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Novel mutation in *SMPD1* gene found by whole-exome sequencing in Niemann-Pick disease patient

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ABSTRACT

Niemann-Pick diseases (NPD) are heterogeneous and autosomal recessive disorders and are classified into four types: type A, type B, type C1, and type C2 that type A and B Niemann–Pick is induced via *SMPD1* gene mutations. Accumulation of sphingomyelin in the central nervous system is the first and prominent cause of the classical infantile type (type A) of NPD which is the most extreme form of NPD and causes severe central nervous system decay, hepatosplenomegaly, and fatal disorder. Our aim with this study was to propose that the novel likely pathogenic variant, c.1586G>C (p.529Trp>Ser) can cause Niemann-Pick disease type A. Present study involves a 2 years-old child as a result of a consanguineous marriage that has been diagnosed with clumsiness and gait disturbance at the beginning, and include hypotonia, microcephaly, severe hepatosplenomegaly in infancy, speech and developmental delay, dysphagia during the disease course. Considering the symptoms of Niemann-Pick disease, the patient's DNA was extracted and used for molecular studying and sequencing. Performing WES on proband identified a novel likely pathogenic, c.1586G>C (p.529Trp>Ser) mutation of the *SMPD1* gene. This gene has an autosomal recessive inheritance pattern. To date, c.1586G>C (p.529Trp>Ser) likely pathogenic variant has not been observed or reported in the world. This mutation locates in the 6415659 positions and disrupts the function of the *SMPD1* gene, causing symptoms of Niemann-Pick disease type A. The mutation reported in this article is associated with the NPD type A, disease phenotype, molecular findings and clinical observations underscore it.

1. Introduction

Niemann–Pick disease (NPD), a group of rare lysosomal storage disorders with an autosomal recessive inheritance pattern, is a heterogeneous disorder classified into four types: type A, type B, type C1, and type C2. NPD types A and B result from the inadequacy of acid sphingomyelinase (ASM), accountable for the hydrolysis of the sphingolipid sphingomyelin. In NPD types C1 and C2, a transmembrane and soluble nonenzymatic protein are inadequate, respectively, both concerned with transporting intracellular cholesterol. Secondary GM2-ganglioside storage in NPC may partly describe the related neurodegenerative aspects. In Niemann–Pick disease, the accumulation of gangliosides (GGs) and glycosphingolipids (GSLs) is prominent phenomenon (Breiden and Sandhoff, 2020). Types A and B occur about 1 in 250,000 in the population per live birth, and type C occurs approximately 1 in 120,000 in the general population (Abghari et al., 2019; Miller et al., 2000). Type A and B Niemann–Pick (also known as acid sphingomyelinase deficiency or ASMD) are induced via *SMPD1* (sphingomyelin phosphodiesterase 1, the critical enzyme in sphingolipid metabolism, EC 3.1.4.12) mutations (Cheema et al., 2020). *SMPD1* gene locates on 11p15, contains six exons with approximately ~6 kilobases length, encodes a protein of 631 amino acids and produces several types of transcripts (Abghari et al., 2019). This enzyme is a glycoprotein with five N-glycosylation sites (Breiden and Sandhoff, 2020), hydrolyzes sphingomyelin. As a result, phosphocholine and ceramide are made, note that this reaction is necessary for sphingomyelin turnover and also cell membrane homeostasis. *SMPD1* plays an influential role in other signaling pathways such as cell survival, proliferation, and differentiation (Ancien et al., 2021).

Accumulation of sphingomyelin in the central nervous system is the first and prominent cause of the classical infantile type (type A) of NPD

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(NPA; MIM# 257200) which is the most extreme form of NPD and causes severe central nervous system decay, hepatosplenomegaly, fatal disorder (Abghari et al., 2019; Nasereddin and Ereqat, 2018; Lipiński et al., 2019), short stature, low body weight, cherry-red maculae (50 %), gray, granular-appearing maculae, diffuse reticular or finely nodular infiltrations, protuberant abdomen, splenomegaly, osteoporosis, hypotonia, muscle weakness, hyporeflexia, psychomotor retardation, mental retardation, spasticity (later), rigidity (later), athetosis (later), microcytic anemia, large vacuolated foam cells (NP cells) on bone marrow biopsy and 'Sea blue' histiocytes (Miller et al., 2000; Qureshi et al., 2022; Hamosh et al., 2005). It is notable that in the brain extracts of NPD type A sufferers, the improved stages of glucosylceramide, dihexoside, trihexoside, GM2, and GM3 were found (Breiden and Sandhoff, 2020).

In NPD type B patients (NPB, MIM# 607616), the central nervous system is not involved, and unlike NPA usually presents in infancy or early childhood and is slowly progressive, NPB may occur in childhood or adulthood. Progressive hypersplenism, along with gradual decay of pulmonary function, happens in NPB (Abghari et al., 2019; Nasereddin and Ereqat, 2018; Lipiński et al., 2019). The most frequent symptoms of NPB are dyspnea, frequent respiratory infections, decreased pulmonary diffusion secondary to alveolar infiltration, diffuse reticular or finely nodular infiltrations, hepatomegaly, splenomegaly, absence of neurologic manifestations, cherry-red maculae (less common) and short stature (less common) (Hamosh et al., 2005). NPA and NPB are also linked to aging and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease (Ancien et al., 2021), and major depression (Kornhuber et al., 2005).

Studies show that one of the important causes of Niemann–Pick disease is consanguineous marriages and the rate of this kind of marriages is high in Iran (Abghari et al., 2019). Autosomal recessive genetic diseases have an equal effect on both females and men in the family. Therefore, complete clinical records and examinatioans and experiments can show quintessential scientific phenotypic patterns (Qureshi et al., 2022). So, molecular studies are important in finding new cases of NPD and lowering the cost and time in this way (Abghari et al., 2019).

Here, for the first time, we report a case that contains c.1586G>C (p.529Trp>Ser) mutation of the *SMPD1* gene that was identified by the whole exome-sequencing. Our aim with this study was to propose that the novel likely pathogenic variant c.1586G>C (p.529Trp>Ser) can cause Niemann-Pick disease type A.

2. Material and method

2.1. Subject

The present study involves a 2 years-old child as a result of a consanguineous marriage that is affected by Niemann-Pick disease (Fig. 1). The symptoms were clumsiness and gait disturbance at the beginning, and include hypotonia, microcephaly, severe hepatosplenomegaly in infancy, speech and developmental delay, dysphagia during the disease course. Severe central nervous system involvement such as agenesis of the corpus callosum, prominence of sulci, and brain atrophy in bilateral frontal temporal regions have been seen on MRI. In addition, mega cistern Magna with prominence of the fourth ventricle and connection of fourth ventricle with cistern Magna hypogenesis of interior is seen (dandy walker variant) (Fig. 2). Local ethics committees received informed consent from the subjected family.

2.2. Mutation analysis

Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using a filter-based methodology and quantified. A total amount of 1.0 μg genomic DNA per sample was used as input material for the DNA sample preparation. Sequencing libraries were generated using Agilent SureSelect Human All ExonV7 kit (Agilent Technologies, CA, USA) following the manufacturer's recommendations and x index

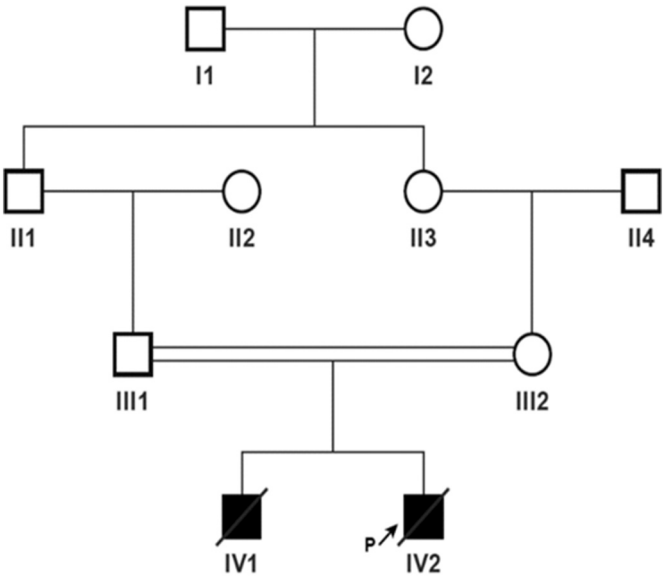

Fig. 1. Pedigree of the family.

codes were added to attribute sequences to the sample. Briefly, fragmentation was carried out by a hydrodynamic shearing system (Covaris, Massachusetts, USA) to generate 180–280 bp fragments. The remaining overhangs were converted into blunt ends via exonuclease/polymerase activities and enzymes were removed. After adenylation of 3′ ends of DNA fragments, adapter oligonucleotides were ligated. DNA fragments with ligated adapter molecules on both ends were selectively enriched in a PCR reaction. Captured libraries were enriched in a PCR reaction to add index tags to prepare for hybridization. Products were purified using the AMPure XP system (Beckman Coulter, Beverly, USA) and quantified using the Agilent high sensitivity DNA assay on the Agilent Bioanalyzer 2100 system. The qualified libraries are fed into NovaSeq 6000 Illumina sequencers. Then data quality control, analysis, and interpretation were run on G9 generation of HP server using Unix based operating system.

Sanger sequencing was performed by ABI prism 3730 sequencer (Applied Biosystems, Waltham, MA, USA) to validate the likely pathogenic mutation and segregation of the mutation in this family. Mutation Surveyor program version 4.0.9 was used to analyze the sequences (SoftGenetics, State College, PA).

We employed the 48-well thermocycler device (BioRad) in this reaction. The materials utilized in PCR as well as their concentration and amount were as follows: 6 μL of master (1×), 2 μL of template DNA, 0.5 μL of (10 Pmol) forward primer, 0.5 μL of (10 Pmol) reverse primer, and 3.5 L of sterile distilled water (total: 12.5 μL). To prepare the PCR solution, we used 0.2 mL microtubes. We poured the mentioned materials into the tubes and stirred them by pipetting. We amplified the noted gene segment with primers.

To amplify the 393 bp segment, the sequence of forward and reverse primers was GAGACCTACATCCTGAATCTG and CCCTTTCCTACATCAAGAACT, respectively. The utilized primers were manufactured by Tag Copenhagen Co. The 393 bp segment was amplified in the thermocycler as follows: Cycle 1 for the initial denaturation: once for 5 min at 94 °C; Cycle 2 including three steps: denaturation, binding the primer to the template strand, and polymerase expansion: 35 times, each for 30 s at 94, F: 54.93/R:54.92, and 72 °C, respectively; Cycle 3 for the final expansion: once for 10 min at 72 °C; Cycle 4 for maintaining the products: once at 4 °C.

3. Results

Performing WES on proband identified a novel homozygous missense variant, c.1586G>C (p.529Trp>Ser) of the *SMPD1* gene

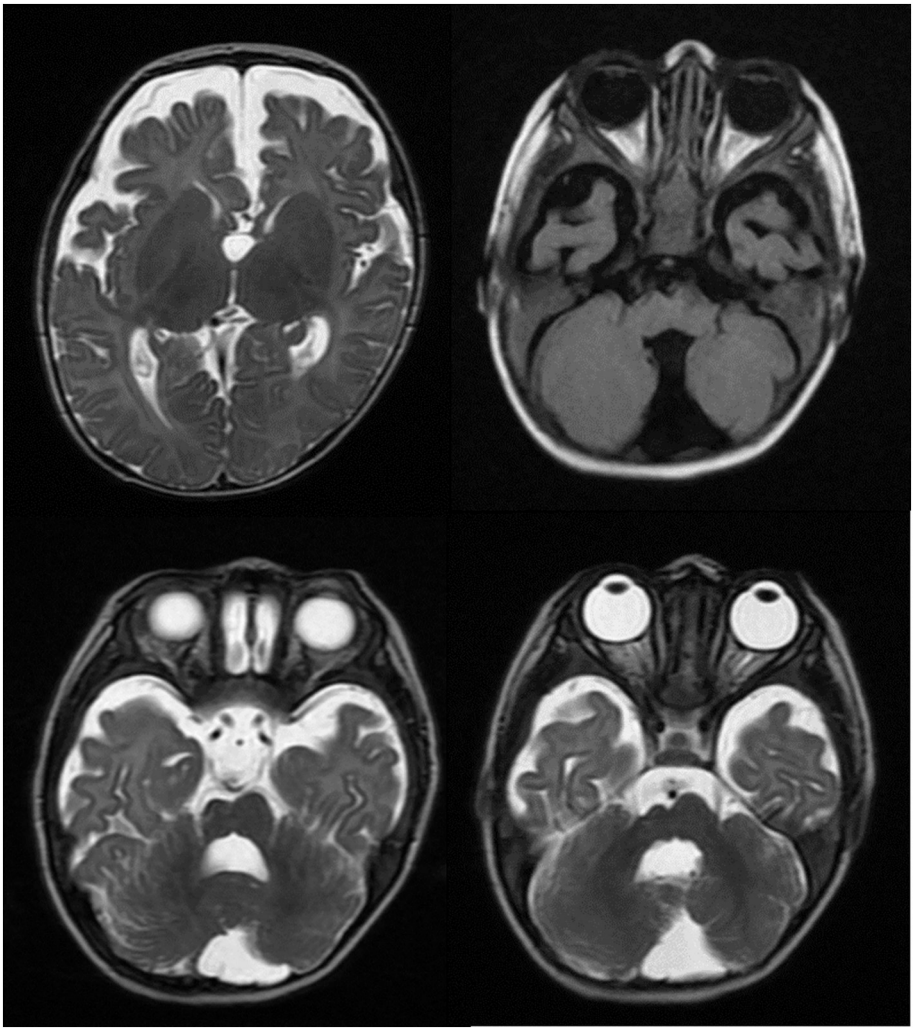

Fig. 2. Axial T2W brain MRI of proband. Images show prominence of sulci, and brain atrophy in bilateral frontal temporal regions and agenesis of the corpus callosum.

(Fig. 3).

This gene has AR inheritance at OMIM. In this family, there was another child that suffered and died from Niemann-Pick disease. With the confirmation, it is obvious that inheritance is AR in the mentioned family, the likely pathogenic c.1586G>C (p.529Trp>Ser) mutation is approved and can be found in Fig. 4.

There is no report of likely pathogenic c.1586G>C (p.529Trp>Ser) mutation in the *SMPD1* gene as we know in 6415659 in ExAC, 1000G, and other control datasets. This variant does not have a gnomAD exomes entry, but its locus is covered in gnomAD exomes as follows. c.1586G>C (p.529Trp>Ser) DANN score (Quang et al., 2015) was 0.9877 and was found as a pathogenic mutation in the EIGEN predictor (Ionita-Laza et al., 2016). This variant was found as a damaging variant in FATHMM-MKL (Shihab et al., 2015), BayesDel noAF (Tian et al., 2019), and BayesDel addAF (Tian et al., 2019) meta-predictors through searching dbNSFP v4 (Liu et al., 2020). Also, it is notable that a likely pathogenic c.1586G>C (p.529Trp>Ser) mutation was found as a disease-causing mutation in the taster predictor (Schwarz et al., 2014). We have submitted c.1586G>C mutation to ClinVar database, the accession number is SCV002556344.

4. Discussion

Loss-of-function mutations in the *SMPD1* gene lead to reduced acid sphingomyelinase catalytic activity, which is manifested in the recessive lysosomal storage disorder Niemann-Pick disease. Until now, there have been 200 pathogenic variants found just in the *SMPD1* gene (Lipiński et al., 2019) including point mutations, small deletions, different numbers of repeated nucleotides, and splice site mutations (Gabandé-Rodríguez et al., 2014) and missense mutations have been reported more than other mutations (Lipiński et al., 2019). The main disease-causing variants involved missense (65.4 %) and frameshifts (19 %) mutations (Zampieri et al., 2016). Several mutations have been found and reported in different exons of *SMPD1* causing Niemann-Pick disease in different populations all over the world. In Iran, there are not big enough sources of NPD mutations and the rate of consanguineous marriages is higher than most worldwide populations; so we expect an increasing rate of NPD in Iran.

In accommodation with our studies, c.1522G>A, c.106_107insCGCTGG, and c.107T>C are as a frequent mutant allele in patients of Iranian descent (Manshadi et al., 2015; Abedini et al., 2016). The most frequent mutations of *SMPD1* in the Ashkenazi Jewish population that causes NPA are c.911T>C (p.Leu304Pro), and c.1493G>T (p.Arg498Leu) (Abghari et al., 2019). Also, c.1829_1831delGCC (p.Arg610del) variant is a well-known mutation among NPB sufferers (Rodríguez-Pascau et al., 2009). In Saudi Arabia population, c.1267C>T (p.His423Tyr) appears to be a relatively frequent mutation in *SMPD1* gene (Jones et al., 2008). Studies clearly revealed ethnic diversities in mutation frequency.

After all these efforts to find the reason and treatment for Niemann-

Fig. 3. Bam file shows that a change of G to C has occurred in the 1586 position in the *SMPD1*gene.

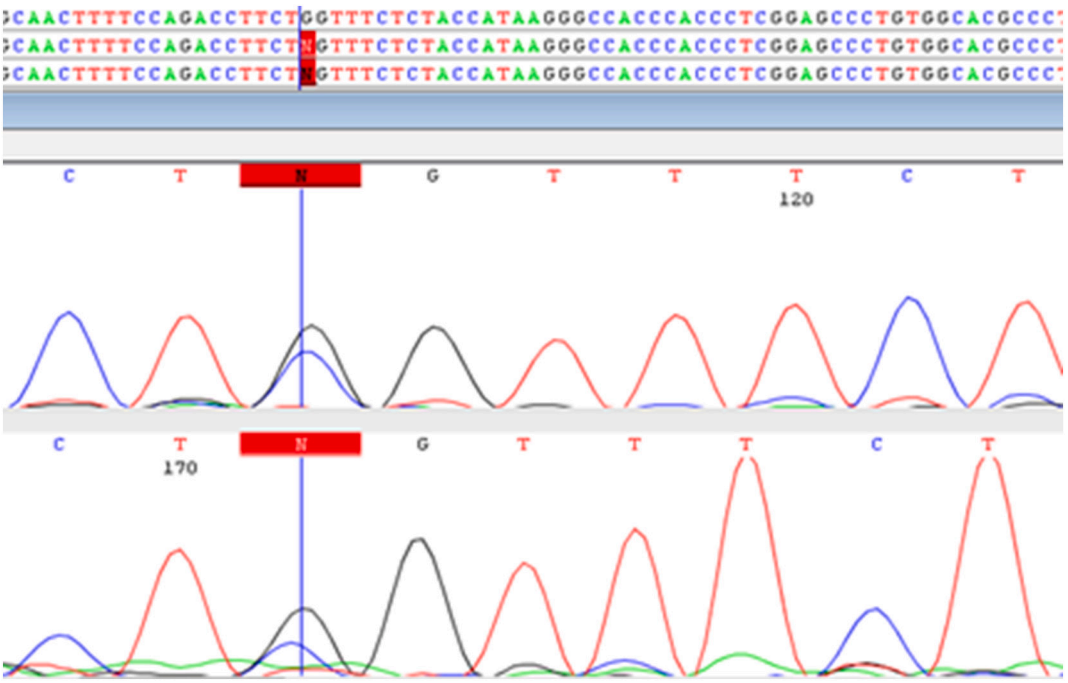

Fig. 4. Heterozygous electropherogram from father (upper panel) and from mother (lower panel) showing the nucleotide exchange. The variant position is marked with red.

Pick disease through these years, there is no actual treatment available for it. However, there is good news, some sufferers from Niemann-Pick type B were treated by using bone marrow transplantation. Also, after enzyme replacement therapy approaches developed, scientists found new ways to increase NPD sufferers' life quality. Recently, gene therapy based on adeno-associated viral vector serotype 9-based by using animal models showed successful treatments for type A (Breiden and Sandhoff, 2020). It is notable that because NPA is typically fatal within the first few life years, there are fewer studies about its treatments (Breiden and Sandhoff, 2020). Finding the history of patients and their family pedigrees and using physical therapy is very important in increasing the quality level of NPD patients lives because this disease develops and its

symptoms will worsen as time goes on (Olson et al., 2013).

In a study conducted by Desnick JP and his colleagues, 8 novel pathogenic variants c.631T>C (p.W211R), c.757G>C (p.D253H), c.940G>A (p.V314M), c.1280A>G (p.H427R), c.1564A>G (p.N522S), c.1575G>C (p.Q525H), and c.1729A>G (p.H577R) have been identified in the *SMPD1* gene associated with NPA/B. Further investigation of these mutations indicates the genotype/phenotype correlation and is consistent with the clinical manifestation in patients (Desnick et al., 2010).

Based on the results, our proband that is homozygote for c.1586G>C (p.529Trp>Ser) variant as a novel mutation of the *SMPD1* gene is a likely pathogenic variant that has a great risk factor for Niemann-Pick disease. By performing mutation analysis and identification of this variant in the proband, genotype/phenotype correlation is revealed, which is a confirmation of the clinical findings. Proband has been diagnosed with clumsiness and gait disturbance at the beginning, and include hypotonia, microcephaly, severe hepatosplenomegaly in infancy, speech and developmental delay, dysphagia during the disease course.

This finding prognosticates the clinical phenotypes of the patient. Results emphasize the genetic heterogeneity of the mutation causing NPA and supply further information for predicting the clinical phenotypes in newly diagnosed infants and children with NPA. To our knowledge, this is the first report of c.1586G>C mutation of the *SMPD1* discovery. Consequently, the results of the present study may be of importance in genetic counseling. Hence, this new mutation supplies new genotype/phenotype correlations and record the genetic heterogeneity in NPA.

5. Conclusions

In present study we describe a 2-years-old child as a result of a consanguineous marriage that has been diagnosed with clumsiness and gait disturbance at the beginning, and include hypotonia, microcephaly, severe hepatosplenomegaly in infancy, speech and developmental delay, dysphagia during the disease course. Further investigation revealed a homozygous variant, identified as c.1586G>C (p.529Trp>Ser), in exon 5 of the *SMPD1* gene (NM_001365135) of our proband. This is a novel mutation for NPA.

Abbreviations

NPD	Niemann-Pick disease
NPA	Niemann-Pick disease type A
NPB	Niemann-Pick disease type B
NPC	Niemann-Pick disease type C
ASM	Acid sphingomyelinase enzyme
ASMD	ASM deficiency disease
SMPD1	Sphingomyelin phosphodiesterase-1 gene
bp	base pair

Ethics approval and consent to participate

In this study, internal consent has been prepared, adjusted, and available in the Laboratory of Sistan and Balochestan. Also, the article has ethical approval code = 94170.

Consent for publication

Informed consent was obtained from all human adult participants and the parents or legal guardians of minors in the Laboratory of Sistan and Balochestan.

CRediT authorship contribution statement

Study conception and design: All authors.

Data collection: Elaheh Shahabi and Ali Khajeh.

Analysis and interpretation of the results: All authors.

Writing, reviewing, editing, visualization, and supervision: Elaheh Shahabi and Dor Mohammad Kordi-Tamandani.

Project administration: Dor Mohammad Kordi-Tamandani.

Declaration of competing interest

The authors declare that they have no competing interests or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Data availability

Data will be made available on request.

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