

## The Demographics and Distribution of Type B Niemann-Pick Disease: Novel Mutations Lead to New Genotype/Phenotype Correlations

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We have collected demographic and/or mutation information on a worldwide sample of 394 patients with type B Niemann-Pick disease (NPD). The disorder is panethnic, with the highest incidence occurring in individuals of Turkish, Arabic, and North African descent. Only five of the 394 patients were Ashkenazi Jewish, revealing that, unlike the type A form of NPD, type B NPD does not occur frequently within this population. Mutation analysis of the acid sphingomyelinase (ASM) gene (designated “*SMPD1*”) was performed on 228 patients (324 unique alleles), and several novel, “common” mutations were found. Among these were the L137P, fsP189, and L549P mutations, which accounted for ~75% of the alleles in Turkish patients, the H421Y and K576N mutations, which accounted for ~85% of the alleles in Saudi Arabian patients, the S379P, R441X, R474W, and F480L mutations, which accounted for ~55% of the alleles in Portuguese/Brazilian patients, and the A196P mutation, which accounted for ~42% of the alleles in Scottish/English patients. The previously reported  $\Delta$ R608 mutation occurred on ~12% of the alleles studied. Overall, a total of 45 novel mutations were found, and several new genotype/phenotype correlations were identified. In particular, the L137P, A196P, and R474W mutations were consistent with a less severe form of type B NPD, whereas the H421Y and K576N mutations led to an early-onset, more severe form that was specific to Saudi Arabia. These data provide the first extensive demographic assessment of this disorder and describe several new mutations that can be used to predict phenotypic outcome and to gain new insights into the structure and function of ASM.

### Introduction

Types A and B Niemann-Pick disease (NPD [MIM 257200]) are lysosomal storage disorders resulting from the deficient activity of acid sphingomyelinase (ASM) and the subsequent accumulation of sphingomyelin, cholesterol, and other lipids within cells and tissues of affected individuals (Schuchman and Desnick 2001). Patients with type A NPD are usually diagnosed early in infancy with organomegaly and follow a rapid neurodegenerative course that leads to death by ~3 years of age. In contrast, patients with type B NPD have little or no CNS involvement and often survive into adulthood. However, the type B form of NPD is clinically heterogeneous and can present with a variety of findings that may include hepatosplenomegaly, growth retardation, frequent respiratory infections, fatigue, and hematologic abnormalities such as high levels of LDL cholesterol and

triglycerides, low levels of HDL cholesterol, and low numbers of platelets. In addition, several patients with type B NPD have been reported who had an intermediate phenotype that included neurodegeneration (see, e.g., Elleder and Cihula 1983; Elleder et al. 1986). Both forms of NPD are panethnic, although most reported cases of type A NPD occurred among Ashkenazi Jewish individuals.

The full-length cDNA and genomic sequences encoding human ASM have been isolated (Schuchman et al. 1991, 1992), and a total of 23 mutations causing type A or B NPD have been published (gene designation “*SMPD1*”). Of these, 15 are missense mutations (Ferlinz et al. 1991; Levran et al. 1991*a*, 1992; Takahashi et al. 1992*a*, 1992*b*; Schuchman 1995; Schuchman and Miranda 1997), 2 are nonsense mutations (Schuchman 1995; Schuchman and Miranda 1997), 4 are frameshift mutations (Levran et al. 1991*a*, 1993*b*), and 1 results from an in-frame 3-bp deletion that results in the removal of a single amino acid from the ASM polypeptide (Levran et al. 1991*b*). One splice-site alteration also has been described (Levran et al. 1993*a*). Notably, three common mutations account for >90% of the mutant alleles in patients of Ashkenazi Jewish ancestry who had type A NPD (Levran et al. 1991*a*, 1992, 1993*b*). Two are missense mutations, R496L and L302P, and the

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third is a 1-bp deletion that causes a frameshift and the introduction of a premature stop codon within the ASM open reading frame (fsP330). The combined carrier frequency for these mutations among Ashkenazi Jewish individuals is between 1/80 and 1/100 (Li et al. 1997). Only one other mutation has so far been identified in the Ashkenazi Jewish population with type A NPD (Schuchman 1995); this mutation, designated “N389T,” was identified as one of the mutant alleles in a single patient with type A NPD; the other allele was fsP330. In contrast to the Ashkenazi Jewish population, each of the non-Jewish patients with type A NPD studied has had unique, or “private,” ASM mutations.

The first mutation (designated “ $\Delta$ R608”) in a patient with type B NPD was found in a mildly affected Ashkenazi Jewish individual who carried a 3-bp deletion in exon 6 that predicted the removal of an arginine residue from position 608 of the ASM polypeptide (Levrant et al. 1991b). This patient carried the R496L mutation on her other allele, suggesting that the presence of the  $\Delta$ R608 mutation was “neuroprotective.” A second Ashkenazi Jewish type B patient was subsequently found to be heteroallelic for  $\Delta$ R608 and a new mutation, designated “H575L.” In addition to these two patients, the  $\Delta$ R608 mutation was frequently found in North African patients with type B NPD who originated from the Maghreb region (i.e., Tunisia, Algeria, and Morocco) (Vanier et al. 1993), where it may account for almost 90% of the mutant alleles. To date, only four other mutations have been reported in patients with type B NPD (Takahashi et al. 1992a, 1992b; Vanier et al. 1993).

In this article, we report demographic and/or molecular data on a worldwide sample of 394 patients with type B NPD. Acid sphingomyelinase mutation analysis was performed on 228 of these patients, and 45 novel mutations were identified, including several common mutations that could be used for genetic screening in specific populations. In addition, we have identified several new genotype/phenotype correlations for this disorder that should be useful to genetic counselors, physicians, and families affected with type B NPD.

## Subjects and Methods

### Subjects

A total of 394 patients with type B NPD were included in this study. Of these, 64 patients were evaluated at the Mount Sinai Clinical Research Center as part of an ongoing natural history study. Information on the remaining 330 patients was obtained from either the treating physicians or major diagnostic laboratories throughout the world. Blood was obtained from 228 of the 394 patients and was used to prepare DNA for mutation analysis (see below). For inclusion in the study, the diagnosis of NPD had to be confirmed by *SMPD1* mu-

tation analysis and/or by determining markedly reduced ASM activity (i.e., <5% of normal) in white blood cells or cultured skin fibroblasts. Cholesterol loading studies were performed on a small subset of patients to rule out type C NPD. Type C NPD was ruled out in the remaining patients by demonstrating either *SMPD1* mutations or very low, residual ASM activity. Patients also needed to be  $\geq 3$  years of age and without evidence of neurodegeneration (to rule out type A NPD).

### *SMPD1* Mutation Analysis

DNA was prepared from whole blood and was used for mutation analysis as described elsewhere (Schuchman and Miranda 1997). Putative mutations were confirmed by sequencing duplicate PCR products, restriction enzyme analysis, and by family studies whenever possible.

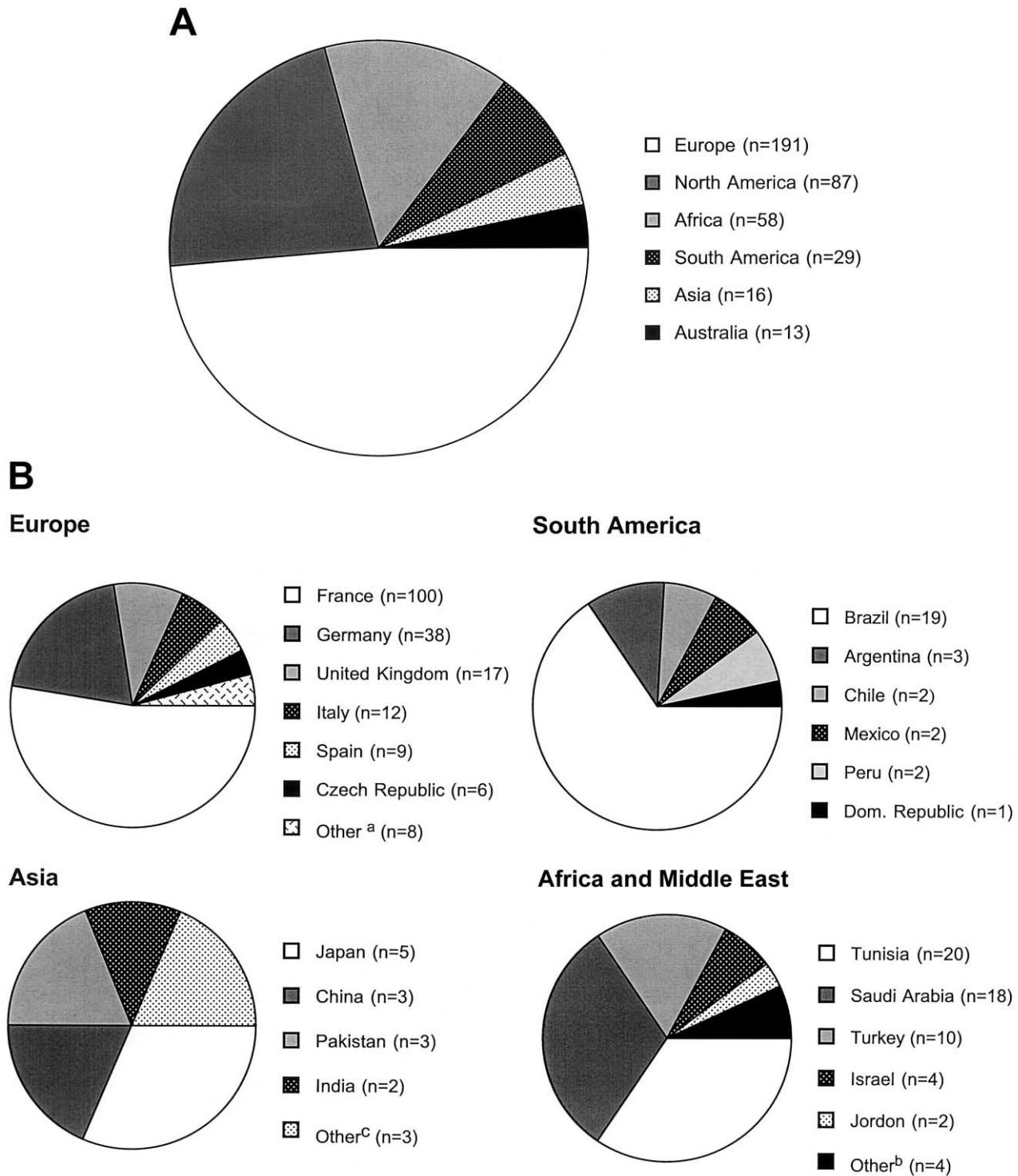
## Results

### *Distribution and Ethnicity of Type B NPD*

Information was collected on a worldwide sample of 394 patients with type B NPD. Mutation analysis was performed on 228 of these patients (see below). No patients were included in this study unless the diagnosis of NPD was confirmed by *SMPD1* mutation analysis and/or ASM enzyme testing. The distribution of these patients by country is shown in figure 1. Of these patients, 87 were residing in the United States or Canada. Other countries with significant numbers of patients in this data set were France (100), Germany (38), Tunisia (20), Brazil (19), Saudi Arabia (18), the United Kingdom (17), Italy (12), and Turkey (10). In total, patients were identified in 29 countries. The most common ethnic group among these type B NPD patients was Turkish, followed by Arabic and Scottish. Only five of the patients were Ashkenazi Jewish.

### *Mutation Analysis and Genotype/Phenotype Correlations*

Mutation analysis was carried out on 228 of the type B NPD patients (324 unique alleles), leading to the identification of 45 novel mutations (table 1). Among these were several “common” mutations that occurred within specific ethnic groups (table 2), including the L137P, fsP189, and L549P mutations, which accounted for ~75% of the alleles among Turkish patients; the H421Y and K576N mutations, which accounted for ~85% of the alleles among Saudi Arabian patients; the S379P, R441X, R474W, and F480L mutations, which accounted for ~55% of the alleles among Portuguese/Brazilian patients; and the A196P mutation, which accounted for ~42% of the alleles among Scottish/English patients. Overall, the previously reported  $\Delta$ R608 mu-



<sup>a</sup>Belgium, Finland, Hungary, Poland, Portugal, Slovakia  
<sup>b</sup>Algeria, Libya, Senegal, Syria  
<sup>c</sup>Indonesia, Korea, Malaysia

**Figure 1** Worldwide distribution of patients with type B NPd. *A*, Distribution by continent of 394 patients with type B NPd. *B*, Distribution by country of patients with type B NPd. The distribution within North America is not shown, but 78 patients were residing in the United States, and 9 were residing in Canada.

**Table 1**  
**Novel Mutations in the ASM Gene**  
**Causing Type B NPD**

Exon and Mutation	cDNA Alteration <sup>a</sup>	Genomic Alteration <sup>b</sup>
1:		
G21X	C→T, nt 61	nt 61
D49V	A→T, nt 156	nt 146
C92W	C→G, nt 276	nt 276
2:		
L137P	T→C, nt 409	nt 875
C157R	T→C, nt 469	nt 934
fsD169	delG, nt 507	nt 970
W174X	G→A, nt 522	nt 986
A196P	G→C, nt 586	nt 1051
R200C	C→T, nt 598	nt 1063
L225M	C→A, nt 673	nt 1138
R228C	C→T, nt 682	nt 1147
G232D	G→A, nt 695	nt 1160
G245S	G→A, nt 733	nt 1198
S248R	C→A, nt 744	nt 1209
R289H	G→A, nt 866	nt 1331
fsS321	delG, nt 962	nt 1427
P323A	C→G, nt 967	nt 1432
P330R	C→G, nt 989	nt 1454
A357D	C→A, nt 1070	nt 1535
3:		
fsF366	insT, nt 1096	nt 2621
R376H	G→A, nt 1128	nt 2652
R376L	G→T, nt 1128	nt 2652
S379P	T→C, nt 1135	nt 2661
fsL380	delC, nt 1140	nt 2663
4:		
A413V	C→T, nt 1239	nt 2762
H421Y	C→T, nt 1261	nt 3014
C431R	T→C, nt 1291	nt 3045
L432P	T→C, nt 1295	nt 3049
W435C	G→C, nt 1305	nt 3059
5:		
A452V	C→T, nt 1355	nt 3311
G456D	G→A, nt 1367	nt 3323
R474W	C→T, nt 1420	nt 3376
P475L	C→T, nt 1424	nt 3380
F480L	C→A, nt 1440	nt 3396
A485V	C→T, nt 1454	nt 3410
Y488N	T→A, nt 1462	nt 3432
6:		
G494S	G→A, nt 1480	nt 3603
R496C	C→T, nt 1486	nt 3609
H514Q	T→G, nt 1542	nt 3665
E515V	A→T, nt 1544	nt 3667
fsI530	delA, nt 1589	nt 3702
W533R	T→C, nt 1609	nt 3709
L549P	T→C, nt 1666	nt 3758
K576N	G→C, nt 1728	nt 3840
R600H	G→A, nt 1799	nt 3911
R600P	G→C, nt 1799	nt 3911

<sup>a</sup> Numbered according to Schuchman et al. (1991).

<sup>b</sup> Numbered according to Schuchman et al. (1992).

tation occurred on ~12% of the NPD alleles studied (~20% of the alleles among patients living in North America and Brazil).

On the basis of these molecular studies, several new genotype/phenotype correlations were identified. In particular, the L137P, A196P, and R474W mutations were associated with a mild disease course (table 3), whereas the H421Y and K576N mutations were consistent with an early onset form of type B NPD that led to childhood death. Several other frame-shift or termination mutations were also found that predicted a severe disease course when homoallelic or present in combination with another such “null” mutation (see table 1).

#### ASM Structure/Function Insights

Several of the novel *SMPD1* point mutations revealed new structure/function insights (table 4). Among these, the G21X mutation was particularly interesting, since it occurred before the second in-frame translation start site in the ASM coding region. There are two in-frame start codons in the ASM sequence (Schuchman et al. 1991), and this mutation reveals that the first ATG must be used in vivo. In addition, the H421Y mutation was interesting, because it occurs within the putative active-site region of ASM and at a histidine residue that might be involved in zinc binding (He et al. 1999). There are also three mutations occurring at cystine residues that might be involved in intra- or intermolecular disulfide bond formation (C92W, C157R, and C431R). Two of these cystine residues are within the saposin domain of ASM and are conserved among all four of the saposin polypeptides. Finally, two independent mutations oc-

**Table 2**

#### Common Type B NPD Mutations in Specific Ethnic Groups

Ethnic Group (No. of NPD Alleles Studied) and Mutation	Allele Frequency (%)
Turkish (20):	
L137P	37
L549P	20
fsP189	16.6
Saudi Arabian (28):	
H421Y	71.4
K576N	13.2
Scottish/British (30):	
A196P	42.3
Brazilian/Portuguese (24):	
ΔR608	20.8
S379P	16.6
R441X	12.5
R474W	12.5
F480L	12.5
Other (222):	
ΔR608	20

**Table 3****Genotype/Phenotype Correlations among Patients with Type B NPD Carrying Common ASM Mutations**

GENOTYPE	NO. OF PATIENTS	AVERAGE AGE (years)	FINDING FOR PHENOTYPE <sup>a</sup>		
			Neurodegenerative Course	Organomegaly	Pulmonary Involvement
L137P/L137P	4	14	–	+	+/-
L137P/fsP189	2	20	–	+	+/-
L549/L549	3	5	–	++	++
fsP189/fsP189	1	3	–	++	++
H421Y/H421Y	11	5	+/-	++	++
K576N/K576N	2	6	+/-	++	++
A196P/A196P	2	37	–	+	+/-
A196P/* <sup>b</sup>	8	30	–	+	+/-
ΔR474W/* <sup>c</sup>	6	18	–	+	+/-
ΔR608/ΔR608	9	17	–	+	+/-
ΔR608/* <sup>d</sup>	21	19	–	+	+/-

<sup>a</sup> + = present; – = absent; ++ = severe; +/- = mild.

<sup>b</sup> Heterozygous for one of the following mutations: D49V, C157R, G245S, R289H, R376H, and C431R.

<sup>c</sup> Heterozygous for one of the following mutations: fsL260, S379P, A485V, or R496L.

<sup>d</sup> Heterozygous for one of the following mutations: Q21X, D49V, L225M, G245S, S379P, R441X, Y446C, P475L R494S, R496L, H575L, or R600H.

curred at R376 (R376H and R376L), a residue that is within a putative sphingomyelin binding domain and is conserved between ASM and the sphingomyelin binding protein, lysenin (Yamaji et al. 1998).

## Discussion

Several conclusions can be drawn from the data presented in this manuscript. First, type B NPD is clearly a panethnic disorder with a worldwide distribution. Most of the patients were living in the United States or Western Europe, where the enzymatic diagnosis can be readily obtained, or in countries with high rates of consanguinity, such as Saudi Arabia, Turkey, and Tunisia. However, patients with type B NPD were identified in 29 different countries, confirming that it is a disorder affecting many distinct populations. Of the 394 patients analyzed in this study, family histories were available for 228. Only 5 of these patients were Ashkenazi Jewish, revealing that unlike the type A form of this disorder, type B NPD does not have an Ashkenazi Jewish predilection. Interestingly, however, each of these five patients carried one copy of a common Ashkenazi Jewish type A mutation (L302P, R496L, or fsP330) in combination with ΔR608. This is the same genotype combination found previously in Ashkenazi Jewish type B patients (Levran et al. 1991b), and it is consistent with past “mixing” of Jewish and North African populations (since ΔR608 represents almost 90% of the type B NPD alleles in the Maghreb region; see Vanier et al. 1993). In contrast to the Ashkenazi Jewish population, in which the disease is quite rare, more than half (~54%) of the 228 patients studied who

had type B NPD were of Turkish, Arabic, or North African ancestry.

Another observation made apparent from this study is that the presentation of type B NPD is remarkably heterogeneous, making its clinical diagnosis particularly difficult. Most of the referring physicians were working within specialized metabolic disease centers, although a significant number were private practice endocrinologists or cardiologists who were managing the patients for growth retardation or abnormal plasma lipids, respectively. The diagnosis was often not made for several years after presentation, and in at least seven docu-

**Table 4****Structure/Function Analysis of Several ASM Mutations**

Residue	Structure/Function Implications
G21X	Reveals that first in-frame ATG is functional
C92W	May affect disulfide bond formation; within saposin domain (conserved aa with saposins A–D)
C157R	May affect disulfide bond formation; within saposin domain (conserved aa with saposins A–D)
R376H and R376L	Within putative sphingomyelin binding domain <sup>a</sup>
H421Y	Within zinc binding domain; within putative active site region <sup>b</sup>
C431R	May affect disulfide bond formation

<sup>a</sup> Putative sphingomyelin binding domain based on homology to the sphingomyelin binding protein, lysenin.

<sup>b</sup> Putative active site region based on homologies to phospholipases C and A2.

mented cases type B NPD patients were misdiagnosed with type 1 Gaucher disease. Two others were misdiagnosed with type C NPD. Thus, it is not possible to accurately estimate the incidence of type B NPD because many patients remain undiagnosed either because of the lack of enzyme testing, variability in presenting symptoms, and/or lack of knowledge about type B NPD among the treating physicians.

Mutation analysis was carried out on 228 of the type B NPD patients. Not surprisingly, within the Turkish and Saudi Arabian populations there were a small number of common ASM mutations that accounted for most of the patients. Three mutations were responsible for >70% of the Turkish NPD alleles, and one mutation accounted for ~70% of the alleles in Saudi Arabia. We also identified a number of type B NPD patients with Scottish heritage. These patients resided in the United States, the United Kingdom, or Canada, and all carried the same point mutation (A196P). Patients carrying at least one copy of this mutation, even when in combination with a termination or other presumably “null” mutation, had an adult form of type B NPD that followed a fairly mild course. Thus, like  $\Delta R608$ , the A196P mutation appears to predict a less severe form of type B NPD. Similar genotype/phenotype analysis suggested that three other mutations (L137P, R474W, and R600H) were also consistent with less severe forms of type B NPD, but more patients carrying these mutations will have to be identified and studied for these observations to be confirmed.

In contrast to these mutations, the H421Y mutation, which occurred on >70% of the type B NPD alleles in Saudi Arabia, was predictive of an early onset, severe form of type B NPD. This is a particularly interesting mutation, since H421 is conserved in several zinc metalloenzymes, including a cGMP specific phosphodiesterase (Francis et al. 1994), and is also conserved among the human, mouse, and *Caenorhabditis elegans* ASM sequences (Lin et al. 1998). Intriguingly, the *C. elegans* sequence in which H421 is conserved is zinc activated, whereas the other *C. elegans* ASM sequence is not (Lin et al. 1998). These observations suggest that H421 is a critical residue in the ASM active site and that the severe H421Y mutation may affect ASM function by disrupting zinc binding. Studies to confirm this observation are currently underway.

It is also notable that three ASM mutations were identified at cystine residues, since previous studies using sulfhydryl reducing reagents had shown that intra- or intermolecular disulfide bonds were important for ASM structure and function (He et al. 1999). Indeed, each of these cystine residues were conserved in both of the *C. elegans* ASM sequences, and two (C92 and C157) occurred within the previously identified saposin homology domain (Linke et al. 2001). It is also of interest

that the C157R mutation had been previously found in an ASM cDNA clone obtained from a commercial, placental cDNA library (Ida et al. 1993). Expression studies using that cDNA sequence had revealed that this mutation led to an inactive ASM polypeptide.

Thus, with these data, we report the first extensive demographic analysis of type B NPD. Forty-five new ASM mutations were identified, including several common mutations occurring within specific ethnic groups. This information should be useful to genetics counselors, physicians, and/or families who are considering prenatal testing for NPD and/or genetic screening to identify carrier individuals. In addition, several new genotype/phenotype correlations were identified. Most important among these is the fact that patients carrying at least one copy of the A196P mutation will develop a less severe form of type B NPD than patients homoallelic for the H421Y mutation, who will develop an early-onset, severe form of the disease. The identification of these new mutations has also provided new structure/function insights about ASM.

These results also have important implications for the design of upcoming enzyme-replacement therapy trials for type B NPD, since several of the mutations identified (e.g., fsP189 and R441X) are frame-shift or termination mutations that will likely lead to the production of rapidly degraded proteins. Thus, individuals carrying these mutations may experience more immunologic reactions to the infused enzyme than those carrying point mutations expressing residual activity (e.g.,  $\Delta R608$ , A196P) (Brooks et al. 1997).

Finally, the data also highlight the fact that type B NPD remains a disorder that is particularly difficult for clinicians to diagnose and suggests that screening programs might be useful in identifying affected patients who might benefit from future therapies. In this regard, it might be useful to screen in heart disease clinics for patients with very low HDL cholesterol levels, since this is a common finding in almost all patients with type B NPD, or in endocrinology clinics, where patients may be seen for growth retardation. The availability of several common mutations should facilitate these screening efforts.

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## Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for NPD [MIM 257200])

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