New Single Nucleotide Deletion In the SMPD1 Gene Causes Niemann Pick Disease Type A in a Child from Southwest Iran: A Case Report

Hamid Galehdari1; Raheleh Tangestani2; Sepideh Ghasemian3

1. Department of Genetics, Shahid Chamran University, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Research Center of Thalassemia and Hemoglobinopathies, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3. Toxicology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Received: Jan 25, 2012; Accepted: Aug 01, 2012; Online Available: Dec 29, 2012

Abstract

Objective: Niemann Pick disease (NPD) type A (NPA: MIM #257200) is a lipid storage disorder with an autosomal recessive inheritance and occurs by defect of the SMPD1 gene encoding sphingomyelinase. Disruption of this enzyme leads to the accumulation of sphingomyelin in brain and liver, which in turn causes dysfunction or damage of tissue.

Methods: We report firstly a 2.5 year old boy with NPA in southwest Iran. Initially, the diagnosis was resulted on the basis of clinical symptoms. The genomic DNA of the suspected individual was subjected to exon sequencing of the SMPD1 gene. According to the human reference sequence NM_000543.4, a novel single guanine deletion resulting in a frameshift mutation (p.Gly247Alafs*9) was observed in the SMPD1 gene that might be causative for the outcome of the disease.

Findings: The present report is the first molecular genetics diagnosis of the NPA in southwest Iran. The detected deletion in the SMPD1 gene is remarkable because of its novelty.

Conclusion: Despite similar morbidity SGA infants exhibited higher lethal complication rates following delayed meconium passage compared to AGA infants.

Key Words: Niemann Pick disease; SMPD1 Gene; Acid sphingomyelinase-1; Mutation

Introduction

NPD is a heterogeneous disease with variations due to molecular and clinical courses. The incidence of the disease is approximately 1:150 000 live births[1]. Because of variations in pathological and clinical features of the disease, NPD is classified into those with deficiency of acid sphingomyelinase activity (types A and B), and those with defective intracellular processing and transporting of LDL cholesterol, which is known as type C. However, NPD type C (NPC) is different at the biochemical and molecular levels with a higher incidence than NPA and NPB[2]. Both types A and B are characterized by Acid sphingomyelinase (ASM) deficiency that leads to excessive sphingomyelin accumulation in all phagocytic cells and in neurons[3], NPB is biochemically similar to NPA, but with less severity[4]. Normally, the enzyme activity of ASM leads to the breakdown of sphingomyelin into ceramide and phosphorylcholine in lysosomes[3]. Generally, multiple organs such as spleen, liver, bone marrow, lymph nodes, lung and central nervous system are affected.
Diagnosis can be achieved at the early childhood with a poor prognosis. Most NPA patients die before reaching the age of 3 years[1,3]. Clinically, NPA is distinguished by failure of thrive, hepatosplenomegaly and progressive neurodegeneration[5]. At molecular genetics level, mutations in the SMPD1 gene on chromosome 11p14.5 are causative for the disease[6]. The SMPD1 gene is approximately 5 kb long and the coding sequence is divided among six exons.

According to the Human Gene Mutation Database (www.hmdb.cf.ac.uk), more than 100 different mutations (including missense, nonsense, deletion, insertion, and splice site mutations) have been reported in the SMPD1 gene.

To date, some clinical investigations have been reported from Iranian patients. For instance, Motamed et al reported a case of NPB in Iran and described the clinical course of his patient[7]. Furthermore, Motamed et al reported a ten year study of liver biopsies in children’s Medical Center in Tehran. They identified numerous patients with storage diseases. Most identified patients were affected with NPD and glycogen storage disease type 1 (GSD-I)[8]. In addition, Majidzadeh et al reported a case of NPD and compared clinical and molecular aspects of the disease[9]. Hoshmand et al presented a novel mutation in the SMPD1 gene (data not published). Here, we present the first molecular genetics diagnosis of NPA in an individual from southwest Iran.

Case Presentation

A 2.5 year-old male, the first born child from first cousin parents was attending our center for genetic counseling and genetic diagnostics. The patient showed typical signs of the Niemann Pick disease, such as hepatosplenomegaly, developmental delay, mental retardation, and foam cells in the bone marrow, hypotonia, and cherry red maculae. Seizure and anorexia at 5-6th month of life was observed. He died as results of his illness. Unfortunately, measurement of the ASM activity in the white blood cells failed and DNA test was the only chance to establish a definite diagnosis.

After obtaining informed consent, blood samples were collected from patient and his parents.

Genomic DNA was extracted from EDTA-anticoagulated whole blood by standard salting out procedures. Selective amplification of all 6 exons was performed in a volume of 25 µl reaction containing 10 pmol of each primer (TAG Copenhagen A/S, Fruebjergvej3, Denmark) and 50ng of genomic DNA. PCR was carried out by a set of designed primers using primer3out software (http://frodo.wi.mit.edu/primer3/). Properties of primers are indicated in Table 1.

Temperature profile of reactions were as follow: initial denaturation at 95°C for 3 min, 35 cycles of 95°C for 30s, 56-60°C for 30s and 72°C for 45s and a final extension at 72°C for 7 min.

| exon  | Primer sequence (5’to 3’)   | length | Tm     |
|-------|----------------------------|--------|--------|
| 1-forward | GAAGCCGCACATGGCCCGCTA      | 445 bp | 60.5°C |
| 1-reverse | AGATGCCACCTCCTCACTAGGA     |        |        |
| 2A-forward | GTTGCACCTAGCTTGGCCTG       | 493 bp | 60.3°C |
| 2A-reverse | GTCTGACAGTACGGTCCCTG       |        |        |
| 2B-forward | TGCTGCTTTGGACAGGCTG       | 1058 bp| 59.4°C |
| 2B-reverse | GCACATAGGGAGCTGCTAGAGCA   |        |        |
| 3-forward | GGCTAGACTGCTGACTCTGGATGAG | 441 bp | 59.1°C |
| 3-reverse | CAAAGTGGGACATGAGCTGGAATCC |        |        |
| 4-forward | GTTGACCTGAGGACACCTCTG     | 354 bp | 58.8°C |
| 4-reverse | CCACCTTACGGGACACAG       |        |        |
| 5-forward | GACAGCGGTAGGTCTAGAGGCTG   | 490 bp | 59.4°C |
| 5-reverse | GTACACAGGGTAACCTGCAAAGGA  |        |        |
| 6-forward | ACGCCGGGCTTATGCTGTTGAG   | 678 bp | 59.6°C |
| 6-reverse | ACCGGATGATCTTGCTGTTGAA   |        |        |
Direct sequencing of PCR products was carried out using ABI automated sequencer 3700 according to the manufacturer's instruction (ABI 3700, PE Applied BioSystems, Foster City, CA, USA). Sequencing reactions were accomplished by the same primers that were used for exon amplification. Sequence analysis was performed with the software BioEdit (version 7.0.5.3). According to the human reference sequence NM_000543.4, a homozygous single guanine deletion, c.740delG, was observed in exon 2 of the SMPD1 gene, which results in a premature stop codon (CTG>TGA) between codons 256-257 (p.Gly247Alafs*9) (Fig 1). Apparently healthy parents were heterozygous for the detected change. They are also obligate carrier. To confirm the pathogenic relevance of the detected deletion, 27 healthy individuals were tested.

**Discussion**

To our knowledge by intensive literature searching, the present report demonstrates firstly the molecular genetics diagnosis of the NPD in southwest Iran. Approximately 1 per 40,000 people of Ashkenazi Jewish descent has NPA[10]. In contrast, there is no information about frequency and distribution of different types of NPD in Iran, particularly in southwest Iran.

The disease refers to a group of disorders with deficiency in lipid storage, causing accumulation of fats in brain and liver that lead to serious damage or dysfunction of mentioned tissues[11]. However, liver enlargement, brain damage, difficulty in walking, speaking and learning are characteristic symptoms for NPA[12]. Diagnosis of this type of disease is usually made by measuring the ASM activity in white blood cells[5]. Regarding some typical symptoms, our patient was suspected to suffer of NPA. He was also referred to our molecular diagnostic center with the aim to find genetic cause of the disease. The SMPD1 gene is the only gene considered to be associated with NPA[6].

Here, we report an unreported deletion of single guanine in exon 2 of the SMPD1 gene, causing a truncated gene product. Exon 2 is unusually large, which encodes for 258 amino acids or approximately 44% of the entire ASM polypeptide. However, the pathogenic nature of the novel deletion was confirmed by screening of healthy individuals.

Three common missense mutations account for
more than 90% of the mutant alleles in individuals of Ashkenazi Jewish ancestry with NPA. But in contrast to the Ashkenazi Jewish population, most affected individuals with NPA showed in previous studies unique mutations in the SMPD1 gene[11]. Consequently, identification of new NPA cases increases the probability to discover new specific mutations for the given population.

The present observations extend the mutation spectrum of the SMPD1 gene in NPA patients worldwide, and can be used for the prenatal or preimplantation genetic diagnosis, at least in southwest Iran.

**Conclusion**

To date, there are no hopeful therapeutic rudiments to cure NPA patients. Therefore, prevention seems to be more effective strategy in the next pregnancy for this family and other families with positive history.

**Conflict of Interest:** None

**References**

1. Niemann-Pick Disease Group UK. Website providing information and support for all types of Niemann-Pick diseases. Available at: www.niemannpick.org.uk.
2. Imrie J, Dasgupta S, Besley GT, et al. The natural history of Niemann-Pick disease type C in the UK. *J Inherit Metab Dis* 2007; 30(1):51-9.
3. Harzer K, Rolfs A, Bauer P, et al. Niemann-Pick disease type A and B are clinically but also enzymatically heterogeneous: pitfalls in the laboratory diagnosis of sphingomyelinase deficiency associated with the mutation Q292 K. *Neuropediatrics* 2003;34(6):301-6.
4. McGovern MM, Wasserstein MP, Giugliani R, et al. A prospective, cross-sectional survey study of the natural history of Niemann-Pick disease type B. *Pediatrics* 2008;122(2):e341-9.
5. Alizon C, Beucher AB, Gourdier AL, et al. Type B Niemann Pick disease: Clinical description of three patients in a same family. *Rev Med Inteine* 2010; 31(8):562-5.
6. Simonaro CM, Park JH, Eliyahu E, et al. Imprinting at the SMPD1 locus: implications for acid sphingomyelinase-deficient Niemann-Pick disease. *Am J Hum Genet* 2006;78(5):865-70.
7. Motamedi MK, Khoddami M, Naseri M, et al. Type B Niemann Pick Disease: report of case with military pattern on chest X-ray. *Arch Iranian Med* 2005; 8 (3):223-5.
8. Motamed F, Monajemzadeh M, Seifrad S, et al. Liver Storage Disease in Iran: A ten year study of liver biopsies in Children Medical Centre Hospital in Tehran-Iran. *Hepat Mon* 2011;11(8):652-5.
9. Majidizadeh T, Dehghan Manshadi M, Rostami M, et al. Niemann Pick disease, a clinical course and molecular findings; a case report of an Iranian patient. *Eurogene* 2009;6(2):234-7.
10. Wasserstein MP, Aron A, Brodie SE, et al. Acid sphingomyelinase deficiency: prevalence and characterization of an intermediate phenotype of Niemann-Pick disease. *J Pediatr* 2006;149(4):554-9.
11. Schuchman EH. The pathogenesis and treatment of acid sphingomyelinase-deficient Niemann-Pick disease. *J Inherit Metab Dis* 2007;30(5):654-63.
12. McGovern MM, Aron A, Brodie SE, et al. Natural history of Type A Niemann-Pick disease: possible endpoints for therapeutic trials. *Neurology* 2006; 66(2):228-32.