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Case Report

Clinical presentation and molecular genetics of Iranian patients with Niemann-pick type C disease and report of 6 NPC1 gene novel variants: A case series

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ABSTRACT

Niemann Pick Type C disease is a rare and progressive neurodegenerative lysosomal storage disorder caused by autosomal recessive mutations in the NPC1 and NPC2 genes. It is characterized by the accumulation of multiple lipid species in the endolysosomal compartment, leading to neurodegeneration and involvement of the liver, spleen, and lungs. Niemann Pick Type C has a wide range of presentations and severities at different ages with different progression rates. According to the Human Gene Mutation Database, to date, 486 disease-causing mutations in the highly polymorphic NPC1 gene and >20 mutations in the NPC2 have been reported. In the present study, we described the clinical, biochemical, and molecular profiles of 18 Iranian patients with Niemann-Pick Type C disease. Also, we describe six novel variants of the NPC1 gene, to our knowledge, not reported to date.

1. Introduction

Niemann Pick type C (NPC) disease is a rare, progressive, and irreversible neurodegenerative lysosomal storage disorder, which is caused by autosomal recessive mutations in the NPC1 gene in approximately 95% of cases (called type C1, OMIM*257220), and NPC2 gene in approximately 5% of cases (called type C2, OMIM*607625) (1–3). It is characterized by impaired intracellular lipid transport and metabolism, which leads to the accumulation of multiple lipid species, including unesterified cholesterol, sphingosine, and a range of glycosphingolipids in the endolysosomal compartment, leading to neurodegeneration and damage in the liver, spleen, and lungs (2–6). The prevalence of NPC disease is estimated to be 1:90.000–104.000 live births (2,7–9). The clinical presentation is heterogeneous, encompassing visceral manifestations (including neonatal cholestasis and hepatosplenomegaly), and neurological and/or psychiatric symptoms (10). Thoroughly, this condition is classified into four groups based on the neurological manifestations and the age of onset: 1. Early-infantile (<2 years, visceral-neurodegenerative form); 2. Late-infantile (2–6 years, neurodegenerative form); 3. Juvenile (6–15 years, neurodegenerative form); and 4. Adult (>15 years, psychiatric- neurodegenerative form) (11). In the adult-onset subgroup, cognitive impairment and neuropsychiatric symptoms are prominent. In addition, their survival can extend even into their seventh decade of life. Therefore, most of these patients are never diagnosed, or an accurate diagnosis is reached after many years of misdiagnosis (12–15). Unlike when the histopathological assessment was used for NPC disease diagnosis (15), gene sequencing and liquid chromatography with mass spectrometry are the leading techniques in NPC diagnosis (16). According to the Human Gene Mutation Database (HGMD), 598 disease-causing mutations in the highly polymorphic NPC1 gene and >20 mutations in the NPC2 have been reported. In addition, 74 possible disease-causing mutations for NPC disease are recorded in this database. In the present study, we described the clinical, biochemical, and molecular profiles of 18 Iranian patients with NPC, as well as six novel variants of the NPC1 gene.

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Table 1
Demographic and clinical characteristics, genotypes, and liquid chromatography-mass spectrometry results of the NPC patients studied.

patient no	Sex / Age of diagnosis (years)	Age of clinical manifestation (years)	Neurological manifestations/age	Systemic and Visceral manifestations	lysoSM-509 ng/ml (normal value: <0.9)	Genotypes
1	Boy/ 2.67	1.5	Neurological regression since 2 years, hypotonia, spastic limbs, swallowing difficulty, VSGP / 18 months of age	jaundice until 2 years of age, cholestasis, Splenomegaly, FTT	2.2	NPC1, Intron 16 c.2515-2A>G Homozygous, Likely pathogenic, Previously unreported
2	Girl/ 2.83	0.5	Neurological regression (since 6 months), tremor, speech problems, pyramidal signs, quadriplegia, Leukodystrophy (PVL), VSGP / 6 months of age	Splenomegaly, FTT	2.5	NPC1 exon20 c.3003_3041 + 24del (p. Phe1002_Gly1014del) Homozygous, Likely pathogenic, Previously unreported
3	Girl/ 3	1.5	Neurological regression, swallowing difficulty, Quadriplegia, VSGP/ 18 months of age	Splenomegaly, FTT	4.6	NPC1 exon 8 c.1171G > T (p. Glu391) pathogenic, Previously unreported
4	Boy/ 1.5	0.5	NDD, hypotonia, VSGP/ 6 months of age	jaundice until 4 months, Huge Splenomegaly, hepatomegaly, Mild liver steatosis, FTT	2	NPC1 exon8 Chr18 (GRCh37): g.21136247 A > C c.1286 T > G p.Val429Gly Pathogenic, Previously unreported
5	Girl/ 3.75	At birth	Neurologic regression, Central hypotonia, spasticity of lower limbs, VSGP / 2 years	Splenomegaly, FTT	3.7	NPC1 homozygous deletion encompassing exon 8 to 9 pathologic, Previously unreported
6	Girl/ 0.33 (4 months)	At birth	–	Jaundice, Cholestasis since 3 days of age, Ascites since 20 days of age, huge hepato-splenomegaly, FTT	1.8	NPC1exon 9, c.1534C > T p.His512Tyr Likely pathogenic, Previously unreported
7	Girl/ 3.5	0.33	Neurological regression, tremor, cataplexy, limb spasticity, increased DTR VSGP / 1.5 years	Splenomegaly (first presentation and since 4 months of age), FTT	2.5	NPC1 exon 9 Chr18(GRCh37): g.21134860 A > G, c.1415 T > C p.Leu472Pro likely pathogenic Pathogenic Carrier state for NPC2
8	Girl / 3.67	<1	NDD, Neurological regression, quadriplegia, Increased DTR, VSGP / before 12 months of age	Mild splenomegaly, FTT	–	NPC1 exon 6 c.852delT p.Phe284fs Likely Pathogenic
9	Boy/ 3.75	1	Neurologic regression, ataxia, tremor, speech problems, swallowing difficulty, spastic limbs, abnormality of movement, VSGP / 2 years of age	Hepato-splenomegaly (first presentation), FTT	4.6	NPC1 exon 3 Chr18 (GRCh37): g.21152039 T > C NM_000271.4: c.286 A > G p.Arg96Gly homozygous, Pathogenic Previously unreported
10	Boy/ 2	0.33	coarse facial features, NDD, tremor, inability to walk, developmental delay, intellectual disability, abnormality of the skeletal system, joint laxity, no speech, VSGP / 1.8 years	jaundice until 4 months, hepato-splenomegaly, mild liver steatosis, macroglossia	3	NPC1 exon 8 Chr18 (GRCh37): g.21136247 A > C NM_000271.4: c.1286 T > G p.Val429Gly homozygous, Pathogenic Previously unreported
11	Girl/ 8.08	0.5	tremor, ataxia, dysarthria, VSGP / 6 years	Splenomegaly	–	NPC1 Chr18 exon 9 c.1433 A > C p.Asn428Thr,

(*continued on next page*)

Table 1 (*continued*)

patient no	Sex / Age of diagnosis (years)	Age of clinical manifestation (years)	Neurological manifestations/age	Systemic and Visceral manifestations	lysoSM-509 ng/ml (normal value: <0.9)	Genotypes
						homozygous, Likely Pathogenic
12	Girl/ 3.67	2	developmental delay, able to sit >1 year, unable to stand or walk until 2 years, tremor, poor hand control / 2 years	Hepato-splenomegaly	–	NPC1 exon 6 c.852delT p.Phe284fs Likely Pathogenic
13	Girl/ 3	1.5	Neurological regression since 1.5 years, abnormality of movement, intellectual disability, swallowing difficulty, spastic limbs / 1.5 years	splenomegaly	4.6	NPC1 Exon8 c.1171G > T, p.Glu391 homozygous, Pathogenic
14	Boy/ 0.08 (1 month)	At birth	–	jaundice, direct bilirubinemia, hepato-splenomegaly	1.5	Previously unreported NPC1 exon20 c.2972_2973delAG (p. Gln991fs), homozygous, likely pathogenic
15	Girl/ 0.33 (4 months)	At birth	–	jaundice since birth, huge hepato-splenomegaly, cholestasis, ascites since 20 days of age	1.8	NPC1 exon 09 c.1534C > T p. His512Tyr homozygous, likely pathogenic
16	Girl / 2.42	2	Falling, leg pain, VSGP / 4 years	Huge Splenomegaly, hepatomegaly	1.9	Previously unreported NPC1 Exon 08 c.1070C > T p.Ser357Leu homozygous, pathogenic
17	Boy / 0.92 (11 months)	0.08	–	jaundice, direct bibilirubinemia, cholestasis, FTT, Steatorrhea since one month of age	1.4	NPC1 Exon 20 c.2974G > T p.Gly992Trp homozygous, pathogenic
18	Girl / 0.58 (7 months)	At birth	NDD, VSGP / 6 months	Jaundice, Cholestasis since birth, Hepatomegaly, splenomegaly since 6 months of age	3.6	NPC1 exon 9 Chr18(GRCh37): g.21134860 A > G, c.1415 T > C p.Leu472Pro homozygous, likely pathogenic

FTT: failure to thrive, VSGP: vertical supranuclear gaze palsy, NDD: neurodegenerative diseases, PVL: periventricular leukomalacia, DTR: deep tendon reflex.

1.1. Patients' data

The demographic and clinical characteristics of 18 Iranian patients with NPC disease presenting to Mofid Children's Hospital are shown in Table 1. Genomic DNA amplification for the NPC1 (OMIM:607623) and NPC2 (OMIM:601015) genes was performed to detect the possible mutations in Niemann-Pick disease type C1 (OMIM: 257220) and Niemann-Pick disease type C2 (OMIM: 607625) patients respectively.

2. Methods

We analyzed the NPC1 and NPC2 genes by PCR, sequencing the entire coding region and the highly conserved exon-intron splice junctions. The reference sequences of the NPC1 and NPC2 genes are NM_000271.4 and NM_006432.3, respectively.

The concentration of the lyso-SM-509 biomarker in a dried blood spot was measured using high-performance liquid chromatography (HPLC) and tandem mass spectrometry; the amount of >0.9 ng/ml was considered pathologic. All patients were tested in the same laboratory. The Ethics Committee approved the study, informed consent was obtained, and our team assured the patients that their data would remain confidential.

3. Results

In addition to the demographic and clinical characteristics, genotypes and liquid chromatography-mass spectrometry results of the NPC patients presenting to Mofid Children's Hospital are shown in Table 1. Out of 18 patients with NPC reported in our study, 12 (66.67%) were female, and 6 (33.33%) were male. The age of diagnosis ranged from 1 month to 8.08 years (median age of diagnosis: 2.56 years).

In our study, 14 patients (77.78%) showed neurological deficits, in which neurodevelopmental regression, neurodegenerative diseases (NDD), developmental delay, and vertical supranuclear gaze palsy (VSGP) were the most common manifestations. The brain imaging was unremarkable for all patients except patient number 2, who showed periventricular leukomalacia. The age of onset of neurological presentations ranged from 6 months to 6 years (median age of onset: 1.88 years).

Systemic and visceral manifestations contained prolonged jaundice (in 8 patients, 44.44%), splenomegaly (in 17 patients, 94.44%), hepatomegaly (in 9 patients, 50%), cholestasis (in 5 patients, 27.78%), ascites (in 2 patients, 11.11%), liver steatosis (in 2 patients, 11.11%), and failure to thrive (FTT) (in 10 patients, 55.56%). Moreover, macroglossia in patient number 10 and steatorrhea in patient number 17 were detected.

The results of liquid chromatography-mass spectrometry assessment of lysoSM-509 concentration were available for 15 patients; all the

measures were more than the optimal limit (0.9 ng/ml) with a range between 1.5 and 4.6 ng/ml (median: 2.78 ng/ml).

Mutation analysis showed mutations in the NPC1 gene in all of the study participants, and merely one patient showed an additional pathogenic carrier state for the NPC2 gene (patient no.7). Out of 18 patients, 13 different mutations in the NPC1 gene were identified, as mentioned in Table 1. p.Phe284fs (c.852delT) was seen in both patients no.8 and no.12. In patient number 5, pathologic homozygous deletion encompassing exon 8 to 9 was detected. Our study describes six novel mutations, to our knowledge not reported to date: c.2515-2A>G in patient no.1; p.Phe1002_Gly1014del (c.3003_3041 + 24del) in patient no.2; p.Glu391 (c.1171G > T) in patients no.3 and no.13; p.Val429Gly (c.1286 T > G) detected in both patients no.4 and no.10; p.His512Tyr (c.1534C > T) in patients no.6 and no.15; p.Arg96Gly (c.286 A > G) in patient no.9.

4. Discussion

NPC disease is a rare lysosomal storage disorder with an autosomal recessive pattern of inheritance, a diverse range of presentations, and severities that appear at different ages with distinct evolution rates (2,17). Manifestations of NPC disease incorporate visceral complications (neonatal cholestasis and hepatosplenomegaly) and neurological impairment such as delay in motor, hypotonia, gait problems, speech delay, cataplexy, school problems, ataxia, seizures, psychiatric problems, dystonia, and dementia. The disease progression is more aggressive in patients for whom neurological involvement presents earlier (15). In our study, the most common neurological manifestation was VSGP shown in 12 patients (66.67% of all patients and 85.71% of patients with neurological involvement), which is highly specific among neurological signs of NPC disease (15,18).

In addition to prolonged jaundice, splenomegaly, and hepatomegaly, as the most common visceral finding of the disease in our study, unexplained neonatal hepatitis may raise concern about the diagnosis of NPC disease, especially if splenomegaly is also present (19).

In mutation analysis for the NPC1 and NPC2 genes, we observed p.Phe284fs (c.852delT) in both patients no.8 and no.12. This likely pathogenic mutation was also reported by Fancello et al. (20). Five other mutations detected in our study were reported previously: p.Leu472Pro (c.1415 T > C) by Morris et al. (21) and even in Iranian patients by Noroozi Asl et al. (22); p.Asn478Thr (c.1433 A > C) by Tonekaboni SH et al. in Iranian patients (23); p.Gln991fs (c.2972_2973delAG) and p.Gly992Trp (c.2974G > T) by Greer et al. (24); p.Ser357Leu (c.1070C > T) by Crespi et al. (25).

Our study describes six novel mutations that, to our knowledge not been reported to date. A previously unreported homozygous mutation in exon 20 of the NPC1 gene, c.3003_3041 + 24del (p.Phe1002_Gly1014del) was detected. This deletion causes a loss of 13 residues. Software analyses (Alamut v.2.7.1) predict an aberrant effect on splicing is likely. To date, this variant has not been described in the Exome Aggregation Consortium, Exome Sequencing Project, or the 1000 Genomes Browser. This is the first time we have detected this variant based on Centogene's mutation/variation database (CentoMD®). It is classified in class 0 (likely pathogenic) according to the recommendations of the Centogene and American College of Medical Genetics (ACMG).

The NPC1 variant in exon 9, c.1534C > T (p.His512Tyr), is located in a highly conserved nucleotide and amino acid positions, with moderate physicochemical differences between the amino acids histidine and tyrosine (Alamut v.2.9). Software analysis by Polyphen-2, MutationTaster, and align-GVGD predict this variant is probably damaging.

The previously unreported homozygous mutation in the exon 8 of the NPC1 gene, c.1171G > T (p.Glu391) creates a premature stop codon. It is classified in class 1 (pathogenic) according to the recommendations of Centogene and ACMG.

Previously unreported homozygous mutation in intron 16 of the NPC1 gene, c.2515-2A>G, is located in the acceptor splice site of intron 16. Software analysis (Alamut v.2.7.1) predicts an aberrant effect on splicing and a skip of exon 17 is likely. It is classified in class 0 (likely pathogenic) according to the recommendations of Centogene and ACMG.

The NPC1 variant c.1286 T > G causes an amino acid change from Valine to Glycine at position 429. Given the pathological result of the biochemical analysis and the clinical presentation, it is considered to be class 1 (pathogenic) according to the recommendations of Centogene and ACMG.

The NPC1 variant c.286 A > G causes an amino acid change from Arginine to Glycine at position 96. The substitution is near the highly conserved donor splice site of exon 3. Together with the clinical manifestation and the pathological increase of the biomarker (lysoSM-509), the variant is classified as pathogenic according to the recommendations of Centogene and ACMG.

In conclusion, we described the demographic and clinical characteristics, genotypes, and biochemical analysis of 18 Iranian patients with NPC. Also, we reported 6 mutations in the NPC1 gene still not reported, to our knowledge.

CRediT authorship contribution statement

Hedyeh Saneifard: Supervision, Project administration, Methodology, Conceptualization. **Marjan Shakiba:** Methodology, Investigation, Conceptualization. **Mohammadreza Alaei:** Resources, Data curation, Conceptualization. **Asieh Mosallanejad:** Validation, Investigation, Data curation, Conceptualization. **Shirin Ghanefard:** Validation, Conceptualization. **Mehrdad Yasaei:** Writing – review & editing, Writing – original draft, Validation. **Kimia Karimi Toudeshki:** Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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