous form *In-silico* analyses using SIFT and Mutation Taster confirmed the damaging effects of this mutations on the function of the proteins. This result enriches knowledge of the *G6PC* gene mutation spectrum and provides genetic data for further studies on glycogen storage disease type Ia in Viet Nam.

Keywords: *G6PC* gene, Glycogen storage disease type Ia, mutation p.His119Leu, rare disease, whole exome sequencing.

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INTRODUCTION

Glycogen storage disease (GSD) is a rare group of genetic metabolic disorders that affects glycogen metabolism. In patients with GSD, while endogenous glucose production is suppressed in postprandial period, exogenous glucose is either metabolized to pyruvate or stored as glycogen in the liver and skeletal muscle (Saltik et al., 2000; Ozen, 2007). Glycogen stores must be metabolized by enzymes before being used. In the absence of enzymes needed for glycogen degradation, the glycogen will accumulate and cause disorders. Glycogen storage disease affect primarily liverand muscles. The incidence rate of GSD I is approximately 1/20.000–1/43.000 live births (Hicks et al., 2011).

Depending on the level of enzyme deficiency and the affected tissues, glycogen storage diseases were classified into twelve type (Wolfsdorf & Weinstein, 2003; Rake et al. 2006). Different GSD types have different symptoms. Most types of GSD affect liver (type 0, I, III, IV, VI and IX). However, some types of GSD have complex signs and symptoms, affecting muscles, liver, and heart. These types of GSD (except GSD type 0) can cause the liver to enlarge due to glycogen being stored in the liver instead of being released as glucose into blood. Common symptoms of GSD are hypoglycemia, hyperlactatemia, hepatomegaly, hypertriglyceridemia. GSD type V and VII affect primarily the skeletal muscles, with muscle weakness and cramps being the most common symptoms. In newborns, some GSD types lead to death within the first year of life, whereas other glycogen storage diseases are relatively asymptomatic or may cause only exercise intolerance (Hicks et al., 2011). Glycogen storage disease type I including type Ia (GSD Ia) and Ib (GSD Ib) characterized by hepatomegaly resulting from accumulation of glycogen in the liver. Among them, GSD type Ia is more common, accounting for about 80% of patients with GSD type I with an estimated annual incidence rate of about 1/100,000 live births (Chou et al. 2002).

Glycogen storage type Ia is an autosomal recessive disorder cause by deficiencies in the activities of glucose-6-phosphatase (G6Pase), an integral resident endoplasmic reticulum (ER) protein. The *G6PC* gene is expressed primarily in the liver, kidneys, and intestines (Chou et al.. 2002). Patients with GSD Ia present many abnormal biochemical symptoms, mainly fasting hypoglycemia, lactic acidosis, hyperlipidemia, hyperuricemia, hepatomegaly, and growth retardation (Gu et al. 2014; Karthi et al. 2019).

The *G6PC* gene is located on chromosome 17q21.31 which is the long arm of chromosome 17 at position 21.31. G6Pase which is a glycoprotein with 357 amino acid, is anchored in the membrane of the ER by 9 transmembrane helices (Pan et al.. 1998). Up to now, approximately 116 mutations in of the *G6PC* gene have been recorded among 550 patients in the Human Gene Mutation Database (HGMD). Almost all previously reported variants were missense. The active center of *G6PC* is proposed to comprise Lys-76, Arg-83, His-119, Arg-170 and His-176 (Stukey & Carman 1997; Hemrika & Wever, 1997). Mutations in active sites were shown to completely abolish *G6PC* enzymatic activity.

In this study, whole exome sequencing was performed on a Vietnamese patient with GSD type Ia. A missense mutation p.His119Leu in *G6PC* gene was found in the patient and members of his family. Information about this mutation will contribute to a better understanding of the disease.

MATERIALS AND METHODS

Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Institute of Genome Research (No. 18/QD-NCHG on 22 March, 2018, Institute of Genome Research Institutional Review Board, Ha Noi, Vietnam).

Patient

The patient with glycogen storage disease type Ia, a boy aged 7 years and 11 months, is the third child in the familiy whilethe second child died at 3 months of age due to unknown coma. The patient presented the first metabolic crisis at 3 months of age after immunization injection. At that time, he presented tachypnea, lethargy, metabolic acidosis (7.05), hypoglycemia (1.9 mmol/l, normal: 3.3−5.5 mmol/l), hyperlactatemia (9.5 mmol/l, normal: 3.3−5.5 mmol/l), hypertriglyceridemia (7 mmol/l, normal: <1,65 mmol/l), ketonuria, elevated transaminase (ALT: 400, normal: <40). After diagnosis, the patient was treated with glucose infusion on metabolic crisis. Over the long term, the patient was treated with applied diet therapy with soymilk, cornstarch, medium-chain triglyceride oil and avoiding long fasting. He showed normal health until 6 years old. He was admitted to Vietnam National Hospital of Pediatrics because of tachypnea and lethargy. The patient presented hepatomegaly 7 cm under costal margin, hyperlactatemia (7.5 mmol/l), elevated transaminase (AST/ALT:1544/950 UI/l), hypertriglyceridemia (8.3 mmol/l).

DNA extraction

Peripheral blood samples from the patient and his family members were provided by Department of Endocrinology, Metabolism and Genetics, Vietnam National Hospital of Pediatrics. Genomic DNA was extracted from peripheral blood samples using QIAamp DNA Blood Mini Kit-QIAGEN following the manufacturer's guidelines.

Whole exome sequencing

The DNA library of patients was prepared using Agilent SureSelect Target Enrichment kit and whole exome sequencing was performed by applying Illumina platform.

Bioinformatics analysis and variants screening

After sequenced by Illumina platform, raw data was assessed and subjected to quality control using FastQC. The paired-end reads were aligned to the reference human genome (GRChr37/hg19) using BWA 0.7.10 (Li & Durbin, 2009). Picard tools (http://broadinstitute.gith-ub.io/picard/) was used to processed post-alignment data. Genome Analysis Toolkit v3.4 was used for variant calling (McKenna et al. 2010). The The effects of variants on genes such as amino acid changes were predicted using SnpEff v4.1 (Cingolani et al., 2012). *In-silico* analyses to confirm the effect of the mutations on the structure and function of the proteins was performed using SIFT (Ng & Henikoff, 2003) and Mutation Taster (Schwarz et al., 2014).

The candidate variants were filtered using four conditions: (i) variants occurring in genes associated with GSD type I; (ii) all variants with a minor allele frequency of 0.1% were excluded; (iii) variants predicted as "Damaging" or "Disease causing" (iiii) all variants reported as benign in ClinVar database were excluded.

Sanger sequencing to validation variants

A fragment of *G6PC* gene was amplified using a specific primer designed using Primer blast (https://www.ncbi.nml.gov/tools/primer-blast/): *G6PC*-2F: 5'-TTCCCAGAGCCTTGC ACAAT-3' and *G6PC*-2R: 5'-AAGCCCT GCTGCTACTTCAC-3'. PCR conditions used for the amplification were: 95^{o}C/12 min; (95^{o}C/45 s; 64^{o}C/45 s; 72^{o}C/45 s) x 35 cycles and 72^{o}C/8 min. The PCR product (639 bp) underwent electrophoresis in agarose 1%. Sanger sequencing was performed on DNA samples of the patient and members of his family for validating the variants of interest identified in bioinformatics analysis.

RESULTS AND DISCUSSION

Bioinformatics analysis revealeda homozygous missense variant c.356A>T (p.His119Leu) in exone 3 of *G6PC* gene. This mutation involves a change from Histidine (His) to Leucine (Leu) at residue

119 (p.His119Leu). The mutation was first identified by Wu et al. (2000) in a Taiwan patient with glycogen storage disease type Ia. This mutation was reported in the dbSNP database (rs1401928680) but not in ClinVar.

Sanger sequencing showed that the patient's parents and sister carried a heterozygous c.356A > T mutation (Fig. 1). The second child of this family, who died at 3 months of age, was not reported in this study.

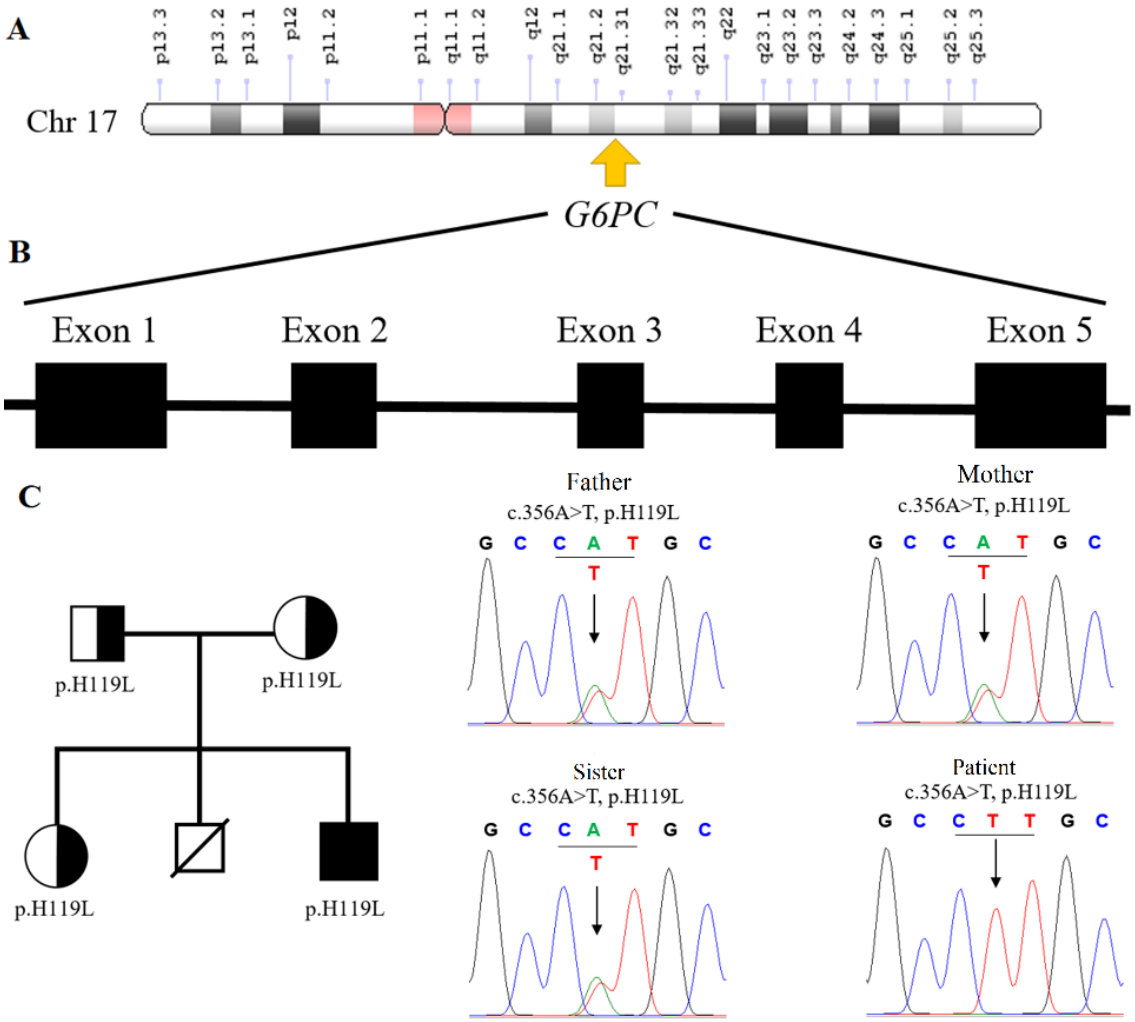

Figure 1. Analysis of p.His119Leu mutation in the patient and his family. (A) *G6PC* gene is located on chromosome 17q21.31 which is the long (q) arm of chromosome 17 at position 21.31. (B) Exon–intron graph of *G6PC* gene. (C) Pedigree of the patient's family and variant p.His119Leu in ther *G6PC* gene

With a SIFT score of 0.012 (Fig. 2A) and MutationTaster2 result as disease-causing, this mutation is predicted to be deleterious. In addition, the His119 residue is located in a conserved amino acid across different species (Fig. 2B).

In this study, the mutation p.His119Leu found in the patient changed hydrophilic amino acid (histidine) to hydrophobic amino acid (leucine). His-119 is an active site residue of G6Pase protein (Hemrika & Wever, 1997; Stukey & Carman, 1997),

providing the proton needed to liberate the glucose moiety (Chou & Mansfield, 2008). The mutation p.His119Leu has been identified in GSD-Ia patients and shown to completely abolish G6PC enzymatic activity (Shieh et al., 2002). The roles of His-119 were confirmed by Lei et al (1995) which substituted this amino acid with either alanine (His119Ala), isoleucine (His119Ile), lysine (His119Lys), methionine (His119Met), asparagine (His119Asn), arginine (His119Arg) and threonine (His119Thr). All of the His-119 mutant have shown a loss of activity in G6PC catalysis.

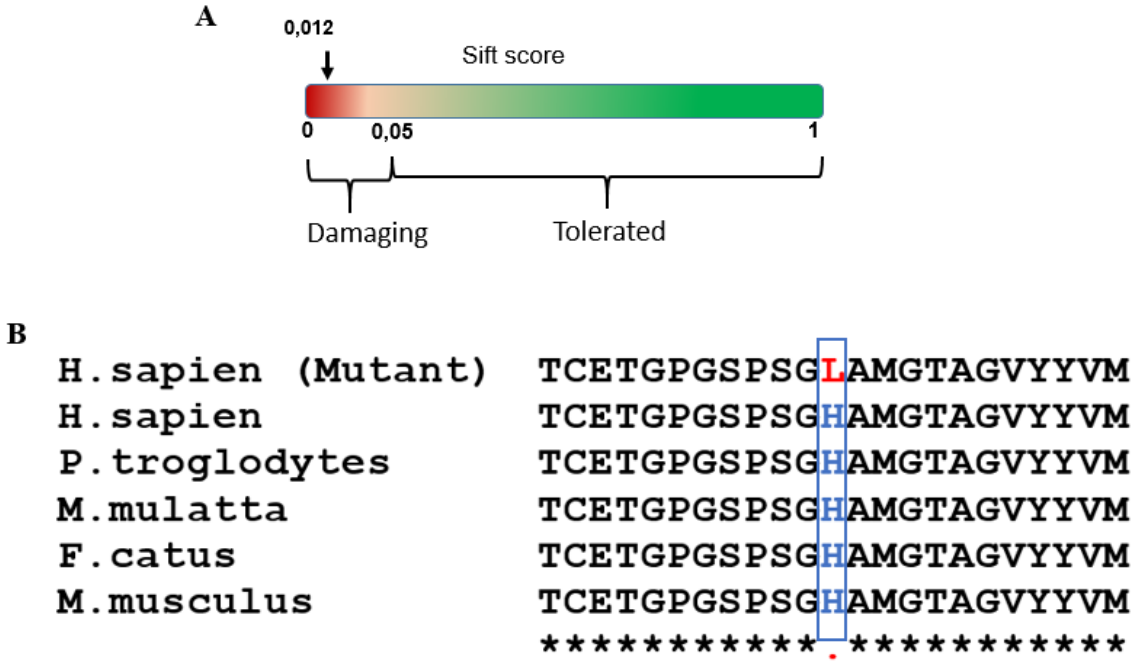

Figure 2. In-silico analysis of the *G6PC* protein. (A) The mutation was predicted to be "Damaging" by SIFT. (B) Conservation of the amino acid changed by p.His119Leu in *G6PC* protein mutation across different species

Signs and symptoms of glycogen storage disease type Ia include low blood sugar (hypoglycemia), which can lead to seizures. Patient can also have a buildup of lactic acid in the body (lactic acidosis), high blood levels of uric acid (hyperuricemia), and excess amounts of fats in the blood (hyperlipidemia). Patients with GSD IA have abnormal enlargement of the liver (hepatomegaly), they may have thinning of bones (osteoporosis), gout, kidney disease, and high blood pressure in the blood vessels (Rake et al., 2002; Froissart et al., 2011). The patient in this study presented hypoglycemia, hyperlactatemia, hepatomegaly, and hypertriglyceridemia ketonuria; biochemical indices were abnormal. Other studies in Chinese and Indian patients with GSD Ia showed similar symptoms (Gu et al., 2014; Zheng et al., 2015; Karthi et al., 2019). This suggests that patients presented with severe hypoglycemia can be clearly diagnosed in early childhood. However, in some studies on mild cases without hypoglycemia and growth retardation, patient can be diagnosed in adolescence or adulthood with complications such as gouty arthritis, hepatitis or tumors called adenomas forming in the liver (Akanuma et al., 2000; Shieh et al., 2012). Therefore, the early diagnosis and identification by genetic analysis is very important for treatment.

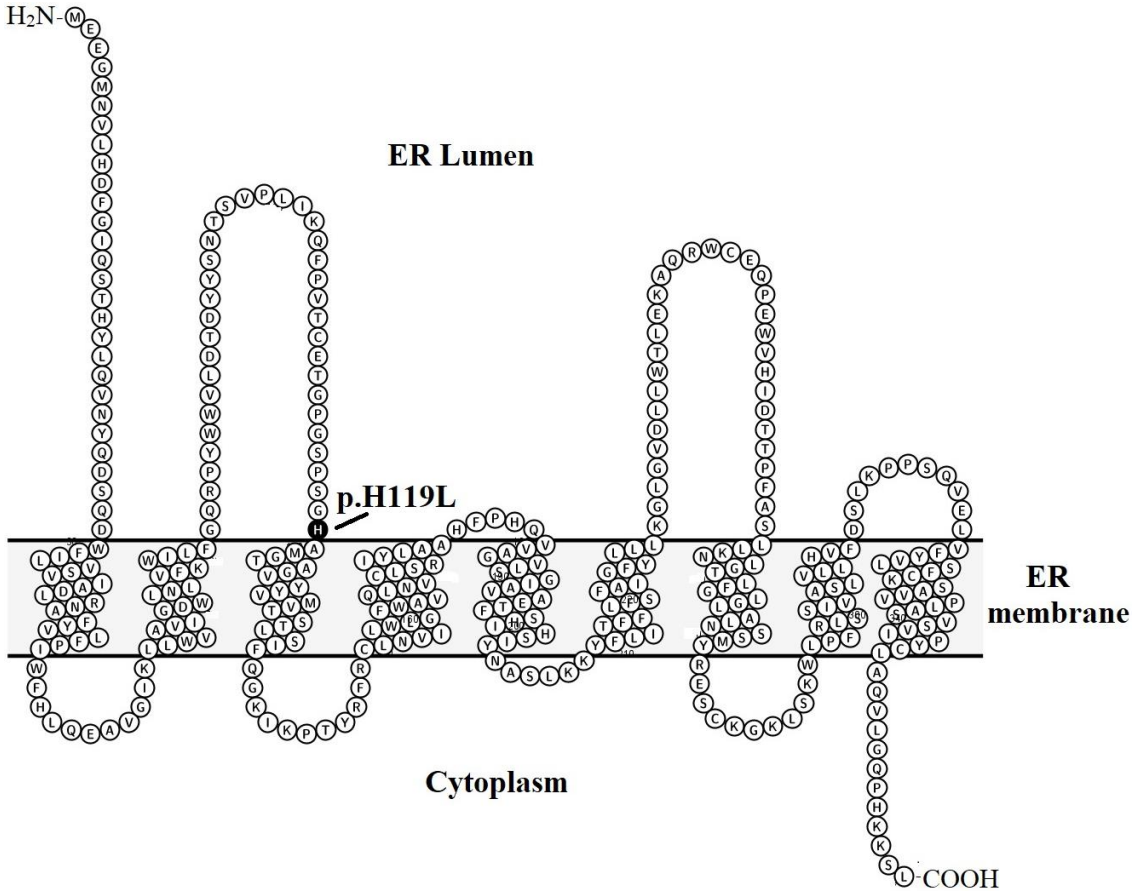

Figure 3. G6Pase protein anchored in the endoplasmic reticulum (ER) by 9 transmembrane helices. The N terminus localized in the ER lumen and the C terminus in the cytoplasm.

CONCLUSION

In conclusion, by applying whole exome sequencing, we identified the p.His119Leu mutation in the *G6PC* gene in a Vietnamese patient with glycogen storage disease type Ia. This is the first report of this mutation in Vietnamese patients with GSD type Ia. The result of this study enriches knowledge of the *G6PC* gene mutation spectrum and provided genetic data for further studies on glycogen storage disease type Ia in Viet Nam.

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