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Genetics

Niemann–Pick disease: A frequent missense mutation in the acid sphingomyelinase gene of Ashkenazi Jewish type A and B patients

(lysosomal hydrolase/sphingomyelin/lysosomal storage disease/polymerase chain reaction/heterozygote detection)

ORNA LEVRAN, ROBERT J. DESNICK, AND EDWARD H. SCHUCHMAN*

Division of Medical and Molecular Genetics, Mount Sinai School of Medicine, New York, NY 10029

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ABSTRACT **Although the A and B subtypes of Niemann–Pick disease (NPD) both result from the deficient activity of acid sphingomyelinase (ASM; sphingomyelin cholinephosphohydrolase, EC 3.1.4.12) and the lysosomal accumulation of sphingomyelin, they have remarkably distinct phenotypes. Type A disease is a fatal neurodegenerative disorder of infancy, whereas type B disease has no neurologic manifestations and is characterized primarily by reticuloendothelial involvement and survival into adulthood. Both disorders are more frequent among individuals of Ashkenazi Jewish ancestry than in the general population. The recent isolation and characterization of cDNA and genomic sequences encoding ASM has facilitated investigation of the molecular lesions causing the NPD subtypes. Total RNA was reverse-transcribed, and the ASM cDNA from an Ashkenazi Jewish type A patient was specifically amplified by the polymerase chain reaction (PCR). Molecular analysis of the PCR products revealed a G → T transversion of nucleotide 1487, which occurred at a CpG dinucleotide and predicted an Arg → Leu substitution in residue 496. Hybridization of PCR-amplified genomic DNA with allele-specific oligonucleotides indicated that the proband was homoallelic for the Arg → Leu substitution and that both parents and several other relatives were heterozygous. This mutation was detected in 32% (10 of 31) of the Ashkenazi Jewish NPD type A alleles studied and occurred in only 5.6% (2 of 36) of ASM alleles from non-Jewish type A patients. Of interest, the Arg → Leu substitution occurred in one of the ASM alleles from the two Ashkenazi Jewish NPD type B patients studied and in none of the ASM alleles of 15 non-Jewish type B patients. In contrast, the mutation was not present in 180 ASM alleles from normal individuals of Ashkenazi Jewish descent. These findings identify a frequent missense mutation among NPD patients of Ashkenazi Jewish ancestry that results in neuronopathic type A disease when homoallelic and can result in the nonneuronopathic type B phenotype when heteroallelic. The identification of this ASM mutation in Ashkenazi Jewish patients should facilitate the prevention of NPD in this population by carrier detection with molecular diagnostic techniques.**

Types A and B Niemann–Pick disease (NPD) are autosomal recessive disorders resulting from the deficient activity of the lysosomal hydrolase, acid sphingomyelinase (ASM; sphingomyelin cholinephosphohydrolase, EC 3.1.4.12) and the accumulation of sphingomyelin, primarily in reticuloendothelial cell lysosomes (1–4). Type A disease is a rapidly progressive neurodegenerative disease of infancy manifested by failure to thrive, severe psychomotor retardation, hepatosplenomegaly, and demise by 2–3 years of age. In comparison, type B disease is characterized primarily by reticuloendothelial system sphingomyelin deposition leading to hepatosplenomegaly and pulmonary involvement, the absence of neurologic manifestations, and survival into adulthood. The nature of the biochemical and molecular defects that underlie the remarkable clinical heterogeneity in the A and B subtypes remains unknown. Although patients with both subtypes have residual ASM activity (≈1 to 10% of normal), biochemical analyses cannot reliably distinguish the two phenotypes. Moreover, the clinical course of type B NPD is highly variable, and it is not presently possible to correlate disease severity with the level of residual ASM activity.

Types A and B NPD occur at least 10 times more frequently among individuals of Ashkenazi Jewish ancestry than in the general population. It is estimated that the incidence of the type A disease among Ashkenazi Jews is ≈1 in 40,000, a gene frequency (q) of ≈1 in 200, and a heterozygote frequency ($2pq$) of ≈1 in 100 (5). The incidence of type B NPD in the Ashkenazi Jewish population is less frequent, perhaps 1 in 80,000 (5). Thus, the combined heterozygote frequency for types A and B NPD has been estimated to be ≈1 in 70 among individuals of Ashkenazi Jewish decent. Although the enzymatic diagnosis of affected patients with either type A or B NPD can be made reliably (4), the enzymatic detection of obligate heterozygotes has proven problematic, particularly using peripheral leukocytes as the enzyme source. Presumably, the occurrence of neutral sphingomyelinases in some sources and/or the presence of residual ASM activity resulting from the mutant allele have contributed to the inability to reliably discriminate carriers for either disease subtype. Even the use of cultured skin fibroblasts, which do not express the neutral sphingomyelinase, has not provided unambiguous results with obligate heterozygotes (D. A. Wenger, personal communication).

Recently, two partial cDNAs encoding human ASM were isolated and sequenced (6). The type 1 cDNA contained an in-frame 172 base pairs (bp) encoding 57 amino acids; in the type 2 cDNA this sequence was replaced by an in-frame 40 bp encoding 13 different amino acids. Of the 92 positive clones identified by cDNA library screening, the type 1 and 2 cDNAs represented ≈90 and 10%, respectively (6). Subsequent isolation and expression of the full-length type 1 and 2 cDNAs demonstrated that only the type 1 transcript was functional (28). The 2347-bp full-length type 1 cDNA had an 87-bp 5′ untranslated region, an 1890-bp open reading frame encoding 629 amino acids, and a 370-bp 3′ untranslated sequence. Analysis of a polymerase chain reaction (PCR)-amplified 1665-bp region of genomic DNA revealed that the type 1 and 2 ASM cDNAs resulted from alternative splicing

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Abbreviations: NPD, Niemann–Pick disease; ASM, acid sphingomyelinase; PCR, polymerase chain reaction; nt, nucleotide; R496L, a G → T transversion of nt 1487 that predicts an Arg → Leu substitution at residue 496.

*To whom reprint requests should be addressed at: Division of Medical and Molecular Genetics, Mount Sinai School of Medicine, 100th Street and Fifth Avenue, New York, NY 10029.

of a single ASM transcript (28). Moreover, the localization of a single ASM-encoding gene (designated *SMPD1*) to the region 11p15.1–p15.4 by somatic cell and *in situ* hybridization techniques, supports the occurrence of a single ASM locus (7). In this communication, we identify a mutation in NPD, a G → T transversion in nucleotide (nt) 1487 that predicts an Arg → Leu substitution at position 496 of the ASM polypeptide (R496L). Notably, this missense mutation was frequently found in type A and B NPD patients of Ashkenazi Jewish ancestry.

MATERIALS AND METHODS

Cell Lines. Primary cultures of fibroblasts and lymphoblasts were established from skin biopsies, and peripheral blood samples were obtained from NPD patients and family members and from normal individuals; informed consent was obtained. NPD cell lines GM00112A, GM00165, GM00370, GM00406, GM00559, GM02895, and GM03252 were obtained from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository (Camden, NJ). Cell lines 444X.F01, 534R.F03, 556X.F01, 888V.F01, 2789X.F01, 4293Q.E02, 4774Z.F01, 5113C.L01, 5115E.F01, and 6791M.F01 were obtained from the Service de Biochimie, Hospice de Lyon (Lyon, France). Cell lines DMN 83.126, DMN 83.133, DMN 84.135, DMN 86.40, DMN 86.49, DMN 87.71, DMN 87.99, DMN 88.9, GJO, and RNS were provided by Peter Penchev (Developmental and Metabolic Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke). The cells were grown in RPMI 1640 medium/10% fetal bovine serum/1% penicillin/streptomycin at 1 mg/ml by standard procedures (8). The diagnosis of types A and B NPD was based on clinical criteria (e.g., age at onset, presence of neurologic involvement, etc.) and by demonstration of markedly deficient ASM activity in cultured cells (9).

Enzyme and Protein Assays. ASM activity was determined in cultured fibroblasts obtained from NPD patients and normal individuals using the fluorescent natural substrate, [*N*-12(1-pyrenesulfonyl)amido dodecanoyl] sphingomyelin as described (9). One unit of activity equals that amount of enzyme that hydrolyzes 1 nmol of substrate per hr. Protein was determined by a modified fluorescamine assay (10).

cDNA and Genomic Amplification and Sequencing of the Mutant Allele. Total RNA and genomic DNA were isolated from cultured skin fibroblasts by standard procedures (11). First-strand cDNA was reverse-transcribed from ≈5 μg of total RNA by using a cDNA synthesis kit according to the manufacturer's instructions (Boehringer Mannheim). The cDNA (≈10% of the total reaction) or genomic DNA (≈0.5 μg) was amplified by PCR with *Thermus aquaticus* (*Taq*) polymerase (Cetus), essentially as described by Saiki *et al.* (12) with the following conditions and modifications. PCR was performed for 30 to 40 cycles and consisted of denaturation for 1 min at 94°C and annealing and extension for 4 min at 66°C or 72°C. To improve the specificity of the PCR amplification for the cDNA, a "PCR boost" procedure was used. In this procedure the concentrations of the primers and *Taq* polymerase were 0.1 μM and 5 units/ml, respectively, for the first 15 cycles. Then each primer was added to a final concentration of 0.5 μM, and an additional 2 units of *Taq* polymerase was added to the reaction mixture. PCR amplification then proceeded for an additional 15–25 cycles.

Pairs of sense and antisense oligonucleotide primers were synthesized on an Applied Biosystems model 380B DNA synthesizer (13) and used to specifically amplify (*i*) the entire coding region of the reverse-transcribed type 1 ASM transcript in three overlapping cDNA fragments, (*ii*) the 1665-bp genomic region containing the alternatively spliced sequences in the type 1 and 2 ASM cDNAs (28), and (*iii*) a genomic region that included the point mutation for confirmation of the candidate missense mutation. To amplify a 984-bp fragment from the 5′ end of the ASM cDNA, the 29-mer sense primer, P1 (5′-AGTAGTCTCGAGACGGGACAGACGAACCA-3′), corresponded to ASM, nt −39 to −23 with an additional 12 nt that included an *Xho* I restriction site and the 31-mer antisense primer, P2 (5′-AGTAGTCTGCAGAGCAGGGTACATGGCACTG-3′), corresponded to ASM nt 926 to 945 with an additional 12 nt containing an *Eco*RI restriction site. To amplify an internal 383-bp fragment of the ASM cDNA, the 29-mer sense primer, P3 (5′-ATCATCAAGCTTGGGTAACCATGAAAGCA-3′), corresponded to ASM nt 947–964 with an additional 12 nt containing a *Hin*dIII restriction site, and the antisense 32-mer primer, P4 (5′-ATCATCGAATTCTACAATTCGGTAATAATTCC-3′), corresponded to ASM nt 1310 to 1330 with an additional 12 nt containing an *Eco*RI restriction site. To amplify a 789-bp 3′ fragment from the ASM cDNA, a 19-mer sense primer, P5 (5′-CTCCACGGATCCCGCAGGA-3′), corresponded to ASM nt 1185 to 1203 and contained an internal *Bam*HI restriction site, and an antisense 32-mer primer, P6 (5′-AGTAGTGTCGACTTGCCTGGTTGAACCACAGC), corresponded to ASM nt 1955 to 1974 with an additional 12 nt containing a *Sal* I restriction site. Primers P3 and P4 also were used to amplify the 1665-bp internal genomic region that contains the alternatively spliced type 1 and 2 cDNA sequences. To confirm the candidate mutation by genomic sequencing and dot-blot analysis (see below), a 27-mer sense primer, P7 (5′-AGTAGTCGACATGGGCAGGATGTGTGG-3′), was used with antisense primer P6 to amplify a 567-bp genomic fragment containing the G → T transversion.

After PCR amplification, the products were isolated from agarose gels and subcloned into either Bluescript KS (+) (Stratagene) or pGEM 7Zf (−) (Promega) vectors. For each amplified product, from four to six independent subclones were sequenced in both orientations by the dideoxynucleotide chain-termination method (14).

Dot-Blot Analysis. Any nucleotide change that occurred in more than two subcloned PCR products was considered a candidate mutation and analyzed by dot-blot hybridization with allele-specific oligonucleotides. In addition, allele-specific oligonucleotides were constructed and used as probes to analyze amplified genomic DNA from normal individuals and NPD patients and family members. From these studies genomic DNA was rapidly isolated from either whole blood or cultured cells by the following procedure. About 0.5 ml of whole blood and 0.5 ml of lysis buffer (10 mM Tris·HCl buffer, pH 7.5/5 mM $MgCl_2$/0.32 M sucrose/1% Triton X-100) were mixed at room temperature. After centrifugation at 13,000 × *g*, the supernatant was removed, and 0.5 ml of PCR buffer (10 mM Tris·HCl buffer, pH 8.3/50 mM KCl/2.5 mM $MgCl_2$/gelatin at 0.1 mg/ml/0.45% Nonidet-P40/0.45% Tween 20/proteinase K at 0.1 mg/ml) was added. For cultured cells, the lysis step was omitted, and the washed cell pellets were resuspended directly in PCR buffer (≈5×10^6 cells per ml). The samples were then incubated at 60°C for 1 hr and boiled for 10 min to inactivate the protease; then 25 μl was removed for PCR amplification. After agarose gel electrophoresis of the PCR products, the concentration of each product was estimated by ethidium bromide staining. For each sample ≈0.5 μg of DNA was used for the dot-blot analysis.

For the analysis of the R496L mutation, the 567-bp PCR product amplified from genomic DNA was analyzed by dot-blot hybridization (11) by using Zetabind nylon membranes (AMF Cuno) and a Bio-Rad dot-blot apparatus. Hybridizations were performed for at least 3 hr at 39°C. After hybridization, the blots were washed at room temperature for 15 min in 6× SSC (1× SSC is 0.15 M sodium chloride/0.015 M sodium citrate, pH 7.0)/0.1% SDS and then for 2 hr in the same solution at either 53°C for the normal (5′-CTATTTG-

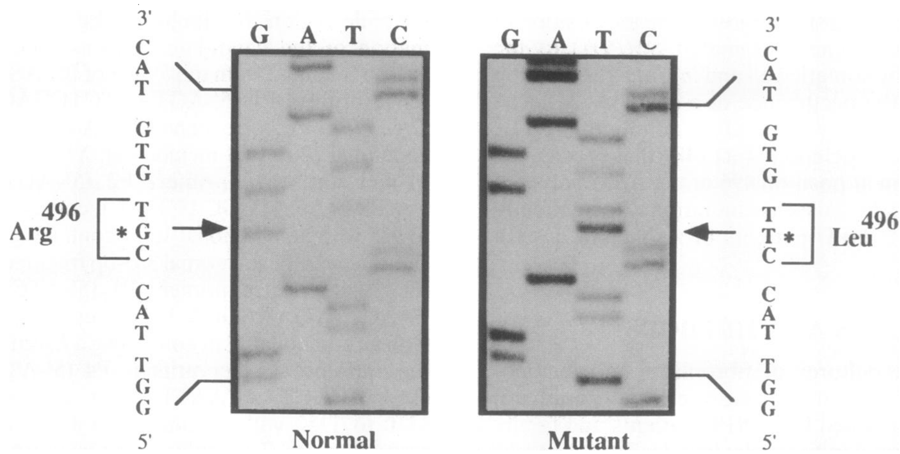

FIG. 1. Partial sequence of the amplified ASM cDNA from an Ashkenazi Jewish type A NPD patient (proband 1) showing the G → T transversion of nt 1487. cDNA synthesis, PCR amplification, and DNA sequencing are described. Arrows indicate the G → T transversion in proband 1 (*Right*) that results in R496L.

GTACACACGG-3′) or 48°C for the mutation-specific (5′-CTATTTGGTACACAAGG-3′) oligonucleotide.

RESULTS

Identification of the R496L Mutation in an Ashkenazi Jewish Type A NPD Patient. To determine the molecular lesions in an Ashkenazi Jewish type A NPD patient (proband 1), who had ≈1% of normal ASM activity in cultured fibroblasts, total RNA was isolated from cultured lymphoblasts and reverse-transcribed into cDNA; then the entire coding region was PCR amplified. Nucleotide sequencing of the subcloned PCR products revealed a single point mutation in a CpG dinucleotide, a G → T transversion of nt 1487 of the full-length cDNA (Fig. 1). This transversion predicted an Arg → Leu substitution in residue 496 of the ASM polypeptide (designated R496L). All other base substitutions occurred in only one or two of the subcloned PCR products analyzed.

To confirm the authenticity of this candidate mutation, a 567-bp region of genomic DNA from the proband, various family members, and 90 normal Ashkenazi Jewish individuals was PCR amplified and then hybridized with normal and R496L-specific radiolabeled oligonucleotides. As shown in Fig. 2, the PCR-amplified genomic DNA from proband 1 hybridized to the mutation-specific, but not to the normal allele-specific oligonucleotide, confirming the authenticity of the transversion and indicating that proband 1 was homoallelic for the R496L mutation. Of the nine other family members studied, both parents, the paternal grandfather, and a paternal aunt were heterozygous for the R496L mutation. The maternal grandmother did not have the mutation, suggesting that the maternal grandfather was heterozygous for this mutation. There was no known consanguinity between the maternal or paternal grandfathers whose ancestors were from different European countries. Moreover, the R496L mutation was not found in 180 ASM alleles studied from a group of normal Ashkenazi Jewish individuals, indicating that the G → T transversion was not a common polymorphism.

Occurrence of R496L in Ashkenazi Jewish and Non-Jewish NPD Type A Families. The occurrence of R496L in other NPD families was determined by dot-blot analysis of genomic DNA from 17 unrelated Ashkenazi Jewish and 18 unrelated non-Jewish type A NPD families. As indicated in Table 1, of the 31 Ashkenazi Jewish NPD alleles studied (i.e., from 6 unrelated patients and 19 unrelated obligate heterozygotes), 32% had the R496L mutation. Only proband 1 was homoallelic for the mutation, whereas three obligate heterozygotes from unrelated families in which material from patients was unavailable were heteroallelic for the R496L mutation. In contrast, only 2 of 36 (5.6%) alleles had the R496L mutation in non-Jewish NPD type A patients. An American of German ancestry was homoallelic for the R496L mutation.

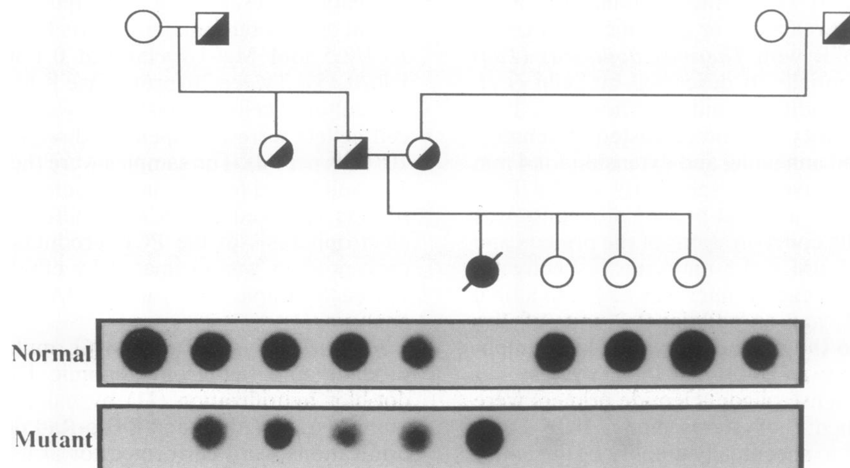

FIG. 2. Identification of the R496L mutation in amplified genomic DNAs from the members of an Ashkenazi Jewish family with type A NPD by dot-blot hybridization with allele-specific oligonucleotides. Note that the patient (proband 1) was homoallelic, and both of her parents were heterozygous for R496L.

Table 1. Frequency of the R496L mutation in Ashkenazi Jewish and non-Jewish families with types A and B NPD

Source	Unrelated families studied, no.	Mutant alleles studied, no.	R496L, %
Type A disease			
Ashkenazi Jewish	17*	31	32
Non-Jewish	18	36	5.6
Type B disease			
Ashkenazi Jewish	2	4	25
Non-Jewish	15	30	0.0

*In three of these families only one obligate heterozygous parent was available for analysis.

Occurrence of the R496L Mutation in Ashkenazi Jewish and Non-Jewish NPD Type B Families. Analysis of genomic DNA from two unrelated Ashkenazi Jewish NPD type B patients revealed the presence of one R496L allele in one patient (designated proband 2). In contrast, the R496L allele was not found in genomic DNAs from 15 non-Jewish NPD type B patients (Table 1).

DISCUSSION

Insights into the molecular nature of the remarkably distinct type A and B NPD phenotypes have been gained by the identification of a mutation in the ASM gene causing this lysosomal storage disease. The G → T transversion of coding nt 1487 occurred at a CpG dinucleotide, a known hotspot for point mutations (15) and predicted the R496L change in the ASM polypeptide. Homoallelism for the R496L mutation resulted in the severe neuronopathic type A phenotype, as evidenced by proband 1, who had ≈1% or normal ASM activity. It is not known whether the substitution of the basic arginine for the more hydrophobic and neutral leucine residue altered the enzyme polypeptide catalytic activity, stability, or both, because monospecific anti-human ASM antibodies useful for immunoblotting are not currently available.

Of the 17 unrelated Ashkenazi Jewish type A families studied, 9 were either homoallelic or heteroallelic for this lesion. In this sample, the frequency of the R496L allele was 32%, indicating that this lesion is an important mutation in type A NPD among Ashkenazi Jewish patients. It is likely that there is another more frequent mutation or, perhaps, multiple mutations, causing type A NPD in Ashkenazi Jewish patients. In contrast, analysis of 18 unrelated non-Jewish type A patients revealed the presence of the R496L allele in only 1 (a frequency of 5.6%). The presence of the R496L allele in this individual may have resulted from an independent mutational event or the occurrence of Jewish ancestors in this family.

One of the two Ashkenazi Jewish type B NPD patients studied was heteroallelic for the R496L mutation. The other allele in this Jewish type B patient had a different ASM mutation, which presumably resulted in the synthesis of a partially functional ASM polypeptide, as this patient had ≈5% residual ASM activity in cultured fibroblasts. The fact that none of the 15 non-Jewish type B patients had the R496L allele suggests that this allele is extremely rare in type B disease outside of the Ashkenazi population. That the R496L mutation was not a common polymorphism in the Ashkenazi Jewish population was supported by the fact that it was not present in 180 ASM alleles analyzed from normal Ashkenazi Jewish individuals.

For the past three decades, the genetic mechanisms responsible for the high frequency of the mutations that cause Tay–Sachs disease, Gaucher disease, and NPD in the Ashkenazi Jewish population (gene frequencies of ≈0.02, 0.02, and 0.005, respectively) have been the subject of interest and debate (16–20). Intrigued by the fact that all three of these disorders are lysosomal diseases resulting from enzymatic defects in the sphingolipid degradative pathway, investigators suggested that there may have been a common selective pressure for their high gene frequencies in the Ashkenazi Jewish population (20). Others argued that the higher gene frequencies in Ashkenazi Jewish individuals could be due to higher mutation rates for these genes (16) or founder effect and genetic drift (19). The recent identification of the mutations causing these three diseases in the Ashkenazi Jewish population has provided insight into this controversy. To date, three mutations in the β-hexosaminidase α chain (localized to chromosomal region 15q23–24) have been identified as the cause of Tay–Sachs disease in almost all Ashkenazi Jewish patients. Two of these mutations result in the infantile form, a 4-bp insertion (21) or a splice-site mutation (22), which account for ≈80 and 20% of the mutant alleles, respectively. Affected Ashkenazi Jewish patients with the less frequent and milder chronic or adult-onset form all have been heteroallelic for a point mutation Gly → Ser at position 269 (23) and one of the two infantile-onset alleles. Type 1 Gaucher disease among Ashkenazi Jewish patients results from multiple mutations in the acid β-glucosidase gene (localized to chromosomal region 1q21–q31), the Asn → Ser (at position 370) allele occurring in ≈75% of the mutant alleles, whereas the other 25% include several other lesions (24). With the identification of the R496L mutation causing NPD, it appears that in the Ashkenazi Jewish population each of these sphingolipidoses results from a common mutation (i.e., ≈70% or more of the mutant alleles) and at least one or more less frequent mutations in their respective genes. The fact that two or more mutant alleles in each gene occur frequently in this population argues for selection, rather than for a higher mutation rate or founder effect and genetic drift, as the major mechanism responsible for their increased frequency. Although it is likely that the major mutation for each disease first became established in the Ashkenazi Jewish population by founder effect and genetic drift, the finding of two or more mutations in each of these genes supports a selective advantage. Because all three disorders involve defects in lysosomal enzymes that degrade sphingolipids, it is tempting to suggest that a common selective agent, such as resistance to an adverse situation (e.g., an infectious disease), could have increased the heterozygote frequency by differential survival (thus, increased fitness) for individuals heterozygous for each of these disorders. Alternatively, heterozygosity for these mutations may have been selected for by unrelated pressures in the past. Although several hypotheses have been advanced (20, 25), the nature of the selective advantages for these mutations remains unknown.

The identification of the R496L allele and other mutations in the ASM gene in types A and B NPD may provide information for genotype–phenotype correlations and permit more accurate genetic counseling for newly diagnosed cases in families without a previously affected individual. Identification of other mutations, particularly those with residual activity that cause type B disease, also may provide structure–function information and may facilitate delineation of the active-site region. Previously, the enzymatic detection of heterozygotes for NPD types A and B was not sufficiently reliable to permit mass voluntary screening in the Ashkenazi Jewish community. Thus, the identification of the R496L and other mutations in types A and B NPD will permit accurate heterozygote identification in families with these lesions as well as heterozygote screening and prevention of NPD in the general Ashkenazi Jewish population, as has been the prototypic experience with Tay–Sachs disease (26). Using molecular techniques, we and others (ref. 27; H. Yoo, personal communication) have already demonstrated the feasibility of molecular heterozygote screening for Tay–Sachs disease in the Ashkenazi Jewish population. The extension of

such molecular screening to include the more common mutations causing Gaucher disease and NPD by the use of multiplex PCR should permit the simultaneous screening and prevention of all three sphingolipidoses in the Ashkenazi Jewish population.

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1. Niemann, A. (1914) *Fahrb. Kinderheikd.* **79**, 1–6.
2. Brady, R. O., Kanfer, J. N., Mock, M. B. & Fredrickson, D. S. (1966) *Proc. Natl. Acad. Sci. USA* **55**, 366–369.
3. Fredrickson, D. S. (1966) in *The Metabolic Basis of Inherited Disease*, eds. Stanbury, J. B., Wyngaarden, J. B. & Fredrickson, D. S. (McGraw-Hill, New York), 2nd Ed., pp. 586–602.
4. Spence, M. W. & Callahan, J. E. (1989) in *The Metabolic Basis of Inherited Disease*, eds. Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D. (McGraw-Hill, New York), 8th Ed., pp. 1655–1676.
5. Goodman, R. M. (1979) *Genetic Disorders Among the Jewish People* (Johns Hopkins Univ. Press, Baltimore), pp. 96–100.
6. Quintern, L. E., Schuchman, E. H., Levran, O., Suchi, M., Ferlinz, K., Reinke, H., Sandhoff, K. & Desnick, R. J. (1989) *EMBO J.* **8**, 2469–2473.
7. Pereira, L., Desnick, R. J., Adler, D., Disteche, C. M. & Schuchman, E. H. (1991) *Genomics* **9**, 229–234.
8. Bernstein, H. S., Bishop, D. F., Astrin, K. A., Kornreich, R., Eng, C. M., Sakuraba, H. & Desnick, R. J. (1989) *J. Clin. Invest.* **83**, 1390–1399.
9. Klar, R., Levade, T. & Gatt, S. (1988) *Clin. Chim. Acta* **176**, 259–268.
10. Bishop, D. F. & Desnick, R. J. (1981) *J. Biol. Chem.* **256**, 1307–1316.
11. Sambrook, J., Fritsch, E. F. & Maniatis, T. A. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
12. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
13. Itakura, K., Rossi, J. J. & Wallace, R. B. (1984) *Annu. Rev. Biochem.* **53**, 323–356.
14. Sanger, F., Nicklen, J. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
15. Coulondre, C., Miller, J. H., Farabaugh, P. J. & Gilbert, W. (1978) *Nature (London)* **274**, 775–780.
16. Knudson, A. G. & Kaplan, W. D. (1962) in *Cerebral Sphingolipidoses*, eds. Aronson, S. M. & Volk, B. W. (Academic, New York), pp. 395–411.
17. Chase, G. A. & McKusick, V. A. (1972) *Am. J. Hum. Genet.* **24**, 339–340.
18. Myrianthopoulos, N. C., Naylor, A. F. & Aronson, S. M. (1972) *Am. J. Hum. Genet.* **24**, 341–342.
19. Fraikor, A. L. (1977) *Soc. Biol.* **24**, 117–134.
20. Myrianthopoulos, N. C. & Melnick, M. (1977) *Prog. Clin. Biol. Res.* **18**, 95–106.
21. Myerowitz, R. & Costigan, F. C. (1988) *J. Biol. Chem.* **263**, 18587–18589.
22. Myerowitz, R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3955–3959.
23. Navon, R. & Proia, R. L. (1989) *Science* **243**, 1471–1474.
24. Tsuji, S., Choudary, P. V., Martin, B. M., Stubblefield, B. K., Mayor, J. A., Barranger, J. A. & Ginns, E. I. (1987) *N. Engl. J. Med.* **316**, 570–575.
25. Myrianthopoulos, N. C. & Aronson, S. M. (1972) in *Advances in Experimental Medicine and Biology*, eds. Volk, B. W. & Aronson, S. M. (Plenum, New York), pp. 561–570.
26. Kaback, M. M. (1977) *Prog. Clin. Biol. Res.* **18**, 1–7.
27. Riggs-Raine, B. L., Feigenbaum, A. S. J., Natowicz, M., Skomorowski, M., Schuster, S. M., Clarke, J. T. R., Mahuran, D. J., Kolodny, E. H. & Gravel, R. A. (1990) *N. Engl. J. Med.* **323**, 6–12.
28. Schuchman, E. H., Suchi, M., Takahashi, T., Sandhoff, K. & Desnick, R. J. (1991) *J. Biol. Chem.*, in press.