Short Report

Identification of seven novel SMPD1 mutations causing Niemann–Pick disease types A and B


Niemann–Pick disease (NPD) types A and B are autosomal, recessively inherited, lysosomal storage disorders caused by deficient activity of acid sphingomyelinase (E.C. 3.1.4.12) because of mutations in the sphingomyelin phosphodiesterase-1 (SMPD1) gene. Here, we present the molecular analysis and clinical characteristics of 15 NPD type A and B patients. Sequencing the SMPD1 gene revealed eight previously described mutations and seven novel mutations including four missense [c.682T>C (p.Cys228Arg), c.1159T>C (p.Cys387Arg), c.1474G>A (p.Gly492Ser), and c.1795C>T (p.Leu599Phe)], one frameshift [c.169delG (p.Ala57Leufs*20)] and two splicing (c.316+1G>T and c.1341delG). The most frequent mutations were p.Arg610del (21%) and p.Gly247Ser (12%). Two patients homozygous for p.Arg610del and initially classified as phenotype B showed different clinical manifestations. Patients homozygous for p.Leu599Phe had phenotype B, and those homozygous for c.1341delG or c.316+1G>T presented phenotype A. The present results provide new insight into genotype/phenotype correlations in NPD and emphasize the difficulty of classifying patients into types A and B, supporting the idea of a continuum between these two classic phenotypes.

Conflict of interest

The authors declare no conflict of interests related to this study.

Keywords: chitotriosidase – genotype/phenotype correlations – genotyping – Niemann–Pick disease – SMPD1 – sphingomyelinase

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Niemann–Pick disease types A (NPA) and B (NPB) are caused by deficient activity of acid sphingomyelinase (ASM; E.C. 3.1.4.12). NPA (MIM# 257200) is a fatal infantile neurodegenerative disorder characterized by massive hepatosplenomegaly and a rapidly progressive neurologic course. NPB (MIM# 607616) is a non-neuronopathic disorder characterized by hepatosplenomegaly and pulmonary involvement. Broad phenotypic variability has been reported within both types, with the recognition of a continuum between them; a large number of patients have been classified as ‘intermediate or atypical type’ (IT) (1).

ASM or sphingomyelin phosphodiesterase-1 is encoded by SMPD1 (MIM# 607608, GenBank accession number M81780.1) (2). To date, more than 100 mutations causing NPD have been reported (Human Gene Mutation Database: http://www.hgmd.org). Most mutations are ‘private’, occurring in one or a few families; however, some mutations have been reported to occur with substantial frequencies in certain ethnic groups (3, 4).

This study reports the molecular and enzymatic characterization of 15 NPD patients, as well as an analysis of the genotype/phenotype relationship.

Materials and methods
We studied 15 patients (12 apparently unrelated) from different parts of Spain; three were immigrants from North Africa and two were of Turkish origin. Patients 7, 10, and 12 were siblings of Patients 8, 11, and 13, respectively. All patients or their guardians gave informed consent. The study protocol was approved by the Aragon Experimental Ethical Committee (CEICA), Spain, and developed following the ethical standards of the Helsinki declaration of 1975, as revised in 2000. Patients were classified as type A, B or IT according to Pavlů-Pereira et al. (1). NPD diagnosis was established by the analysis of sphingomyelinase activity in leukocytes and/or cultured skin fibroblasts using C14:choline-methyl-[14C]sphingomyelin (5) or the fluorimetric enzyme assay (6). Plasma chitotriosidase (CT) activity was measured according to Pavlů-Pereira et al. (1) and was established by the analysis of sphingomyelinase activity in leukocytes and/or cultured skin fibroblasts using C14:choline-methyl-[14C]sphingomyelin (5) or the fluorimetric enzyme assay (6). Plasma chitotriosidase (CT) activity was measured as previously described (7). Plasma pulmonary and activation regulated chemokine (CCL18/PARC) concentration was analyzed by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) (8). Genomic DNA was extracted from peripheral blood using a standard protocol; the SMPD1 fragments were amplified by using the polymerase chain reaction (PCR) and sequenced on a MegaBACE 500 DNA Analysis System (GE Healthcare, Amersham, UK). Figure 1 presents the amplification strategy. Mutations were confirmed with two independent PCR products and with parental DNA when available. Hundred control alleles from the general healthy Spanish population were also sequenced. All mutations were described according to the Human Genome Variation Society (HGVS) recommendations (http://www.hgvs.org/mutnomen). Several software tools were used to predict the effects of amino acid changes on protein function and HUMAN SPLICING FINDER (http://www.umd.be/HSF/), NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/) and NNSPLICE (http://www.fruitfly.org/seq_tools/splice.html) to analyze potential splicing mutations. Total RNA was extracted from cultured fibroblasts using the RNasy Mini kit (Qiagen, Chatsworth, CA), treated with RNase-Free DNase Set (Qiagen, Chatsworth, CA) to remove the genomic DNA and reverse transcribed using random hexamer primers and the RevertAid H minus first strand cDNA synthesis kit (Thermo Fisher Scientific Inc., Waltham, MA). The SMPD1 cDNA was amplified by PCR using specific primers and sequenced. Primers used are listed in Table S1, Supporting Information. Genotyping for c.1049_1072dup24 in CHIT1 was performed as previously described (9).

Statistical analysis was carried out using the ssps software package (SPSS Inc., Chicago, IL). Variable distribution normality was analyzed by the Kolmogorov–Smirnov test and mean comparison by non-parametric Mann–Whitney U test. Differences with p < 0.05 were considered statistically significant.

Results
Clinical and biochemical data of patients at baseline are presented in Table 1.

We sequenced SMPD1 in 15 NPD patients identifying 15 different mutations, 7 of them were previously undescribed (Table 2). The most prevalent mutations were p.Arg610del and p.Gly247Ser, accounting for 21% (5/24) and 12% (3/24) of total alleles, respectively. None of the novel mutations was found in any of the 100 control alleles. Parental genotyping was possible in all cases except for Patients 2, 8, 11 and 14 in order to ensure that the mutations were located in different alleles.
The amplification strategy. The SMPD1 gene was amplified by polymerase chain reaction in a group of four overlapping fragments (comprising 983 nucleotides of promoter zone, exons 1 and 2, intron 1, and partial intron 2) and a second group of three overlapping fragments (comprising exons 3, 4, 5, and 6, 447 bp into the 3′ untranslated region (UTR), partial intron 2, and full introns 3, 4, and 5).

Table 1. General characteristics of patients at baseline

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Onset age (years)</th>
<th>Sex</th>
<th>NPD type</th>
<th>% ASM&lt;sup&gt;a&lt;/sup&gt;(L/Fb)</th>
<th>SMPD1 genotype</th>
<th>CT activity (nmol/ml/h)</th>
<th>CT genotype dup 24 bp</th>
<th>CCL18 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>F</td>
<td>B</td>
<td>15/0.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.(Gly29Aspfs<em>48);[Trp32</em>]</td>
<td>0</td>
<td>Homo</td>
<td>862</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>F</td>
<td>B</td>
<td>15/0.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.[Arg610del];[Arg610del]</td>
<td>455</td>
<td>Neg</td>
<td>1024</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>F</td>
<td>B</td>
<td>9/ND</td>
<td>p.[Gly247Ser];[Arg610del]</td>
<td>1666</td>
<td>Neg</td>
<td>645</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>M</td>
<td>B</td>
<td>17/ND</td>
<td>p.[Phe333Serfs*52];[Arg476Trp]</td>
<td>1410</td>
<td>Neg</td>
<td>719</td>
</tr>
<tr>
<td>5</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>M</td>
<td>A</td>
<td>0/0/3.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.[Gly247Ser];[Cys387Arg]</td>
<td>520</td>
<td>Het</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>M</td>
<td>B</td>
<td>0/1.4/8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.[Gly247Ser];[Gly492Ser]</td>
<td>48</td>
<td>Neg</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>M</td>
<td>B</td>
<td>0/0</td>
<td>p.[Gly247Ser];[Gly492Ser]</td>
<td>96</td>
<td>Neg</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>F</td>
<td>A</td>
<td>ND/4.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.[Cys228Arg];[Ala57Leufs*20]</td>
<td>ND</td>
<td>Het</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>M</td>
<td>B</td>
<td>22/ND</td>
<td>p.[Leu599Phe];[Leu599Phe]</td>
<td>112</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>F</td>
<td>B</td>
<td>20/ND</td>
<td>p.[Leu599Phe];[Leu599Phe]</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>F</td>
<td>IT</td>
<td>ND/5.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.[Arg610del];[Arg610del]</td>
<td>ND</td>
<td>Neg</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>F</td>
<td>A</td>
<td>0/1.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>c.[1341delG];[1341delG]</td>
<td>855</td>
<td>Neg</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>F</td>
<td>A</td>
<td>2.8/ND</td>
<td>c.[1341delG];[1341delG]</td>
<td>878</td>
<td>Neg</td>
<td>1417</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
<td>F</td>
<td>IT</td>
<td>ND/2.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.[Arg230Cys];[Arg378His]</td>
<td>ND</td>
<td>Het</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>1.1</td>
<td>M</td>
<td>A</td>
<td>4/ND</td>
<td>c.[316+1G&gt;T];[316+1G&gt;T]</td>
<td>792</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

CT, chitotriosidase; F, female; IT, intermediate or atypical type; M, male; ND, not determined; NPD, Niemann–Pick disease.

<sup>a</sup>Residual enzymatic activity determined in either fibroblasts (Fb) or leukocytes (L) and expressed as the percentage of normal values.

<sup>b</sup>Deceased patient.

<sup>c</sup>Using the fluorimetric enzyme assay described by van Diggelen et al. (6).

<sup>d</sup>Using C14:choline-methyl-[14C]sphingomyelin (52 mCi/mm; Perkin Elmer, Waltham, MA) as a substrate with the method described by Vanier et al. (5).

Patients 9 and 10, homozygous for the novel mutation p.(Leu599Phe), presented a moderate splenomegaly but no hepaticomegaly, ocular manifestations, or pulmonary or neurological symptoms. Patients 12 and 13, homozygous for the novel mutation c.1341delG, exhibited hepatosplenomegaly, pulmonary involvement, failure to thrive, deafness, feeding difficulties, lack of concentration, and cherry red maculae (CRM). Patient 15, homozygous for the novel mutation c.316+1G>T, presented hepatosplenomegaly, pulmonary involvement, coarse facial appearance, mild hypotonia, delayed development and CRM. Patients homozygous for p.Arg610del presented with diverse symptoms. Patient 2 had only a severe splenomegaly. In contrast, Patient 11 showed hepatosplenomegaly, gastrointestinal symptoms, delayed development, and after an initial period of normal neurologic development presented with regression and neurological signs (hypotonia and convergent squint).

The remaining mutations appeared as compound heterozygous. Patient 5, heterozygous for p.Gly247Ser and the novel p.(Cys387Arg) mutations, displayed hepatosplenomegaly, failure to thrive, vomiting, axial hypotonia and CRM. In contrast, p.Gly247Ser in heterozygosis with the novel p.(Gly492Ser) mutation (Patients 6 and 7) caused a mild phenotype. One of them was asymptomatic and the other had been splenectomized. The combination of p.Gly474Ser and p.Arg610del (Patient 3) caused splenomegaly, mild dyspnea, headache and dizziness. Patient 1, heterozygous for p.Trp32* and the frameshift mutation p.Gly29Aspfs*48, showed splenomegaly and interstitial pulmonary infiltration. Patient 4, heterozygous for p.Arg476Trp and p.Phe333Serfs*52, suffered from organomegaly. Patient 8, heterozygous for two novel mutations, p.(Ala57Leufs*20) and p.(Cys228Arg), presented hepatosplenomegaly, coarse facial appearance, development delay, axial hypotonia,
decreased muscle bulk, absent deep tendon reflexes in lower extremities and CRM. Patient 14, heterozygous for p.Arg230Cys and p.Arg378His, presented a large hepatosplenomegaly and pulmonary involvement during her first 5 years of life, with neurological regression causing inability to walk. Bronchitis and recurrent pneumonia were present until their disappearance at the age of 14. The spleen size reduced in puberty and since then she has had normal development.

Mean plasma CT activity was significantly higher in NPD patients than controls (683 ± 550 vs 58 ± 33 nmol/ml/h; p = 0.001) but there was no relationship between CT activity and clinical severity (Table 1). The available CCL18/PARC plasma concentrations showed a marked elevation for patients (933 ± 307 ng/ml) vs controls (64 ± 33 ng/ml), p = 0.001.

### Discussion

The mutational analysis of 15 NPD patients using overlapping fragments was a good approach to identify all the mutant alleles. The p.Arg610del mutation was the most frequent in our cohort as previously reported (3, 4), followed by the p.Gly247Ser mutation. Surprisingly, the p.Ala484Glu mutation, previously reported as the second most frequent in Spain (3), was not found in any patient.

We found two patients homozygous for p.Arg610del. Patient 2 presented a mild NPB in agreement with previous studies (10, 11) and with previous results of in vitro expression (3). In contrast, Patient 11 showed a more severe phenotype and was reclassified as an IT because of the onset of neurological signs. However, a patient with similar symptoms classified as NPB (12) and other intermediate cases have also been reported (1, 13). The fact that patients with the same genotype showed different symptoms indicates that there is not a clear genotype/phenotype association, and suggests that there must be other factors that modify the clinical presentation.

The novel mutation p.(Leu599Phe) found in homozygosis was associated with a mild NPB with significant residual ASM activity (approximately 20%), in agreement with the predicted effect on protein function (Table 3). Another novel mutation found in homozygosis, c.1341delG, was associated with a severe NPA. The sequence of the mutant fibroblasts c-DNA showed that this deletion produced an alternative splicing with the introduction of the 3′ thirty nucleotides of intron 4. The novel mutation c.316+1G>T in homozygosis was associated with a severe NPA. The splicing analysis tools each consistently identified the donor splicing site in the wild-type sequence but not in the mutated sequence. The compound genotype of two novel mutations, p.(Ala57Leufs*20) and p.(Cys228Arg), resulted in a severe NPA. The latter mutation affects a disulfide bond, leading to misfolding or impaired protein stability. The predicted effect of this combination (frameshift + folding/stability problems) was consistent with the observed phenotype. The p.Gly247Ser mutation was found in homozygosis with three different mutations. Homoaallelism for Gly247Ser has previously been unequivocally associated with NPA (14); the same phenotype was observed in homozygosis with the novel p.(Cys387Arg) mutation, which seems to affect a disulfide bond, leading to misfolding or decreased protein stability. In contrast, the homozygosis for p.Gly247Ser and p.Arg610del mutations was associated with NPB, in agreement with the mild effect previously described for p.Arg610del. The homozygosis for p.Gly247Ser and the novel p.(Gly492Ser) mutation is intriguing.
Additional Supporting information may be found in the online version of this article.

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References

Identification of mutations causing NPA and NPB


