Characterization of Niemann-Pick diseases genes mutation spectrum in Iran and identification of a novel mutation in SMPD1 gene

Fateme Zahedi Abghari**,1, Fatemeh Bayat**,1, Masoumeh Razipour1, Morteza Karimipoor1, Maryam Taghavi-Basmenj1, Sirous Zeinali1, Elham Davoudi-Dehaghani*1

Received: 5 Dec 2018                     Published: 25 Nov 2019

Abstract

Background: Niemann-Pick diseases are rare inherited lipid storage disorders caused by mutations in the SMPD1, NPC1, and NPC2 genes. The aim of this study was to assess the mutation spectrum of a cohort of Iranian Niemann-Pick patients.

Methods: A consanguineous couple with a child suspected of having Niemann-Pick disease type A (died at age 2) was screened for gene mutations in the SMPD1 gene. Sanger sequencing was performed for all exons and exon-intron boundary regions. A literature review on SMPD1, NPC1, and NPC2 genes mutations in Iran was conducted using published original papers on this subject.

Results: A novel frameshift c.762delG (p.Leu256fs*) at a heterozygous state was identified in the parents. According to the review study, identified mutations in 39 Iranian patients were concentrated in exon 2 of the SMPD1 gene and exons 8 and 9 of the NPC1 gene.

Conclusion: Niemann-Pick diseases genes mutation analysis (SMPD1, NPC1, and NPC2) in Iran shows the genetic heterogeneity of these diseases in this country. More studies with larger sample sizes should be conducted to further examine genetic changes associated with Niemann-Pick diseases in Iran.

Keywords: Niemann-Pick disease type A, Type B, Type C NPD, SMPD1, NPC1, NPC2

Introduction

Niemann-Pick diseases (NPD) are a heterogeneous group of autosomal recessive disorders with common features of hepatosplenomegaly and neurologic deficits. These disorders are classified into 4 main types: type A, type B, type C1, and type C2 (1-4). The prevalence of types A and B is approximately 1/250 000 in general population and the incidence of type C is about 1/120 000 per live birth (5, 6).

Niemann-Pick type A and B
Type A and B (also called acid sphingomyelinase deficiency or ASMD) are caused by different SMPD1 (sphingomyelin phosphodiesterase 1) mutations (7-9). The SMPD1 gene, on 11p15, consists of 6 exons and produces several types of transcripts, which (NM_000543) encodes the lysosomal enzyme acid sphingomyelinase with 631 amino acids. This enzyme hydrolyzes sphingomyelin to

↑What is “already known” in this topic:
Niemann-Pick disease (NPD) is a heterogeneous group of neurodevelopmental disorders which are characterized by severe damage in various fields of growth. A high rate of consanguineous marriages in countries such as Iran can be associated with a higher incidence of autosomal recessive diseases, including NPD.

→What this article adds:
Here, we present a novel mutation in SMPD1 gene and review reports on molecular studies on SMPD1, NPC1, and 2 genes in Iran.
A novel mutation in \textit{SMPD1} gene

Phosphocholine and ceramide. The defect in acid sphingomyelinase results in lysosomal accumulation of sphingomyelin. The progressive deposition of this phospholipid in the central nervous system leads to the classical infantile form (type A) of NPD (NPA; MIM# 257200), which is a very severe subtype of Niemann-Pick disease. NPA usually occurs in infancy or early childhood and is characterized by hepatosplenomegaly, failure to thrive, and progressive neurodegeneration (3, 4, 10, 11). NPD type B patients (NPB, MIM# 607616) display no central nervous system involvement (1, 12). Variety of symptoms, including hepatosplenomegaly, respiratory problems, hyperlipidemia, and thrombocytopenia, may be presented in childhood or adulthood (12).

\textbf{Niemann-Pick type C1 and C2}

NPD types C1 and C2 have very similar clinical manifestations. They are caused by mutations in \textit{NPC1} (in 95\% of cases) and \textit{NPC2} genes, respectively. \textit{NPC1} gene has 25 exons and located in 18q11.2. This gene produces several transcripts, one of which (NM_000271) encodes NPC1 protein with 1278 amino acids. NPC intracellular cholesterol transporter 2 or \textit{NPC2} gene (14q24.3) has 5 exons and produces different types of transcripts, one of which (NM_006432) encodes NPC2 protein with 151 amino acids (13-19).

Mutations in either gene result in impaired intracellular lipids trafficking, leading to overaccumulation of lipids in cells (20, 21). NPD types C1 and C2 show a highly variable clinical presentation, including progressive neurodegeneration, liver and lung disease, which can develop at any age (22, 23).

The Islamic Republic of Iran is a country in the Middle East with a high rate (~ 38\%) of consanguineous marriages (24). Previous studies have shown that consanguineous marriages are associated with an increased risk of autosomal recessive disorders. Therefore, a higher incidence of rare diseases such as NPD is expected in Iran, showing the importance of molecular studies on NPD and other autosomal recessive disorders in developing cost- and time-saving diagnostic approaches for these diseases (25, 26).

Here, we report a novel mutation in the \textit{SMPD1} gene in an Iranian family with a child affected with NPA. We also review molecular studies on NPD in Iran and describe the spectrum of NPD mutations in Iranian population.

\textbf{Methods}

\textbf{Mutational analysis}

Screening of the \textit{SMPD1} gene was performed for a consanguineous couple with a child suspected of NPA who had died at the age of 2. The affected child was noted to have hepatosplenomegaly, hypotonia, developmental delay, cherry-red maculae, and frequent respiratory infections. The parents completed and signed an informed consent form. Peripheral blood samples were collected from the couple. Genomic DNA was extracted using the salting out method according to the standard protocol. Primers were designed for amplification of exons and exon-intron boundaries of \textit{SMPD1} gene by Primer3 online software. The amplification reaction was performed in a \textsc{MyCycler} thermal cycler (Bio-Rad, USA). Then, 5\µL of the PCR products were loaded on a 1.5\% agarose gel. All exons and exon-intron boundary regions of the \textit{SMPD1} gene were sequenced on both strands (ABI3130 Genetic Analyzer). The sequences were compared against reference sequence of \textit{SMPD1} gene using nucleotide BLAST.

\textbf{Search strategy}

PubMed/MEDLINE, Scopus, Google Scholar, SID, Medlib, and Magiran databases were searched up to August 2018 using the keywords: Niemann-Pick diseases in all types A, B and C, mutation, Iran, \textit{SMPD1}, \textit{NPC1}, and \textit{NPC2}. After removal of duplicates, reviews and unrelated articles, 9 articles and abstracts were included in the current study.

\textbf{Results}

\textbf{Molecular analysis}

A novel heterozygous frameshift deletion (c.762delG)
(p.Leu256fs*) and a previously reported benign variant (c.107T>C) were identified in the SMPD1 gene in the couple. Examining the potential consequences of a single nucleotide deletion (G) at position 762 showed that this change results in a premature stop codon at position 256 (Fig. 1).

**Overview of the studies in Iran**

PubMed/MEDLINE, Scopus, Google scholar, SID, Medlib, and Magiran databases were searched and 20 related articles and abstracts retrieved, among which 9 studies that presented the results of mutation analysis of NPD genes in 39 patients were selected for a more detailed analysis. The spectrum and frequency of the NPD genes mutations among Iranian reported patients are presented in Table 1. A total of 17 pathogenic variants were found in the investigated studies. Four of 7 pathogenic mutations detected in the SMPD1 gene were in exon 2 (27-29). Also, more than half of the identified mutations in the NPC1 gene were in exons 8 and 9 (30-33). There was only 1 report of NPC2 mutation in Iran, which was reported in 2 Iranian siblings (34). Figure 2 shows the position of reported mutations in the NPD genes.

**Discussion**

Previous studies have shown that NPD diseases are rare disorders and more prevalent in populations with a high rate of consanguinity such as Ashkenazi Jewish, French Canadians, and Arabs in Saudi Arabia (35, 36). Unfortunately, there is no information about the prevalence of these diseases in Iran, but due to the high frequency of consanguineous marriages in this country, an increased frequency of NPD diseases is expected. Therefore, the development of a stepwise strategy based on common mutations for rapid and cost-effective NPD molecular genetic testing is necessary in Iran to confirm the diagnosis of the NPD diseases, carrier detection, prenatal diagnosis (PND), and preimplantation genetic diagnosis (PGD) of these diseases in families (26). The aim of this study was to take a step in this direction by reporting a new case of NPA and reviewing all reported NPD genes mutations in Iran.

In the molecular analysis, we found a novel frameshift deletion at position 762 of the SMPD1 gene, which results in a premature stop codon TGA. The variant may lead to an unstable mRNA, which can be degraded by nonsense-mediated decay, or produce a truncated nonfunctional protein of 256 amino acid residues. Pathogenicity of this variant can be supported by the pathogenic effect of downstream nonsense mutations such as p.Glu260Ter (CM961340) and p.Leu263Ter (CM920623) reported in NPA patients (7-9). To date, more than 100 mutations responsible for NPA and NPB have been reported in the SMPD1 gene (Human Gene Mutation Database: http://www.hgmd.org/). Mutations such as small deletions or nonsense mutations in SMPD1 (NM_000543) gene, which result in truncated proteins, can produce type A (7-9).

So far, several common NPD mutations have been

| Nucleotide changes | Protein changes | rs number | Genes | Exon/Intron | Number of alleles in Iran | MAF | References |
|-------------------|----------------|-----------|-------|-------------|--------------------------|-----|------------|
| c.762delG         | p.Leu256fs*    | -         | SMPD1 | E2          | 2                        | -   | Our cases  |
| c.740delG         | p.Gly247Alafs*9| -         | SMPD1 | E2          | 2                        | -   | (Galehdari et al. 2015) |
| c.1033-1034insT   | p.Glu345Valfs*46| -         | SMPD1 | E2          | 2                        | -   | (Manshadi et al. 2015)  |
| c.573delT         | p.Ser192Alafs*6| rs727504167| SMPD1 | E2          | 2                        | -   | (Manshadi et al. 2015)  |
| c.946-961del16    | p.Pro316Metfs*64| -         | SMPD1 | E2          | 2                        | -   | (Mikaeli et al. 2016)   |
| c.1155T>A         | p.Asn385Lys    | -         | SMPD1 | E3          | 1                        | -   | (Manshadi et al. 2015)  |
| c.1417-1418delCT  | p.Leu473Glufs*20| rs398123476| SMPD1 | E5          | 2                        | -   | (Manshadi et al. 2015)  |
| c.1166G>T         | p.Arg389Leu    | -         | NPC1  | E8          | 2                        | -   | (Karimzadeh et al. 2013) |
| c.1192C>T         | p.His398Tyr    | -         | NPC1  | E8          | 2                        | -   | (Tonekabonii et al. 2015) |
| c.1415T>C         | p.Leu472Pro    | -         | NPC1  | E9          | 4                        | -   | (Noroozii et al. 2017)   |
| c.1433A>C         | p.Asn478Thr    | -         | NPC1  | E9          | 2(2sister)               | -   | (Tonekabonii et al. 2015) |
| c.1547G>A/C       | p.Cys516Tyr/c.516Tyr| rs751951695/768999208| NPC1 | E9/E18      | 1+1²                    | -   | (Rohanizadeh et al. 2017) |
| c.2278G>A         | p.Gly910Ser    | -         | NPC1  | E18         | 2                        | -   | (Karimzadeh et al. 2013) |
| c.2657dupG        | p.Pro887Serfs*31| -         | NPC1  | E18         | 2                        | -   | (Karimzadeh et al. 2013) |
| c.2925-2928delCTGC| p.Cys976Phefs*6| -         | NPC1  | E20         | 2                        | -   | (Karimzadeh et al. 2013) |
| c.3478-6T>A       | -              | -         | NPC1  | I22         | 2                        | -   | (Karimzadeh et al. 2013) |
| c.358C>T          | p.Pro120Ser    | rs104894458| NPC2  | E3          | 2                        | -   | (Alavi et al. 2013)      |

1: MAF: Minor allele frequency (Highest population MAF: < 0.01)  2: Patient was compound heterozygote for two variants. E: Exon, I: Intron. The Ser357Leu/Tyr394His (NPC1) and Leu137Pro (SMPD1) were excluded from our table because of ambiguous nomenclatures and sequence position respectively (Tonekabonii et al. 2015 and Abedini et al. 2016).
A novel mutation in **SMPD1** gene

found in different populations. Two pathogenic variants in **SMPD1**, c.911T>C (p.Leu304Pro), and c.1493G>T (p.Arg498Leu) have been identified as the most common cause of NPA in Ashkenazi Jewish population (7, 37). On the other hand, c.1829_1831delGCC (p.Arg610del) variant is known as a common mutation in NPB patients in different populations (38). A mutation c.1267C>T (p.His423Tyr) in **SMPD1** is also reported as the most common cause of NPB in Saudi Arabia (37). Two variants, c.3019C>G (p.Pro1007Ala) and c.3182T>C (p.Ile1061Thr), are also known as the most common genetic causes of NPC in different populations (39). A review of all reported NPD mutations in Iran showed that the majority of them are private. These mutations are mainly located in exon 2 of the **SMPD1** gene and exons 8 and 9 of the **NPC1** gene. These findings can be useful in developing a stepwise strategy to molecular diagnosis of the NPD in Iran. The most frequent benign variants reported in NPD genes in Iran are c.1522G>A, c.106_107insCGCTGG, and c.107T>C in **SMPD1** (28, 40). Several benign variants have been incorrectly classified as pathogenic or likely pathogenic in some reports (28). These variants are not shown in Table 1.

**Conclusion**

A novel mutation in **SMPD1** gene has been reported in this study. A review on all NPD genes mutations reported from Iran has also been presented here. However, more studies should be conducted on larger groups of NPD patients to characterize mutations in NPD genes in Iran.

**Acknowledgements**

We thank the investigated family for participating in this study. This work was supported by Pasteur Institute of Iran.

**Conflict of Interests**

The authors declare that they have no competing interests.

**References**

1. Schuchman EH. The pathogenesis and treatment of acid sphingomyelinase-deficient Niemann-Pick disease. J Inherit Metab Dis. 2007;30(5):654-63.
2. Vance JE. Lipid imbalance in the neurological disorder, Niemann-Pick C disease. FEBS Lett. 2006;580(23):5518-24.
3. Schuchman EH, Wasserstein MP. Types A and B Niemann-Pick disease. Best Pract Res Clin Endocrinol Metab. 2015;29(2):237-47.
4. Schuchman EH, Desnick RJ. Types a and B Niemann-pick disease. Mol Genet Metab. 2017;120(1-2):27-33.

---

**Fig. 2.** Schematic illustration and overall distribution of pathogenic variants in coding regions of **SMPD1/NPC1/NPC2** genes in Iranian patients with NPD disease (19, 20, 23). aa: amino acid. A: SMPD1 protein, SP: Signal peptide (aa1–46), SAPD: a saposin-like (SAP) domain (aa89–165), PRD: pro-rich domain (aa166–198), MCD: metallophoesterase/catalytic domain (aa199–461), CTD: C-terminal domain (aa462–629): Exon 2 had the highest percentage of mutations. B: NPC1 protein, SS: signal sequence (aa1-46), SBD: sterol-binding domain or luminal loop-1(aa25-264), L2: luminal loop-2 (aa371–615), SSD: sterol-sensing domain (aa 616-791), PHD: patched homology domain (aa 1085-1253), LTS: a lysosomal targeting signal at the C terminus (aa 1275–1278). NPC2 binds to the L2 of NPC1. L2 region had the highest percentage of mutations in Iranian studies. C: NPC2 protein, SS: signal sequence (aa 1-19), ECR: evolutionary constrained regions (A-D). ECR-D is the cholesterol binding region.
Vanier MT, Niemann-Pick disease type C. Orphanet J Rare Dis. 2010;5:16.

Fuller M, Meikle PJ, Hopwood JJ. Epidemiology of lysosomal storage diseases: an overview. InFabry disease: perspectives from 5 years of FOS 2006. Oxford PharmaGenesis.

Zampieri S, Filocamo M, Pianta A, Lualdi S, Gort L, Coll MJ, et al. SMPD1 Mutation Update: Database and Comprehensive Analysis of Published and Novel Variants. Hum Mutat. 2016;37(2):139-47.

Desnec JP, Kim J, He X, Wasserstein MP, Simonoar CM, Schuchman EH. Identification and characterization of eight novel SMPD1 mutations causing types A and B Niemann-Pick disease. Mol Med. 2010;16(7-8):316-21.

Rodriguez-Pascua L, Gort L, Schuchman EH, Vilageliu L, Grinberg D, Chabás A. Identification and characterization of SMPD1 mutations causing Niemann-Pick type A and B in Spanish patients. Hum Mutat. 2009;30(7):1117-22.

Gorelik A, Illes K, Heinz LX, Superti-Furga G, Nagar B. Crystal structure of mammalian acid sphingomyelinase. Nature communications. 2016;7:12196.

McGovern MM, Aron A, Brodie SE, Desnec RJ, Wasserstein MP. Natural history of Type A Niemann-Pick disease: possible endpoints for therapeutic trials. Neurology. 2006;66(2):228-32.

Pavlu-Pereira H, Astew B, Poupctova H, Ledvinova J, Sikora J, Vanier MT, et al. Acid sphingomyelinase deficiency. Phospholipid accumulation in the infantile form of Niemann-Pick disease type C. Brain. 2007;130(Pt 1):120-33.

Newton J, Milsten S, Spiegel S. Niemann-Pick type C disease: The atypical sphingolipidosid. Adv Biol Regul. 2018;70:82-88.

McKay Bounford K, Gissen P. Genetic and laboratory diagnostic approach in Niemann Pick disease type C. J Neurol. 2014;261 Suppl 2:S569-75.

Cruz JC, Sugii S, Yu C, Chang TY. Role of Niemann-Pick type C1 protein in intracellular trafficking of low density lipoprotein-derived cholesterol. J Biol Chem. 2000;275(6):4013-21.

Infante RE, Radhakrishnan A, Abi-Mosleh L, Kinch LN, Wang ML, Grishin NV, et al. Purified NPC1 Protein II. Localization of sterol binding to a 240-amino acid soluble luminal loop. J Biol Chem. 2000;275(6):4013-21.

Vanier MT, Millat G. Structure and function of the NPC2 protein. Biochim Biophys Acta. 2004;1685(1-3):14-21.

Evans WR, Hendrickz CJ. Niemann-Pick type C disease—the tip of the iceberg? A review of neuropsychiatric presentation, diagnosis and treatment. BJ Psych Bull. 2017;41(2):109-14.

Rosenbaum AI, Maxfield FR. Niemann-Pick type C disease: molecular mechanisms and potential therapeutic approaches. J Neurochem. 2011 Mar;116(5):789-95.

Millard EE, Gale SE, Dudley N, Zhang J, Schaffer JE, Ory DS. The sterol-sensing domain of the Niemann-Pick C1 (NPC1) protein regulates trafficking of low density lipoprotein cholesterol. J Biol Chem. 2005;280(31):28581-90.

Storch J, Xu Z. Niemann-Pick C2 (NPC2) and intracellular cholesterol trafficking. Biochim Biophys Acta. 2009;1791(7):671-8.

Yanjanin NM, Velez JI, Groopman A, King K, Bianconi SE, Conley SK, et al. Linear clinical progression, independent of age of onset, in Niemann-Pick disease, type C. Am J Med Genet B Neuropsychiatr Genet. 2010;153B(1):132-40.

Sevin M, Lesca G, Baumann N, Millat G, Lyon-Caen O, Vanier MT, et al. The adult form of Niemann-Pick disease type C. Brain. 2007;130(Pt 1):120-33.

Saadat M, Ansari-Lari M, Farhoud DD.Consanguineous marriage in Iran. Ann Hum Biol. 2004;31(2):263-9.

Gialluisi A, Pippucci T, Anikster Y, Ozbek U, Medlej-Hashim M, Megarbane A, et al. Estimating the allele frequency of autosomal recessive disorders through mutational records and consanguinity: the Homozygosity Index (HI). Ann Hum Genet. 2012;76(2):159-67.

Akrami SM. Genetics of consanguineous marriage: Impact and importance of counseling. J Pediatr Genet. 2012;1(4):217-20.

Galehdari H, Tangestani R, Ghasemian S. New single nucleotide deletion in the SMPD1 gene causes niemann pick disease type A in a child from Southwest Iran: a case report. Iran J Pediatr. 2013;23(2):233.

Manshadi MD, Kamaldeghlan B, Keshavarzi F, Aryani O, Dadgar S, Arastehkani A, et al. Four novel p. N385K, p. V36A, c. 1033–1034insT and c. 1417–1418delCT mutations in the sphingomyelin phosphodiesterase 1 (SMPD1) gene in patients with types A and B Niemann-Pick disease (NPD). Int J Mol Sci. 2015;16(4):6688-76.

Mikaeel S, Rabbbani B, Bady RS, Maleki M. A novel mutation in SMPD1 gene in a patient with Niemann-Pick disease. International Conference on Medicine, Public Health and Biological Sciences (MHPBS). https://www.scienceboard.net/Paper-MHPBS01-MHPBS01_159html2016.

Karimzadeh P, Tonekaboni SH, Ashrafi MR, Shafeghati Y, Rezayi A, Salehpour S, et al. Effects of miglustat on stabilization of neurologic disorder in Niemann-Pick disease type C: Iranian pediatric case series. J Child Neurol. 2013;28(12):1599-606.

Tonekaboni SH, Aryani O, Karimzadeh P, Zaman T, Ashrafi MR, Salehpour S, et al. Clinical and Molecular Study of NPC in Iran. Report of 5 Novel Mutations. Iran J Child Neurol. 2015;9(4):8-9.

Noroozi Asl S, Vakili R, Ghaemi N, Eshraghi P. The Report of Three Rare Cases of the Niemann-Pick disease in Birjand, South Khorasan, Eastern Iran. Iran J Child Neurol. 2017;11(3):53-6.

Rohanizadegan M, Abd SM, O'Donnell-Luria A, Mihalek I, Chen P, Sanders M, et al. Utility of rapid whole-exome sequencing in the diagnosis of Niemann-Pick type disease C presenting with fetal hydrops and acute liver failure. Cold Spring Harb Mol Case Stud. 2017;3(6):a002147.

Alavi A, Nafassi S, Shamshir H, Nejad MM, Elahi E. Identification of mutation in NPC2 by exome sequencing results in diagnosis of Niemann-Pick disease type C. Mol Genet Metab. 2013;110(1):139-44.

Simonoar CM, Desnec RJ, McGovern MM, Wasserstein MP, Schuchman EH. The demographics and distribution of type B Niemann-Pick disease: novel mutations lead to new genotype/phenotype correlations. Am J Hum Genet. 2002;71(6):1413-9.

Spiegel R, Raas-Rothschild A, Reish O, Regev M, Meiner V, Bargal R, Sury V, Meir K, Nadari M, Hermann G, Iancu TC. The clinical spectrum of fetal Niemann–Pick type C. Am J Med Genet A. 2009 ;149(3):446-50.

Jones I, He X, Katouzian F, Darroch PI, Schuchman EH. Characterization of common SMPD1 mutations causing types A and B Niemann-Pick disease and generation of mutant-specific mouse models. Mol Genet Metab. 2008;95(3):152-62.

Rodriguez-Pascua L, Gort L, Schuchman EH, Vilageliu L, Grinberg D, Chabás A. Identification and characterization of SMPD1 mutations causing Niemann-Pick types A and B in Spanish patients. Hum Mutat. 2009;30(7):1117-22.

Millat G, Marcas C, Tomasetto C, Chilh K, Fensom AH, Herzer K, et al. Niemann-Pick C1 disease: correlations between NPC1 mutations, levels of NPC1 protein, and phenotypes emphasize the functional significance of the putative sterol-sensing domain and of the cysteine-rich luminal loop. Am J Hum Genet. 2001;68(6):1373-85.

Abbedi E, Mohaddes Ardelimi S, Hosseinpour Feizi AA. Sphingomyelinase gene mutation common in patients with type A and B Niemann Pick disease in North West, Iran. article in persian with an abstract in English. J Urmi Univ Med Sci. 2016;26(12):1041-53.