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Evaluation of the Genetic Background of Patients with Niemann-Pick Disease

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Abstract

Background: Congenital liver disease refers to a group of heterogeneous diseases from a clinical genetic point of view. The most crucial features are hepatosplenomegaly and elevated liver enzymes. This study aims to identify genetic variants causing the disease in three Iranian families with congenital liver disease using molecular techniques.

Methods: Patients were referred to Next Generation Genetic Polyclinic (NGGC) in Mashhad after confirmed congenital liver disease diagnosis by gastroenterologists. Following informed consent signed by participants, DNA was extracted from blood samples. Whole exome sequencing (WES) was performed for three probands. After the analysis of raw data, candidate variants were confirmed in the patients and their parents.

Results: We have found the possible disease-causing variant as the c.1718G>C variant (p. Trp573Ser) in the SMPD1 gene in the F-1 patient and c.1718G>C (p. Trp573Ser) in the SMPD1 gene in the F-3 patient. Moreover, we have found the c.3175C>T variant (p. Arg1059Ter) in the NPC1 gene in the F-2 patient.

Conclusions: In this study, disease-causing variants were identified in three probands suspected of Niemann-Pick disease. Such results show the relatively high power of molecular techniques to assist clinicians with disease management, therapeutic strategies, and preventive options such as preimplantation genetic diagnosis and prenatal diagnosis.

Keywords: Genetics, Liver disease, Niemann-Pick disease Whole exome sequencing.

Introduction

The key symptoms of congenital liver disease are elevated liver enzymes and hepatosplenomegaly, and the only proper treatment is a liver transplant. Niemann-Pick disease (NPD) causes mutations related to liver disease (1). NPD is a genetic disease with a broad spectrum of symptoms that vary in severity, affecting several organ systems. Based on genetic etiology, clinical signs, and presenting symptoms, NPD is classified into four types: A, B, C1, and C2 (2).

NPD types A and B are known due to SMPD1 gene mutations, which exhibit different clinical phenotypes because of a primary sphingomyelin storage disorder caused by acid sphingomyelinase deficiency (3-5). The classic phenotype of NPD is often characterized by hepatosplenomegaly, along with progressive ataxia, dystonia, and dementia. NPD type A is the most common in infants, particularly among Eastern and Central European populations (6). It is characterized by jaundice, hepatomegaly, failure to thrive, progressive nervous system deterioration, and profound brain damage, often leading to death before 18 months of age (7).

In NPD type B patients, symptoms of severe liver failure may or may not be present despite hepatosplenomegaly. Some patients with type B may have a distinctive cherry red spot in the macula (8). Type B patients rarely have central nervous system involvement but often experience

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compromised pulmonary function (6).

NPD type C typically develops in late childhood, and most patients do not survive until the second decade of life (3-5). Patients with NPD type C may present with severe ascites due to severe liver disease, respiratory failure, and renal failure (9, 10). If they do not have liver or lung disease, they often present with hypotonia and developmental delay (2). Other phenotypes of this disease include delayed motor development, neonatal hypotonia, and fatal neonatal liver disease. Adult-onset NPD phenotypic changes may include dementia, juvenile/adult dystonic lipidosis, DAF syndrome (downgaze paresis, ataxia, foam cells), and hepatosplenomegaly (10-12).

Here, we describe three cases to illustrate NPD's clinical manifestations and biochemical and molecular genetic findings.

Materials and Methods

We selected patients from families with congenital liver disorders who were referred to NGGC for genetic counseling and diagnostic genetic tests. After obtaining informed consent from participants, we collected peripheral blood samples in Ethylenediaminetetraacetic acid (EDTA) tubes. DNA was extracted using the conventional Salting out method. Following quantification and qualification of DNA samples, whole exome sequencing (WES) was performed for three probands (Macrogen Co., Korea).

Sanger Sequencing and Co-Segregation Analysis: Specific primers were designed (Metabion, Germany) for each candidate genetic variant using the online NCBI Primer designing tool (13). Polymerase chain reactions (PCR) were performed under standard conditions and then analyzed through Sanger sequencing on an ABI 3500 genetic analyzer (Applied Biosystems, Codon Co., Iran). Sequencing data were analyzed using NCBI BLAST and compared with wild-type samples and reference sequences from NCBI Gene and dbSNP databases (14-16).

Results

In this study, we present three families with five affected children, two of whom died at the ages of 11 months and four months, respectively. All patients' symptoms began at birth, including hypotonia, elevated liver enzymes, and developmental regression. Unfortunately, the older sibling died before the research was completed. In the F-2 family, another affected child with similar manifestations was reported. The F-3 family lost both affected children before genetic counseling, but based on clinical and laboratory data, the primary diagnosis suggested Niemann-Pick disease**.**

We identified two different mutations in the SMPD1 gene in the F-1 and F-3 patients. Since the F-3 patients had deceased and samples were unavailable, we conducted WES on the mother's sample. Data analysis revealed a heterozygous c.3175C>T variant (p. Arg1059Ter) in the NPC1 gene (Table 1).

Table 1. Clinical information of the studied subjects.

Proband ID	F-1	F-2	F-3	
Parameters				
Sex	Female	Female	Male	Male
Age	3y	1y	11m	4m
Age at onset	at birth	at birth	at birth	at birth
Alive	Yes	Yes	No	No
Parents' Consanguinity	Yes	Yes	Yes	Yes
Clinical Findings				
Hepatosplenomegaly	+	+	+	+
Elevated Liver Enzymes	+	+	+	+
Developmental regression	+	+	+	+
Hypotonia	+	+	+	+
Icterus	-	-	-	-
Cardiac abnormalities	-	-	-	-
Hyperglycemia	-	-	-	-
Skeletal dysplasia	+	-	-	-
Weight loss	+	-	-	-

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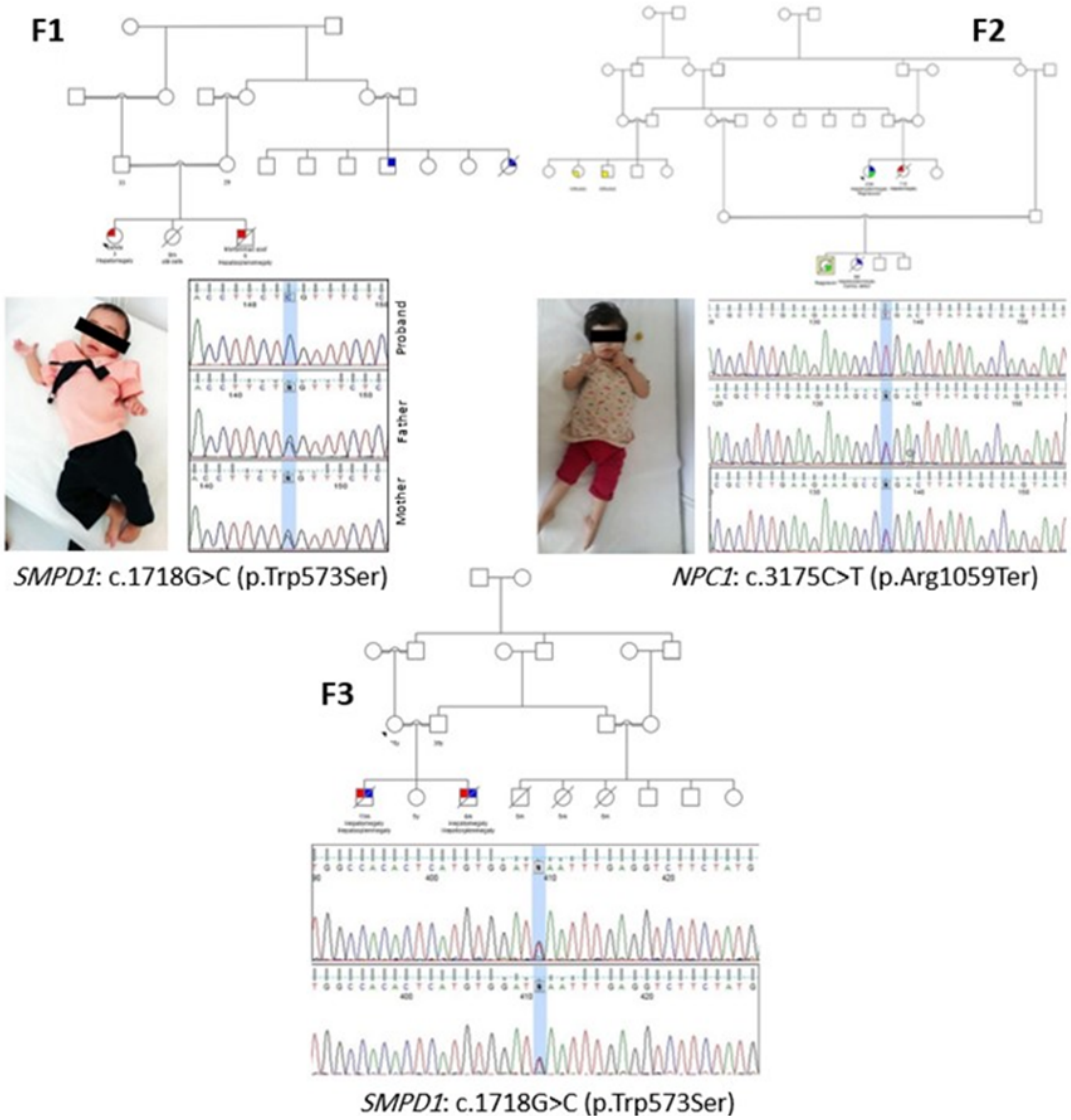

Fig. 1. Family pedigrees and chromatograms of Sanger sequencing results on family members.

All variants were confirmed in patients and parents in the F-1 and F-2 families. Due to the lack of samples from the F-3 family's patients, we confirmed the variant in the parents, both of whom were heterozygote carriers (Fig. 1 and Table 2).

Table 2. Variants identified in the families**.**

Proband's ID	Zygosity	Gene	ACMG Classification	Nucleotide/ Amino acid change
L-1	Mutated Homozygote	*SMPD1* NM_000543.5	Likely pathogenic	c.1718G>C (p. Trp573Ser)
L-2	Mutated Homozygote	*NPC1* NM_000271.5	Pathogenic	c.3175C>T (p. Arg1059Ter)
L-3	Heterozygote	*SMPD1* NM_000543.5	Likely pathogenic	c.1718G>C (p. Trp573Ser)

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Results

Here, we identified two patients who had the c.1718G>C variant in exon 6 of the SMPD1 gene. Moreover, our F-2 patient had the c.3175C>T variant in exon 21 of the NPC1 gene. This difference may occur due to population heterogeneity in Iran and the heterogeneous nature of NPD. In contrast to our report, a novel c.762delG frameshift (p. Leu256fs*) was identified in the heterozygous state in one of our proband's parents' samples. Interestingly, it has been shown that mutations reported from 39 Iranian patients are predominantly located in exon 2 of the SMPD1 gene and exons 8 and 9 of the NPC1 gene (17, 18).

The SMPD1 gene provides instructions for making an enzyme called acid sphingomyelinase. This enzyme is found in lysosomes, small compartments in the cell that digest and recycle molecules. Acid sphingomyelinase converts a fat (lipid) called sphingomyelin into another type of lipid called ceramide. Sphingomyelin also binds to a fat called cholesterol and helps form other fats involved in various cellular processes. The formation of these lipids is vital for the typical structure and function of cells and tissues. The coded protein also has phospholipase C activity. A defect in this gene is the cause of NPD type A and B (6).

The NPC1 gene encodes a large protein in the endosomes and lysosomes membrane and mediates intracellular cholesterol transport through cholesterol binding to its N-terminal domain. It is predicted to have a cytoplasmic C-terminus, 13 transmembrane domains, and three large loops in the endosomal lumen, the last loop is located at the N-terminus. This protein transports low-density lipoproteins to late endosomal/lysosomal compartments, which are hydrolyzed and released as free cholesterol. Defects in this gene cause NPD type C disease, a rare autosomal recessive disorder characterized by excessive accumulation of cholesterol and glycosphingolipids in late endosomal/lysosomal compartments (19). In a study of a patient with NPD type A, his cDNA was examined, and molecular analysis showed a c.1487T>G nucleotide change in the SMPD1 gene, which caused the replacement of Arg. Variant confirmation in the parents showed a heterozygous status for this mutation (20).

A study on SMPD1 gene sequencing identified eight previously described mutations and seven new mutations, including four missense mutations: c.682T>C (p.Cys228Arg), c.1159T>C (p.Cys387Arg), c.1474G>A (p.Gly492Ser), c.1795C>T (p.Leu599Phe), and a frameshift variant as c.169delG (p.Ala57Leufs20)(21). Molecular assessment of patients susceptible to NPD demonstrated eight types A and 13 types B patients. All SMPD1 mutant alleles were identified, including 17 different mutations, 10 of which were novel. Expression studies on six identified mutations confirmed their pathogenicity due to their low enzymatic activity. An allele with a mutation affecting a non-canonical splicing site produced only aberrant mRNAs, corresponding to previously reported non-functional SMPD1 partial transcripts. This study is the first comprehensive mutational analysis of Spanish NPD type A/B patients (22). In a study conducted in 2018, an infant with hepatosplenomegaly, hypotonia, delayed motor development, and bilateral cherry red spots was diagnosed with NPD type A, having a pathogenic frameshift mutation in the SMPD1 gene: c.573 del T (p. Ser192Alafs) (17).

A study of ten Japanese affected cases (seven patients in late infancy, two juveniles, and one adult) and one Caucasian patient with NPD type C was examined for mutations in the NPC1 gene. This study found 14 new mutations, including small deletions and point mutations. Three patients were homozygous, five were compound heterozygous, and the other three were suspected to be compound heterozygous with an unknown error in one of their NPC1 alleles. Of the 14 mutations, the Gly1553Ala substitution causing one splicing error in exon 9 was relatively common in Japanese patients, as two patients were

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homozygous and one was compound heterozygous for this mutation (18). Nowadays, by using high-throughput sequencing technologies and NGS analysis, any changes in whole candidate genes can be identified (23). Identifying the underlying cause of the disease in these people makes it easier to manage NPD. Considering that consanguineous marriage is common in Middle East countries such as Iran, genetic counseling, especially for families with an affected child, is necessary. From another standpoint, more studies on the role of proteins encoded by these genes will aim to develop therapeutic methods using targeted drug treatments along with high-powered molecular techniques to help clinicians manage the disease and offer preventive options such as preimplantation genetic and prenatal diagnoses.

Discussion

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Conflicts of interest

There is not any conflict.

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