MUTATION UPDATE

Molecular Pathology of NEU1 Gene in Sialidosis

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Lysosomal sialidase (EC 3.2.1.18) has a dual physiological function; it participates in intralysosomal catabolism of sialylated glycoconjugates and is involved in cellular immune response. Mutations in the sialidase gene NEU1, located on chromosome 6p21.3, result in autosomal recessive disorder, sialidosis, which is characterized by the progressive lysosomal storage of sialylated glycopeptides and oligosaccharides. Sialidosis type I is a milder, late-onset, normosomatic form of the disorder. Type I patients develop visual defects, myoclonus syndrome, cherry-red macular spots, ataxia, hyperreflexia, and seizures. The severe early-onset form, sialidosis type II, is also associated with dysostosis multiplex, Hurler-like phenotype, mental retardation, and hepatosplenomegaly. We summarize information on the 34 unique mutations determined so far in the sialidase gene, including four novel missense and one novel nonsense mutations found in two Czech and two French sialidosis patients. The analysis of sialidase mutations in sialidosis revealed considerable molecular heterogeneity, reflecting the diversity of clinical phenotypes that make molecular diagnosis difficult. The majority of sialidosis patients have had missense mutations, many of which have been expressed; their effects on activity, stability, intracellular localization, and supramolecular organization of sialidase were studied. A structural model of sialidase allowed us to localize mutations in the sialidase molecule and to predict their impact on the tertiary structure and biochemical properties of the enzyme. Hum Mutat 22:343–352, 2003.

KEY WORDS: NEU1; sialidase; neuraminidase; sialidosis; cathepsin A; protective protein; mutation analysis

DATABASES:
NEU1 – OMIM: 256550; GenBank: NM_000434

INTRODUCTION

Lysosomal storage diseases are inherited metabolic disorders caused by defects in the synthesis, processing, targeting, or function of lysosomal enzymes and proteins involved in the biogenesis of this organelle. The diseases are characterized by the massive accumulation of undegraded substrates of deficient enzymes in the lysosomes of the affected tissues. The biggest group of lysosomal storage diseases is associated with deficiencies of glycosidases involved in the catabolism of the sugar chains of glycolipids, oligosaccharides, and glycoproteins. This group includes single-enzyme deficiencies: the most frequent being Gaucher disease (MIM# 230800), caused by deficiency of acid β-glucosidase; Fabry disease (MIM# 301500), caused by deficiency of α-galactosidase A; and Tay-Sachs disease (MIM# 272800), caused by deficiency of β-hexosaminidase A); as well as the functional deficiencies of multiple enzymes such as I-cell disease (MIM# 252500), caused by genetic mutations in enzymes responsible for posttranslational modification of multiple lysosomal glycosidases [reviewed in Kornfeld and Sly, 2001].

Clinically related autosomal recessive disorders, sialidosis (MIM# 256550) and galactosialidosis (MIM# 256540), are of particular interest because the same underlying biochemical defect, deficiency of lysosomal sialidase, is caused in these disorders by two different primary genetic defects. Sialidosis (mucolipidosis I or “cherry-red spot myoclonus syndrome”) is an autosomal recessive lysosomal storage disease caused by mutations in the sialidase gene [reviewed in Thomas, 2001], whereas galactosialidosis results from mutations in the gene coding for a lysosomal carboxypeptidase, cathepsin A [reviewed in d’Azzo et al., 2001]. Extensive biochemical studies have shown that in the lysosome these two enzymes form a high molecular weight complex, necessary for the functional activity of sialidase [reviewed in Pshezhetsky and Ashmarina, 2001]. Cathepsin A stabilizes

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the catalytically active conformation of sialidase and protects it against rapid proteolytic degradation in the lysosome [d’Azzo et al., 1982; van der Horst et al., 1989; Vinogradova et al., 1998]. Cathepsin A was cloned and its mutations in galactosialidosis were first characterized 15 years ago [Galjart et al., 1988], but cloning of sialidase has been hampered for almost two decades by the low tissue content and instability of this enzyme. Three groups simultaneously identified the human sialidase cDNA and gene by a homologous search in the Expressed Sequence Tags Database (dbEST, National Center for Biotechnology Information) and direct sequencing of human chromosome 6 [Bonten et al., 1996; Milner et al., 1997; Pshezhetsky et al., 1997]. These studies paved the way for the characterization of the molecular basis of sialidosis.

Sialidosis is subdivided into two main clinical variants with different ages of onset and severity. Sialidosis type I, or the nondysmorphic type, is a relatively mild, late onset form. Patients usually present on the second or the third decade of life and develop gait abnormalities, progressive impaired vision, bilateral macular cherry-red spots, and myoclonus syndrome [Durand et al., 1977; Rapin et al., 1978; O’Brien, 1979]. Ataxia and seizures have also been reported [reviewed in Thomas, 2001]. Sialidosis type II, or the dysmorphic type, is the early-onset form, which is also associated with Hurler-like phenotype, dystostosis multiplex, short stature, developmental delay, mental retardation, and hepatosplenomegaly [Kelly and Graetz, 1977; Winter et al., 1980]. Sialidosis type II patients are classified as those having the infantile-onset form who are relatively normal at birth, and those having the congenital-onset form that manifests prenatally and is associated with ascites and hydrops fetalis [Aylsworth et al., 1980; Beck et al., 1984]. Some authors have also used the term “juvenile sialidosis” to describe a form that manifests in late childhood, with a relatively mild clinical phenotype [Itoh et al., 2002; Bonten et al., 2000].

Sialidase deficiency disrupts the catabolic pathways for degradation of sialylated glycoconjugates, causing their accumulation in the lysosome and excretion in urine [Thomas, 2001]. For all clinical subtypes of sialidosis, storage products mostly consist of sialylated oligosaccharides and glycoproteins [Thomas, 2001]. For some patients, a several-fold increase of G\textsubscript{M3}- and G\textsubscript{D3}-gangliosides was reported in systemic organs [Ulrich-Bott et al., 1987] and in the brain [Yoshino et al., 1990], suggesting that sialidase is also involved in degradation of these molecules; however, more recent work has described a separate enzyme, ganglioside sialidase, encoded by the NEU3 gene [Wada et al., 1999; Monti et al., 2000]. Since both NEU3 and NEU1 sialidases were able in vitro to desialylate G\textsubscript{M2}- and G\textsubscript{M3}-gangliosides into the corresponding asialo-derivatives, the question of which enzyme is implicated in the intralysosomal catabolism of gangliosides remains open [Igdoura et al., 1999; Li et al., 2001].

The human sialidase gene NEU1 is located inside the locus of the major histocompatibility complex (6p21.3) [Oohira et al., 1985], and contains five introns and six exons (Fig. 1). A single-splice product, 1,245-bp SIAL mRNA, codes for a 415-amino acid precursor. After the cleavage of a 47–amino acid N-terminal signal peptide and glycosylation, it becomes a 48.3-kDa mature active enzyme similar to that found in the multienzyme lysosomal complex. Sialidase is targeted to the endosomal-lysosomal compartment as an integral membrane protein by vesicular transport, which involves association of the adapter proteins with a tyrosine-containing internalization signal at the C-terminus of the enzyme [Lukong et al., 2001]. It is likely that the transmembrane

FIGURE 1. Location of sialidosis mutations in the sialidase gene. The exons are represented by boxes. The positions of missense mutations are shown above the gene. Other mutations (nonsense, insertions, deletions, and splice-site mutations) are shown below the gene. Clinical phenotypes associated with mutations are shown in Table 1.
domain in the lysosome is cleaved similarly to that of acid phosphatase, resulting in the appearance in the cell of two pools of lysosomal sialidase, soluble and membrane-associated, which are both absent in cultured cells of sialidosis patients [Verheijen et al., 1983; Miyagi et al., 1990, 1992, 1993]. Immunoelectron microscopy demonstrated that, in addition to the lysosomal membrane and lysosomal lumen, NEU1 sialidase is present on the plasma membrane and in intracellular (possibly endocytic) vesicles [Vinogradova et al., 1998].

**MUTATIONS AND THEIR BIOLOGICAL RELEVANCE**

A total of 34 mutations in the sialidase gene causing sialidosis have been identified so far by a variety of molecular techniques (Table 1). (The numbers for the nucleotide changes are reported in accordance with GenBank entry NM_000434). In addition to mutations identified in humans, a c.625C>A (p.L209P) change in the sialidase gene was reported in the SM/J mouse strain, characterized by a reduction of sialidase activity to 20–30% of normal [Carrillo et al., 1997; Rottier et al., 1998]. Polymorphisms within the coding sequence of the sialidase gene have never been reported.

**splice-Site Mutations**

A transversion, c.1020+1G>C, in the last intron of the sialidase gene, disrupts the consensus sequence between exon 5 and intron 5 and causes complete deletion of exon 5 from the transcript, as detected by RT-PCR [Penzel et al., 2001]. Skipping exon 5 would result in a frameshift and the appearance of a premature stop codon at position 268. In accordance with this prediction, a patient homozygous for the c.1020+1G>C mutation developed a severe prenatal form of sialidosis and showed a complete loss of sialidase activity in cultured skin fibroblasts [Penzel et al., 2001].

**Insertions and Deletions**

Three deletions and two insertions were identified so far in the sialidase gene (Table 1 and Fig. 1). One deletion (c.625delG) [Lukong et al., 2000] and one duplication (c.4_7dupACTG) [Pshezhetsky et al., 1997] cause frameshifts and the appearance of premature truncation codons, probably leading to nonsense-mediated decay of the mRNA and a complete loss of sialidase activity in the affected cells. Patients homozygous for c.625delG and c.4_7dupACTG mutations belong to a severe infantile-onset form of sialidosis associated with dysostosis multiplex, dysmorphic phenotype, hepatosplenomegaly, and ascites [Pshezhetsky et al., 1997; Lukong et al., 2000]. Another deletion, c.1209delC [Bonten et al., 1996], caused a frameshift that extended the sialidase protein by 69 amino acids. Transient expression of the mutant protein showed that it does not have enzymatic activity and is not transported to the lysosome [Bonten et al., 1996]. Duplication c.1193_1198dupACCCT [Bonten et al., 2000] caused an in-frame duplication of H399 and Y400 amino acid residues and produced a mutant protein with about 30% of residual activity. Finally, Uhl et al. [2002] found an 11-kb interstitial deletion that removed the entire coding region of the sialidase gene. Two Turkish patients homozygous for the deletion had a severe prenatal form of sialidosis and died shortly after birth [Uhl et al., 2002].

**Nonsense Mutations**

Three nonsense mutations have been reported: c.838C>T (p.R280X), c.1129G>T (p.E377X)1 [Bonten et al., 2000], and c.87G>A (p.W29X) [Sergi et al., 2001]. All of them should result in a synthesis of truncated proteins without catalytic activity. In the case of the c.1129G>T (p.E377X) mutation, truncated protein expressed in human fibroblasts or COS cells lacked both lysosomal localization and catalytic activity [Bonten et al., 1996]. Siblings carrying this mutation in one allele showed a mild clinical phenotype because of the V54M missense mutation in the other allele that reduced enzymatic activity of the mutant sialidase in vitro to only ~40% of the normal level [Bonten et al., 2000]. R280X and W29X mutations were found in the most severely affected patients with a congenital form of sialidosis [Bonten et al., 2000; Sergi et al., 2001].

In this study, the novel nonsense mutation c.1039C>T, which changes the codon of R347 into a stop codon, was found in a sialidosis patient of Czech origin. The patient developed a clinical phenotype typical for infantile type II sialidosis. Developmental delay, coarse facial features, and enlarged head circumference were noted at the second month of age. A skeletal x-ray at the age of 13 months revealed dysostosis multiplex. At this age hepatomegaly and vacuolated lymphocytes were also observed. The mutation was identified by the DHPLC analysis of PCR-amplified exons and flanking intron regions, followed by direct sequencing of the fragments that showed abnormal DHPLC elution profiles (not shown). Presence of the mutation in the mRNA of patients’ cells was confirmed by sequencing of cDNA amplification products (not shown). A premature termination codon should lead to the nonsense mediated decay of the mRNA, but even if the truncated sialidase protein is synthesized, 66 amino acid residues comprising the sixth β-sheet of the sialidase structure [Lukong et al., 2000] will be missing, and therefore the protein will not be properly folded. In addition, the mutant protein should lack two active site residues (Y370 and E394) and the C-terminal tyrosine-containing signal necessary for sialidase targeting [Lukong et al., 2001]. The patient is heterozygous for this mutation, and because we could not determine the mutation in the second allele, we cannot

1 Authors reported mutation as c.1127G>T (p.G378X) [Bonten et al., 2000]. However a codon GGC coding for G378 cannot be changed into a stop codon by a G>T mutation. So we assume that authors meant the c.1129G>T mutation causing a change of the codon for E377 to a stop codon.
## TABLE 1. Mutations in the Sialidase Gene Causing Sialidosis

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Base change</th>
<th>Exon</th>
<th>Effect</th>
<th>2nd allele</th>
<th>Exon</th>
<th>Base change</th>
<th>Exon</th>
<th>Phenotype</th>
<th>Origin</th>
<th>Reference</th>
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<tr>
<td><strong>Splicing site mutations</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>c.1020+1G&gt;C</td>
<td>c.1020+1G&gt;C</td>
<td>Intron 5</td>
<td>Skip of exon 5, frameshift</td>
<td>c.1020+1G&gt;C</td>
<td>Intron 5</td>
<td></td>
<td>Type II congenital</td>
<td>Not reported</td>
<td>Penzel et al. [2001]</td>
<td></td>
</tr>
<tr>
<td><strong>Insertions and deletions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.1020+1G&gt;C</td>
<td>c.1020+1G&gt;C</td>
<td>Intron 5</td>
<td>Deletion of entire coding sequence</td>
<td>c.1020+1G&gt;C</td>
<td>Intron 5</td>
<td></td>
<td>Type II congenital</td>
<td>Not reported</td>
<td>Penzel et al. [2001]</td>
<td></td>
</tr>
<tr>
<td>L231H</td>
<td>c.692T&gt;A</td>
<td>4</td>
<td></td>
<td>R294S</td>
<td>4</td>
<td>c.880C&gt;A</td>
<td>2</td>
<td>Type II infantile</td>
<td>American</td>
<td>Bonten et al. [2000]</td>
</tr>
<tr>
<td>W240R</td>
<td>c.718T&gt;C</td>
<td>4</td>
<td></td>
<td>P80L</td>
<td>4</td>
<td>c.293C&gt;T</td>
<td>2</td>
<td>Type II congenital</td>
<td>Japanese</td>
<td>Bonten et al. [2000]</td>
</tr>
<tr>
<td>G243R</td>
<td>c.727G&gt;A</td>
<td>4</td>
<td></td>
<td>G243R</td>
<td>4</td>
<td>c.727G&gt;A</td>
<td>2</td>
<td>Type II infantile</td>
<td>American</td>
<td>Bonten et al. [2000]</td>
</tr>
<tr>
<td>E377X^a</td>
<td>c.160G&gt;A</td>
<td>4</td>
<td></td>
<td>E377X</td>
<td>4</td>
<td>c.1129G&gt;T</td>
<td>2</td>
<td>Type II infantile</td>
<td>Italian</td>
<td>Pshezhetsky et al. [1997]</td>
</tr>
<tr>
<td>G270F</td>
<td>c.808C&gt;T</td>
<td>5</td>
<td></td>
<td>L270F</td>
<td>5</td>
<td>c.808C&gt;T</td>
<td>2</td>
<td>Type II congenital</td>
<td>Spanish</td>
<td>Lukong et al. [2000]</td>
</tr>
<tr>
<td>V275A</td>
<td>c.824T&gt;C</td>
<td>5</td>
<td></td>
<td>V275A</td>
<td>5</td>
<td>c.407G&gt;A</td>
<td>2</td>
<td>Type II infantile</td>
<td>Spanish</td>
<td>Rodríguez Criado et al. [2003]</td>
</tr>
<tr>
<td>R294S</td>
<td>c.880C&gt;A</td>
<td>5</td>
<td></td>
<td>L231H</td>
<td>5</td>
<td>c.692T&gt;A</td>
<td>2</td>
<td>Type I adult</td>
<td>French</td>
<td>Bonten et al. [2000]</td>
</tr>
<tr>
<td>A298V</td>
<td>c.893C&gt;T</td>
<td>5</td>
<td></td>
<td>A298V</td>
<td>5</td>
<td>c.893C&gt;T</td>
<td>2</td>
<td>Type II infantile</td>
<td>Jewish</td>
<td>Bonten et al. [2000]</td>
</tr>
<tr>
<td>P316S</td>
<td>c.946C&gt;T</td>
<td>5</td>
<td></td>
<td>P316S</td>
<td>5</td>
<td>c.946C&gt;T</td>
<td>2</td>
<td>Type I adult</td>
<td>Japanese</td>
<td>Bonten et al. [2000]</td>
</tr>
</tbody>
</table>

### Notes
- "^a" indicates a novel mutation.
- "^b" indicates a conserved change.
- "^c" indicates a proline residue.
- "^d" indicates a threonine residue.
- "^e" indicates a glutamate residue.
- "^f" indicates a serine residue.
- "^g" indicates a glycine residue.
- "^h" indicates a histidine residue.
- "^i" indicates a lysine residue.
- "^j" indicates a glutamine residue.
- "^k" indicates a methionine residue.
- "^l" indicates a valine residue.
- "^m" indicates an alanine residue.
- "^n" indicates a cysteine residue.
- "^o" indicates a tyrosine residue.
- "^p" indicates a tryptophan residue.
- "^q" indicates a proline residue.
- "^r" indicates a threonine residue.
- "^s" indicates a glutamate residue.
- "^t" indicates a glycine residue.
- "^u" indicates a histidine residue.
- "^v" indicates a lysine residue.
- "^w" indicates a glutamine residue.
- "^x" indicates a methionine residue.
- "^y" indicates a valine residue.
- "^z" indicates an alanine residue.
- "^A" indicates a proline residue.
- "^B" indicates a glycine residue.
- "^C" indicates a histidine residue.
- "^D" indicates a lysine residue.
- "^E" indicates a glutamine residue.
- "^F" indicates a methionine residue.
- "^G" indicates a valine residue.
- "^H" indicates an alanine residue.
- "^I" indicates a cysteine residue.
- "^J" indicates a tyrosine residue.
- "^K" indicates a tryptophan residue.
- "^L" indicates an arginine residue.
- "^M" indicates a glutamine residue.
- "^N" indicates a methionine residue.
- "^O" indicates an isoleucine residue.
- "^P" indicates a valine residue.
- "^Q" indicates a glutamine residue.
- "^R" indicates a methionine residue.
- "^S" indicates an alanine residue.
- "^T" indicates a cysteine residue.
- "^U" indicates a tryptophan residue.
- "^V" indicates an arginine residue.
- "^W" indicates a glutamine residue.
- "^X" indicates a methionine residue.
- "^Y" indicates an isoleucine residue.
- "^Z" indicates a glutamine residue.
**Amino acid change** | **Nucleotide change** | **Nucleotide change**
--- | --- | ---
V54M | c.159G→A | c.160G→A
L19A | c.271T→G | c.272T→G
L231H | c.690T→A | c.692T→A
R280X | c.836C→T | c.838C→T
G328S | c.980G→A | c.982G→A
P335Q | c.1004C→A | c.1004C→A
Y370C | c.1109A→G | c.1109A→G

Fs, frame shift; PTC, premature termination codon; Non-conserv, non-conservative amino acid change; Conserv, conservative amino acid change.

All reported mutations were experimentally verified at both the RNA and genomic DNA level.

*The numbers for the nucleotide changes are reported in accordance with published DNA sequence [Pshezhetsky et al., 1997] and GenBank entry for lysosomal sialidase (accession number NM_000434), the A of the initiator codon denoted as nucleotide +1. The following changes were made to mutations originally reported by Bonten et al. [1996, 2000].

*Reported as G378X (c.1127G→T) by Bonten et al. [2000]. Since this combination is impossible, we presume E377X (c.1129G→T).

*Reported as G218A (c.654G→A) by Bonten et al. [2000]. The 218th amino acid residue is cystein, we presume G219A (c.656G→A).

*Reported as R294S (c.878C→T) by Bonten et al. [2000]. C→T change cannot result in R294S mutation, we presume c.880C→A.
speculate about the genotype-phenotype correlation in this patient.

**Missense Mutations**

This is the most numerous group of sialidase mutations, with 24 changes identified so far. Several mutations are common. For example, the c.G679>A mutation, causing the G227R change and almost complete loss of enzyme activity, was found in four unrelated patients of Mexican, North American (Caucasian), Italian, and Greek origin [Bonten et al., 2000; Lukong et al., 2000]. All patients were homozgyous for the mutation and developed similar clinical symptoms (e.g., dysmorphic features, dysostosis), consistent with a type II sialidosis. Exceptionally, the patient of Greek origin was diagnosed as having type I sialidosis [Bonten et al., 2000]. A c.928G>A mutation resulting in a p.G328S change was found in two unrelated patients of Italian and Dutch origin [Bonten et al., 2000; Lukong et al., 2000]. The mutant protein retained about 40% of normal enzymatic activity, which correlated well with the mild clinical phenotype of the patients. A c.808C>T (p.L270F) mutation was found in two siblings and in an unrelated individual from Spain whose ancestors all came from a small area to the east of the city of Seville, suggesting a founder mutation [Lukong et al., 2000; Rodríguez Criado et al., 2003]. The patients were all homozygous for the mutation; however, they showed significant clinical variability from congenital to infantile type II sialidosis. In vitro expression studies demonstrated that the mutant enzyme is targeted to the lysosome and retains about 10% of residual activity [Lukong et al., 2000]. Finally, the c.880C>T (p.R294S) mutation was found in two unrelated individuals of African-American origin affected with type I sialidosis [Bonten et al., 2000], and a combination of c.649G>A (p.V217M) and c.727G>A (p.G243R) mutations were found in two unrelated sialidosis type I patients from Japan [Naganawa et al., 2000]. All other missense mutations detected in the sialidase gene were individual (Table 1).

Using homology modeling, a potential effect of missense mutations on the sialidase tertiary structure was estimated and was correlated with the residual activity, folding, and intracellular localization of the enzyme, as well as with the clinical phenotype [Bonten et al., 2000; Lukong et al., 2000, 2001; Naganawa et al., 2000; Itoh et al., 2002]. For example, Lukong et al. [2000] built the structural model of human sialidase using the atomic coordinates of homologous sialidases from *Micromonospora viridifaciens*, *Salmonella typhimurium*, and *Vibrio cholerae*. The model demonstrated that despite the low sequence identity (15% with viral sialidases and ~30% with bacterial sialidases), the topology of secondary structural elements as well as of the catalytic domain was strictly conserved between all enzymes. Localization of the missense mutations on the sialidase structural model divided them into three groups [Lukong et al., 2001]. The first group included mutations that affected sialidase active site residues (Y370C) or could interfere with their correct positions (L91R, P80L, 399insHY, P316S, and P335Q) (Fig. 2). In addition, the L363P mutation could potentially affect the β-strand adjacent to the strand containing the active site residue Y370, and therefore could also have an effect on the active site. The second group included mutations (V54M, G68V, S182G, and G328S) that were found on the periphery of the sialidase molecule, usually in long flexible loops, and that did not cause obvious changes in the sialidase structure (Fig. 2). Three of those mutations (V54M, S182G, and G328S) gave rise to a properly-targeted protein with high residual activity and were found in sialidosis type I patients with a very mild phenotype [Bonten et al., 2000; Lukong et al., 2000]. Surprisingly, the G68V mutation completely inactivated the enzyme and caused a severe congenital form of sialidosis [Tyłki-Szymanksa et al., 1996].

The third group (V217M, G219A, G227R, L231H, W240R, G243R, F260Y, L270F, R294S, and A298V) included mutations found on the surface of the sialidase molecule in proximity to βM, βN, βO, βQ, βR, βS, and βT strands (Fig. 2). The majority of these mutations resulted in complete or almost complete inactivation of the enzyme and caused severe type II phenotype. Three of these mutants (F260Y, L270F, and A298V) after expression in vitro could not form a complex with cathepsin A that activates and stabilizes sialidase in the lysosome, suggesting that this group of mutations affects
a cathepsin A-binding domain on sialidase, which would lead to enzyme inactivation [Lukong et al., 2001].

In the current study, we have identified four novel amino acid substitutions in one sialidosis type I patient of Czech origin and two sialidosis type I patients of French origin. The Czech patient, with a previously described clinical case [Ledvinova et al., 1994], was homozygous for the c.1034C>T (p.T345I) substitution (Table 1). The T345I change is located in the beginning of the βW strand of the sialidase structure, in proximity to the active site residue R341 (Fig. 3). In all the members of the sialidase superfamily, this position is occupied by a small polar amino acid (threonine residue in the human and mouse lysosomal sialidases [Wang et al., 2001] and the Micromonospora viridifaciens sialidase; glycine residue in the human membrane and cytosolic sialidases and in the Vibrio cholerae sialidase; and serine residue in the Salmonella typhimurium sialidase) (Fig. 3). Replacement of T345 by a bulk hydrophobic isoleucine residue changes the position of the R341 active site residue. That explains the reduction of the enzymatic activity to about 7% of the norm. A mild clinical phenotype of the patient homozygous for the T345I mutation correlated well with the observed high residual activity of the sialidase [Ledvinova et al., 1994].

The first sialidosis type I patient of French origin presented at 18 years of age with ocular myoclonus and myoclonus of the arms and legs; a cherry-red spot was found on ocular examination. Further examination revealed signs of epilepsy and discrete cerebello-pyramidal signs. The patient has normal IQ and leads a nearly normal life. Our analysis revealed that the patient is a

![Figure 3](image)

**Figure 3.** Amino acid sequence alignment of human lysosomal sialidase (NEU1, residues 61–394) with homologous sialidases from *Vibrio cholerae* (KIT, accession number NP_253149, residues 230–380 and 566–795), *Micromonospora viridifaciens* (EUR, accession number NP_005634, residues 1–369), and plasma membrane sialidases (NEU3, accession number NP_005374, residues 15–404). Identical residues are boxed. Active site residues are shown as black on yellow; “Asp-box” repeats are shown as black on blue; and novel missense mutations identified in the human sialidase gene are shown as black on pink. The β-sheets in the structures of bacterial sialidases [Gaskell et al., 1995; Crennell et al., 1993, 1994] are indicated by arrows above the alignment.
compound heterozygote for c.407G>A (p.G136E) and c824T>C (p.V275A) substitutions (Table 1). G136 residue is the next after D135, the active site residue that binds the N-acetyl/N-glycolyl group of the substrate [Lukong et al., 2000]. In all members of the sialidase family, with the exception of the lysosomal sialidase, this position is occupied by a proline residue homologous to glycine (Fig. 3). The G136E change introduces an extra negative charge to the active site, which should affect substrate binding and catalytic activity of the enzyme. In all members of the sialidase superfamily, the position of V275 is occupied by a bulk hydrophobic residue: valine in the Micromonaspora veridifaciens sialidase and in the cytosolic sialidase from human, rat, and hamster; and leucine in the Salmonella typhimurium, Vibrio cholerae, and in the human membrane sialidases (Fig. 3). Replacement of this residue by a smaller alanine in the sialidase structural model should destabilize the βR strand and affect positions of the active site residues E264 and R280. About 5% of the residual sialidase activity was detected in patients’ cells, which explains the mild clinical phenotype.

The second sialidosis type I patient of French origin developed unsteady gait, myoclonus, and tremor at 14 years of age. Six months later, he developed partial complex epilepsy and failure to thrive. Ophthalmologic examination revealed macular cherry-red spots. He had progressive ataxia and became wheelchair-bound at 15 years of age, but remains intellectually normal. A biochemical analysis revealed a massive urinary excretion of sialyloligosaccharides. Residual sialidase activity in cultured fibroblasts was about 7–10% of the normal value.

We have determined that the patient is heterozygous for a missense mutation c.332T>C (p.L111P). The mutation was identified in the cDNA (Table 1) and confirmed in the genomic DNA by sequencing. A new SacII restriction site created by the mutation was identified in exon 2 by the restriction enzyme assay (Table 1). The second mutation in this patient was not determined.

The L111 residue is located in the βD strand close the first aspartic acid box. Leucine at this position is conserved in all mammalian enzymes: lysosomal, plasma membrane, and cytosolic (Fig. 3). Structural modeling shows that the proline at this position destabilizes the β-strand and affects the position of proximal active site residue (D103).

CLINICAL AND DIAGNOSTIC RELEVANCE

The analysis of sialidase mutations in sialidosis reveals considerable molecular heterogeneity, reflecting the diversity of clinical phenotypes. Only five mutations were found in unrelated individuals and a founder mutation likely existed for only three patients from a region near Seville, Spain [Rodriguez Criado et al., 2003]. For diagnosis, this situation suggests that the direct enzyme assay in leucocytes and cultured skin fibroblasts is preferable to molecular testing. The patients’ clinical severity generally correlates with their genotype and with the predicted effect of missense mutations on the tertiary structure of the enzyme. Most of the patients are affected with a severe form of sialidosis (type II). These patients mainly have frameshifts or other mutations resulting in premature truncations; however, 11 of the total 24 missense mutations reported in the sialidase gene also cause sialidosis type II. These 11 mutations (Table 1) affect conserved amino acid residues, which are involved in catalysis (P80L, L91R, Y370C), are important for maintaining the secondary and tertiary structure of the protein (G227R, W240R, P335Q, L363P), or participate in the formation of the sialidase complex with cathepsin A necessary for activation of the enzyme (F260Y, L270F, and A298V). In only one case does a change of the conserved G68 residue for valine not cause significant structural alterations in the sialidase molecule, but results instead in severe congenital type II sialidosis (Table 1). Patients who are genetic compounds, having a combination of a mild and a severe mutation, are clinically milder (sialidosis type I), suggesting that a small percentage of normal sialidase activity can protect against severe phenotypes. This raises the hope that enzyme replacement therapy may eventually be a possible approach for the treatment of sialidosis.

FUTURE PROSPECTS

In addition to its role in the intralysosomal catabolism of glycoproteins and glycolipids, lysosomal sialidase is also involved in signaling during the immune response. Sialidase is overexpressed during the activation of T cells, B cells, macrophages, and neutrophils [Landolfi et al., 1985; Landolfi and Cook, 1986; Cross and Wright, 1991] (N. Stamatos, private communication). The induced enzyme is targeted to the plasma membrane and is responsible for processing different molecules expressed on the cell surface, resulting in their hyposialylation [Chen et al., 1997]. Among those molecules are surface antigen-presenting proteins, such as MHC class I, which are required to render T cells responsive to antigen-presenting cells [Landolfi and Cook, 1986], and G_M3-ganglioside, which modulates Ca2+ immobilization and regulates IL4 production [Chen et al., 2000]. In activated B cells, sialidase participates in converting the so-called vitamin D3-binding protein into a macrophage activating factor (MAF) [Yamamoto and Kumashiro, 1993; Naraparaju and Yamamoto, 1994; Yamamoto and Naraparaju, 1996a,b]. Consequently, immune cells with genetic or induced sialidase deficiency fail to synthesize IL4, and become less responsive for antigen-presenting cells or fail to produce MAF and/or synthesize IL4 [Landolfi et al., 1985; Yamamoto and Kumashiro, 1993; Chen et al., 1997]. In mice, sialidase deficiency results in the well-documented impaired activation of lymphocytes and macrophages [Landolfi et al., 1985; Yamamoto and Kumashiro, 1993; Chen et al., 1997]. Similarly in humans, sialidase deficiency may account for frequent
infections observed in sialidosis patients, which can result from the reduced capacity of immune cells to produce cytokines and antibodies, leading to partial immunodeficiency. Future studies should show if the impairment of the nonlysosomal sialidase function contributes to the clinical phenotype of sialidosis patients.

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