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Niemann-Pick C1 Disease: The I1061T Substitution Is a Frequent Mutant Allele in Patients of Western European Descent and Correlates with a Classic Juvenile Phenotype

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Summary

Niemann-Pick type C (NPC) disease is an autosomal recessive lipid-storage disorder usually characterized by hepatosplenomegaly and severe progressive neurological dysfunction, resulting from mutations affecting either the *NPC1* gene (in 95% of the patients) or the yet-to-be-identified *NPC2* gene. Our initial study of 25 patients with NPC1 identified a T_{3182}→C transition that leads to an I1061T substitution in three patients. The mutation, located in exon 21, affects a putative transmembrane domain of the protein. PCR-based tests with genomic DNA were used to survey 115 unrelated patients from around the world with all known clinical and biochemical phenotypes of the disease. The I1061T allele constituted 33 (14.3%) of the 230 disease-causing alleles and was never found in controls (>200 alleles). The mutation was particularly frequent in patients with NPC from Western Europe, especially France (11/62 alleles) and the United Kingdom (9/32 alleles), and in Hispanic patients whose roots were in the Upper Rio Grande valley of the United States. The I1061T mutation originated in Europe and the high frequency in northern Rio Grande Hispanics results from a founder effect. All seven unrelated patients who were homozygous for the mutation and their seven affected siblings had a juvenile-onset neurological disease and severe alterations of intracellular LDL-cholesterol processing. The mutation was not found (0/40 alleles) in patients with the severe infantile neurological form of the disease. Testing for this mutation therefore has important implications for genetic counseling of families affected by NPC.

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Introduction

Niemann-Pick type C (NPC) disease (MIM 257220) is an autosomal recessive lipid-storage disorder usually characterized by hepatosplenomegaly and severe progressive neurological dysfunction. In cultured cells, lysosomal sequestration of endocytosed LDL-derived cholesterol and accompanying anomalies in intracellular sterol trafficking constitute the hallmark phenotypic features of the disease and are used to assist in the diagnosis of patients. In tissues, the pattern of accumulating lipids is more complex and differs between brain and non-neural organs. Marked variations have been observed in the severity of the clinical course and in the cholesterol processing abnormalities, and these define the clinical and biochemical phenotypes, respectively (for review, see Pentchev et al. 1995; Vanier and Suzuki 1996, 1998). A genetic heterogeneity (*NPC1* and *NPC2*) was established by cell-hybridization and linkage studies (Steinberg et al. 1994; Vanier et al. 1996). The *NPC1* gene (mutated in ~95% of patients) was mapped to 18q11 and was isolated by positional cloning (Carstea et al. 1997). The genomic structure was reported while this manuscript was undergoing editorial review (Morris et al. 1999). The cDNA sequence predicts a 1,278-amino-acid protein with 16 transmembrane regions and a putative sterol-sensing domain (Carstea et al. 1997; Lange and Steck 1998). The protein appears to be located in the late endosomal membranes (Kobayashi et al. 1999; Neufeld et al. 1999) but also seems to interact with lysosomes (Neufeld et al. 1999). Deletion of the four amino acid residues containing the lysosome-targeting motif has been shown to result in the retention of the protein in the endoplasmic reticulum (Watari et al. 1999). Apart from the eight mutations reported together with positional cloning of the gene (Carstea et al. 1997), only the mutation found in the Nova Scotian patients (previously designated as type D) has been published (Greer et al. 1998). Initial studies in the laboratories of

the authors (Yamamoto et al. 1999; G. Millat and M. T. Vanier, unpublished data; J. A. Morris and P. G. Pentchev, unpublished data) disclosed a very large mutational spectrum in patients with NPC1. While nearly all mutations appeared to be private, there was an indication that one particular missense mutation, I1061T, might be frequent, although not in Japanese.

A survey including 115 unrelated patients, covering all known clinical and biochemical phenotypes of the disease, was therefore undertaken, after simple tests on genomic DNA were devised. The largest proportion of patients originated from Western Europe. Remaining patients were from a much wider geographic area—more specifically, North Africa, the Middle East, French Canada, and the isolate of Hispanic Americans from northern Colorado described by Wenger et al. (1977). For each patient, the absence or presence of one or two mutant alleles was compared to the clinical subtype and to the severity of alterations of intracellular LDL-cholesterol trafficking to establish genotype/phenotype correlations.

Subjects and Methods

Subjects

Cultured skin fibroblasts or peripheral blood samples were obtained from 120 patients with NPC who belonged to 115 unrelated families and from the parents in 8 of these families. The geographic or ethnic origin of the families was as follows: France, 31; United Kingdom, 16; Germany, 13; Belgium, 5; Switzerland, 6; Italy, 9; Poland, 1; Czechoslovakia, 3; North Africa, 6; Spain, 3; Portugal, 1; Greece, 2; Turkey, 2; Yugoslavia, 2; Gypsy, 1; Arab Emirates, 2; Costa Rica, 2; Lebanon, 1; Pakistani, 1; Australia, 1; French Canada, 3; and Hispanics from the Northern Rio Grande valley of the United States, 4. The diagnosis was finally assessed on fibroblast cultures by demonstration of intralysosomal storage of unesterified cholesterol by filipin staining and study of LDL-induced cholesterol esterification (Vanier et al. 1991*b*). Cells from all patients were evaluated or reevaluated in a single laboratory (Lyon). In 51 families, genetic complementation analysis (Vanier et al. 1996) had been performed to assess that the patient did belong to the main complementation group (mutation in the *NPC1* gene). Classification of patients into various clinical phenotypes was done as previously proposed by us (Vanier et al. 1988, 1991*a;* Vanier and Suzuki 1996). Neurological patients were categorized by type and age at onset of first neurological symptoms into either a severe infantile form (onset at age <2 years), a late infantile form (onset at age 3–5 years), a juvenile form (onset at age 5–16 years), or an adult form (onset at age >16 years). The denomination "rapidly fatal cholestatic form" applied to patients who died from liver failure in the first months of life. Our criteria for inclusion of patients into three biochemical phenotypes, on the basis of the severity of impairment in intracellular cholesterol processing in cultured fibroblasts, have also been well documented (Vanier et al. 1991*b*). The classic phenotype includes patients with a striking accumulation of free cholesterol in lysosomes, as assessed by filipin staining, together with a severe block in LDL-induced cholesteryl ester formation, studied after a 4.5-h challenge with LDL (<100 pmol cholesteryl ester formed, compared to a normal value of 2,950 ± 1,200). Cells from "variant" patients show only mild changes (filipin stain may be positive only after challenge with pure LDL, with cholesteryl ester formation ranging from 500 pmol to normal). Genomic DNA was further obtained from a control population of 100 unrelated unaffected subjects.

RNA isolation and cDNA reverse transcription.—Total RNA was isolated from fibroblast monolayers by the Trizol reagent (Gibco BRL, Life Technologies), and reverse transcription was performed using the First-Strand cDNA Synthesis kit (Amersham Pharmacia Biotech), according to the manufacturers' instructions.

SSCP and sequencing.—The *NPC1* cDNA was amplified in 18 overlapping fragments. Each PCR was performed in a 20-μl reaction volume containing 0.4 μl of cDNA, 200 μM each dNTP, 1.5 mM $MgCl_2$, PCR buffer, 1 U of Eurobio*Taq*, 4 pmol each primer, and 0.1 μl $\alpha[^{33}P]$dATP, 3000 Ci/mmol (Amersham Pharmacia Biotech). Two microliters of the radiolabeled PCR product was mixed with 15 μl stop solution (95% formamide, 10 mM NaOH, 0.25% bromphenol blue, 0.25% xylene cyanol) and was denatured for 5 min at 95°C. The samples were loaded on 0.5 × mutation detection enhancement gels (FMC BioProducts) with or without 10% glycerol. Electrophoresis was run in 0.6 × Tris-borate EDTA running buffer either at 20 W at +4°C or at 6 W at room temperature.

For sequencing, PCR was performed on cDNA with primers Os and Oas and on genomic DNA with primers F10 and Oas (table 1). After amplification, PCR products were purified and were directly sequenced on Sequagel-6 gels (National Diagnostics) by use of the Thermosequenase cycle sequencing kit with the forward exonic primer GM1s labeled with $\gamma[^{33}P]$-ATP, 3000 Ci/mmol) (Amersham Pharmacia Biotech).

Allele-Specific Oligonucleotide (ASO) Analysis

Genomic DNA was PCR-amplified using primers F10 (sense) and Oas (antisense) (table 1). The 35 PCR cycles each included 1 min at 94°C, 1 min at 60°C, and 2 min at 72°C. The ~3-kb PCR product was laid as dot-blot on a nylon membrane (Hybond-N+, Amersham Pharmacia Biotech). Membranes were prehybridized for 1 h at 65°C in 3M trimethylacetate buffer containing 10 mM $NaHPO_4$, 0.5 M EDTA, 10% SDS, 1% Denhardt, and

Table 1

Oligonucleotide Sequences

Oligonucleotide	cDNA Coordinates[a]	Sequence (5′→3′)
Os	3028/3049	AAGTGTGGCAAAGGGGGACATG
Oas	3309/3286	ACCGAGGTTGAAGATAGTGTCGTC
F10	2949/2967	TCCGGAAGGCAAACAGAGG
GM1s	3043/3062	GGACATGCTGCCTATAGTTC
RS-1061s[b]	3156/3181	TGACGCTCTGAAGAAAGCCCGACGTA
RS-1061as		tcccacccagtgtaggccctttgctg
ASO-I1061I	3191/3172	TTACTGGCTATAAGTCGGGC
ASO-I1061T	3172/3191	GCCCGACTTACAGCCAGTAA

NOTE.—Exonic sequences are given in uppercase and intronic sequences in lowercase characters.

[a] Counted from the adenosine residue of the initiation codon.

[b] The underlined nucleotide generates a *Rsa* I site when mutation I1061T is present.

100 μg salmon sperm DNA/ml. Digoxigenin-labeled wild-type (ASO-I1061I) or mutated (ASO-I1061T) probes (10 pM) (table 1) were added to the prehybridization solution, and hybridization was conducted for 1 h at 65°C. Membranes were washed first for 2 min at room temperature in 1% SSC, 10% SDS, then for 2 min at 65°C in 50 mM Tris-HCl pH 7.5, 3M trimethylacetate, 2 mM EDTA, and 0.1% SDS. The hybridized products were visualized by use of a digoxigenin luminescent detection kit (Roche Diagnostics).

Mutation Detection by Introduction of an Rsa *I Restriction Site*

Genomic DNA was PCR-amplified using the primers RS-1061s and RS-1061as (table 1). The 30 PCR cycles each included steps of 30 s at 92°C for denaturation, at 56°C for annealing, and at 72°C for extension. The 147-bp PCR product was digested for 2 h at 37°C by *Rsa* I and was analyzed on a 3% Metaphor gel (FMC BioProducts).

Results

A T_{3182}→C Transition Leading to an I1061T Substitution Is a Frequent Mutation in Patients with NPC1

In the process of a complete mutational analysis in 25 NPC1 patients covering the entire spectrum of clinical phenotypes, 3 patients with a neurological juvenile clinical presentation studied by sequencing of cDNA and genomic DNA were found to have a T_{3182}→C transition leading to a I1061T substitution. This hydrophobic/hydrophilic amino acid change was located within one of the putative transmembrane domains (Carstea et al. 1997) of the protein. The mutant allele was present in the heterozygous state in one French patient (15.1) (table 4) and in one Hispanic patient (20.1) from northern Colorado (table 4). The homozygous status of another French patient (1.3) (table 3) was definitively assessed from parental studies. Since all other mutations identified appeared to be private, a wider screening for the I1061T allele was performed. Because no information was initially available regarding the genomic structure of NPC1, allele-specific oligonucleotide analysis was first chosen to screen a larger population of patients of European descent as well as a normal population. The initial data were confirmed, and the study of 90 additional patients indicated that I1061T was a frequent mutation in this population. Meanwhile, the intronic sequences flanking the responsible exon, further characterized as exon 21 (Morris et al. 1999; T. Yamamoto and E. Nanba, unpublished data), were determined (fig. 1). An alternative procedure was then devised, by introduction of an *Rsa* I restriction site by means of a sense primer with a G mismatch at the 3′ terminus combined with an intronic antisense primer (table 1). While the normal allele remains uncut (147 bp), the mutant allele appears as a shorter 122-bp band (fig. 2). Combining the results obtained by one method or the other, 115 unrelated patients with NPC (table 2), 16 parents (from families 1, 3, 4, 8, 9, 20, 22, 24) (see table 3 and table 4) and 100 control subjects (data not shown) were studied in total. The I1061T allele was never found in the control population and constituted 33/230 alleles (14.3%) in the global NPC population.

Analysis of the NPC Population

Genotype/phenotype correlations.—The data presented in table 2 give two clear indications. First, the I1061T allele is predominantly found in patients with the most typical NPC presentation—that is, with a ju-

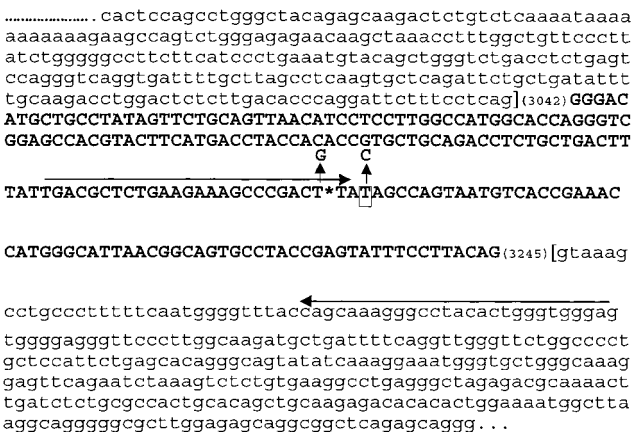

Figure 1 Sequence of *NPC1* exon 21 with intron boundaries, depicting the location of the T_{3182}→C transition and the design of primers for detection of the mutation by creation of a *Rsa* I restriction site. Intronic sequences are in lowercase characters; exonic sequences are in uppercase.The asterisk (*) indicates the base change made in the forward primer to introduce a *Rsa*I restriction site when the adjacent T→C base change is present.

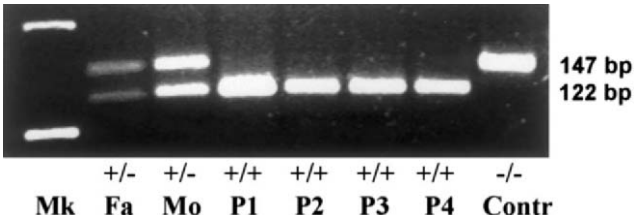

Figure 2 Restriction analysis of the I1061T mutation in a representative family. In presence of the mutation, *Rsa* I digestion of the 147-bp PCR product generates a 122-bp fragment. The results presented are those in family 1 (see table 3). Mk indicates molecular-mass DNA marker (100 and 200 bp); Fa, father; Mo, mother; P1, P2, P3, P4, affected siblings; Contr, normal subject. The plus sign (+) denotes the presence of a I1061T allele; the minus sign (−) denotes its absence.

venile neurological onset and pronounced abnormalities of intracellular LDL-cholesterol processing (the classic biochemical phenotype). In this population, I1061T constituted 30% (25/84) of alleles, and all seven homoallelic patients were included in that group. Second, this mutation was never found in patients with the severe infantile neurological form of the disease (0/40 alleles). On the other hand, a heteroallelic status could be associated either with a slightly more aggressive disease (neurological late infantile form, 2/12 patients), or with an adult neurological (1/8 patients) or nonneurological (2/4 patients) form.

In five of the seven families with homoallelic patients, several siblings were affected, and the clinical history of a total of 15 patients is reported in table 3. The median age of onset of neurological symptoms was relatively late, ~10 years, with learning problems at school as the major first complaint. Later symptoms included cerebellar signs, especially problems with fine hand movements, ataxia, dystonia, and vertical supranuclear ophthalmoplegia. The course of the disease was, in general, quite slow. Two patients died at age 16 years, but three survived until ages 28.5, 34, and 39 years. Several others were still alive at age 20 years.

The clinical pattern of patients who were heteroallelic for the I1061T mutation was much wider, as would be expected for compound heterozygotes. In 12/20 families, patients belonged to the neurological juvenile form and showed classic biochemical alterations. Patients in two additional families were still very young, so their neurological course could not be predicted. It should also be noted that the rapidly fatal cholestatic form of the disease is not correlated with the nature of the NPC1 mutation, since it has often been found to be associated with neurological forms in the same sibship (Vanier et al. 1991*a*). Interestingly, the mutation was also found in three adults—one with a neurological disease and two with isolated splenomegaly. In patient 27.1, the diagnosis had been primarily assessed in the study of splenic lipids (Dr. K. Harzer, Tübingen, Germany). Finally, although the I1061T mutation was clearly associated with a "classic" biochemical pattern, a "variant" pattern (milder alterations) occurred in heterozygotes with about the same frequency as is generally observed in a large population of patients.

Geographic distribution of the patients carrying the mutation.—A striking observation was the fact that patients in three of the four Hispanic American families from the Northern Rio Grande area (families 5, 6, and 7) (table 3) were homozygotes for the mutation. A heterozygote status (patient 20.1) in the fourth family (table 4) could probably be explained by mixed Italian ancestry. Nevertheless, the study of a large European population (table 3 and table 4) disclosed that this mutation was also quite prevalent among patients with NPC originating from France (11/62 alleles), the United Kingdom (9/32 alleles), and, to a lesser extent, Switzerland and Germany (4/38 alleles). From our current data, the mutation would appear less frequent in other European countries (Italy, Spain, Portugal, Greece, Belgium), but the number of families studied is too small for a definitive conclusion. A homozygote family was also observed in French Canadians from Quebec, and a heterozygote was found in an Australian family with an English name. Taken together, the data suggest that the I1061T mutation originated in Europe and is found in the Hispanics of the northern Rio Grande due to a founder effect.

Discussion

Our data demonstrate that an I1061T substitution in the *NPC1* gene is a frequent mutant allele in patients with NPC who have a European background. It was important to ascertain that I1061T is a disease-causing mutation, since unpublished work from our laboratories has given evidence that, in this gene, several common polymorphisms are present, both in patients and in the general population. Interestingly, four of those polymorphisms (H215R, I858V, N931N, and R1266Q) are shared by Europeans and Japanese (Yamamoto et al. 1999; G. Millat and M. T. Vanier, unpublished data). In the absence of a straightforward way to perform expression studies of NPC1, screening of a large control population on genomic DNA provided good evidence that I1061T was a disease-related mutation. The fact that patients homozygous for the mutation share a very homogeneous clinical and biochemical phenotype was another strong argument. In the three families in which parents could also be studied, both were confirmed to carry the mutation, excluding the possibility of a second allele with a large deletion in the patients.

Since I1061T appeared as a potentially frequent *NPC1* mutation, our next aim was to investigate

Table 2

Study of the I1061T Mutation in a Population of 115 Unrelated NPC Patients Covering the Various Clinical and Biochemical Phenotypes of the Disease

Clinical Phenotype	Biochemical Phenotype	No. of Patients with I1061T Mutation Status		
		Homoallelic	Heteroallelic	Not Found
Neurological onset (n = 96):				
Infantile (n = 20)	Classic (n = 20)	0	0	20
Late infantile (n = 12)	Classic (n = 12)	0	2	10
Juvenile (n = 56)	Classic (n = 42)	7	11	24
	Intermediate (n = 6)	0	0	6
	Variant (n = 8)	0	1	7
Adult (n = 8)	Classic (n = 3)	0	0	3
	Variant (n = 5)	0	1	4
No neurological disease (n = 19):				
Cholestatic rapidly fatal (age <1 year) (n = 3)	Classic (n = 3)	0	1	2
Children age <6 years (short follow-up) (n = 12)	Classic (n = 10)	0	2	8
	Variant (n = 2)	0	0	2
Adults (n = 4)	Classic (n = 1)	0	1	0
	Variant (n = 3)	0	1	2

whether clear genotype/phenotypes correlations could be established. Therefore, it was essential to screen a population of patients fully representative of the various clinical and biochemical phenotypes described in our earlier large surveys (Vanier et al. 1988, 1991*a*, 1991*b*). Regarding clinical aspects, a majority of patients with NPC have a neurological disease with a juvenile or late infantile onset; these phenotypes cover 60% of the patients in the present study. However, in Europe, the Middle East, and North Africa, patients with the severe infantile neurological form were found to represent 20%–30% of the patients in an earlier study (Vanier et al. 1991*a*). Such patients clinically differ from the other forms, and we felt that it was important to include them in a proportion similar to that previously reported by us. As predicted, more adult patients have been diagnosed with NPC in recent years (Patterson et al. 1999). Unexpectedly, some of them did not present neurological abnormalities (Vanier and Suzuki 1998; Fensom et al. 1999), and molecular-level studies of such atypical patients are particularly important. The question of symptomatic heterozygotes could have been raised in the two patients with isolated splenomegaly and mild alterations of cellular cholesterol metabolism (Vanier and Suzuki 1998), if not for a pathognomonic profile of splenic lipids (studied by Dr. K. Harzer, Tübingen). Finally, three patients who had died from the neonatal, rapidly fatal cholestatic form were included, although there is good evidence from familial and molecular genetics studies that the perinatal liver disease does not correlate with the mutation (Vanier et al. 1991*a*; Vanier and Suzuki 1998; G. Millat and M. T. Vanier, unpublished data). Regarding the severity of alterations in intracellular LDL-cholesterol processing, we and others (Vanier et al. 1988; 1991*b*; Bowler et al. 1990) have documented a wide range of variation, with only partial correlation to clinical course (Vanier and Suzuki 1998). Of the patients included in the present study, ~20% showed a "variant" or "intermediate" biochemical phenotype, a proportion quite similar to that published earlier by us in a random population (Vanier et al. 1991*b*).

Compared to the course of the disease in a large survey of patients (Vanier et al. 1988), there was a clear trend for the 15 homozygous I1061T patients to show a quite homogeneous phenotype, with late and insidious onset of the neurological disease. Several patients previously reported in the literature (Wenger et al. 1977; Pons et al. 1976) showed only learning problems in school and mental retardation for a long period. The later course of the disease, although typical for NPC (cerebellar involvement, dystonia, vertical supranuclear ophthalmoplegia), was, in general, slowly progressive. Several patients survived in their twenties or even their thirties. This mutation thus appears correlated with a relatively mild neurological phenotype with juvenile onset. Interestingly, it might exclude the most aggressive infantile neurological form, since no copy of this mutant allele was found in 20 such patients. On the other hand, the I1061T mutation was present in one allele of two patients with a neurological late infantile form and of two adult patients with isolated splenomegaly and no neurological disease (Vanier and Suzuki 1998; Fensom et al. 1999). Identification of the other mutation in these patients appears to be critical for better understanding of the genotype/phenotype correlations and is currently under way. It is obvious that the I1061T substitution leads to a severe disruption of cellular LDL-cholesterol processing. The nature and location of the associated mutations may shed light on the mechanism that differentiates severe (classic) and mild (variant) cellular

Table 3

Biochemical Data, Clinical History, and Origin of 15 Patients Homozygous for the I1061T Mutation (Seven Unrelated Families)

Family	Origin	Affected Siblings	Biochemical Pattern	Neurological Involvement			Age at Death (years)	Age at Last Follow-Up (years)	Reference
				Age at Onset (years)	First Manifestation	Later Manifestations			
1[a]	French	1.1, Male	Classic	9–10	Learning problems	Cerebellar and pyramidal signs, VSO	...	24	Carstea et al. (1993); family 13
		1.2, Female	Classic	8–9	Learning problems	Ataxia, dystonia, VSO	...	20	Ceuterik and Martin (1986)
		1.3[a], Female	Classic	6–7	Learning problems	Ataxia, dystonia, VSO	...	20	
		1.4, Male	Classic	11–12	School problems	Ataxia, dysmetria, VSO	16	...	
2	French	2.1, Male	ND	8–10	School problems	Generalized seizures, ataxia, cerebellar and pyramidal signs, VSO, bedridden from age 12 years	16	...	Pons et al. (1976); case 1
		2.2[a], Female	Classic	10	School problems	Ataxia, dysmetria, VSO, seizures, dystonia, pyramidal signs, bedridden from age 22.5 years	28.5	...	Pons et al. (1976); case 2
3[a]	Swiss	3.1[b], Female	Classic	?	Some school problems; employed as charwoman until age 23 years	Ataxia, other cerebellar signs, VSO, extrapyramidal signs, then pyramidal signs	34	...	
		3.2, Female	Classic	14	Mental retardation, VSO, ataxia	Intention tremor, dystonia	39	...	
4[a]	French Canadian (Quebec)	4.1[b], Female	Classic	9	School problems	Problems with fine movements	...	9.5	
		4.2, Female	Classic	Absent			...	3	
5	Northern Rio Grande Hispanic	5.1, Female	ND	?	Age 14 years: mental retardation, VSO	No clear other neurological abnormalities	...	21	Wenger et al. (1977); family 1, patient 1
		5.2, Female	ND	? (> 5.5)	Age 13 years: seizures,VSO	Dysmetria	...	15	Wenger et al. (1977); family 1, patient 2
		5.3[b], Male	Classic	7	Poor coordination	Learning difficulties, lethargy	...	10	Wenger et al. (1977); family 1, patient 3
6	Northern Rio Grande Hispanic	6.1[b], Male	Classic	8	School problems, mental retardation, no neurological abnormalities	Poor fine finger movements	...	9.5	Wenger et al. (1977); family 3, patient 6
7	Northern Rio Grande Hispanic	7.1[b], Female	Classic	11	Slow in school, mental retardation, no neurological abnormalities	Increasing learning problems, VSO, impaired cerebellar function in upper extremities, brisk deep tendon reflexes	...	14.5	Wenger et al. (1977); family 4, patient 7

NOTE.—VSO = vertical supranuclear ophthalmoplegia.

[a] Both parents were also studied and confirmed to be heterozygotes for the mutation.

[b] Patient included in table 2.

Table 4

Origin and Phenotypes of 23 Patients with NPC (20 Families) Heteroallelic for the I1061T Mutation

Patient Identification[a]	Origin	Clinical Phenotype	Biochemical Phenotype	Age at Death	Age at Last Follow-Up (years)
8.1[b]	France	N, Late infantile	Classic	8 years	
8.2		N, Late infantile	Classic		10.5
9.1[b]	United Kingdom	N, Late infantile	Classic	8 years	
9.2		N, Late infantile	Classic		10
10.1	France	N, Juvenile	Classic		7
11.1	France	N, Juvenile	Classic	11 years	
12.1	France	N, Juvenile	Classic		7
13.1	France	N, Juvenile	Classic		10.5
14.1	France	N, Juvenile	Classic		10
15.1	France	N, Juvenile	Classic		32
16.1	United Kingdom	N, Juvenile	Classic		11
17.1	United Kingdom	N, Juvenile	Classic	25 years	
18.1	United Kingdom	N, Juvenile	Classic		7
19.1	United Kingdom	N, Juvenile	Classic		12
20.1	United States, Hispanic[c]	N, Juvenile	Classic		
21.1[b]	United Kingdom	N, Juvenile	Variant	18 years	
21.2		N, Juvenile	Variant		21
22.1	United Kingdom	N, Adult	Variant		32
23.1[b,d]	United Kingdom	Cholestatic rapidly fatal form	Classic	19 d	
24.1	Australia	Non-neurological	Classic		1
25.1[b]	Germany/Italy	Non-neurological	Classic		3
26.1[e]	United Kingdom	Non-neurological	Classic		51
27.1	Germany	Non-neurological	Variant		53

NOTE.—N = neurological.

[a] The number before the dot indicates the family; the number after the dot indicates the individual patient.

[b] The parents of this patient were also studied, and one of them was found to be heterozygous.

[c] Hispanic from Northern Rio Grande with Italian grandmother.

[d] Patient 2, Maconochie et al. (1989).

[e] Fensom et al. (1999).

cholesterol alterations, which seems genetically determined (Vanier et al. 1991*a*).

Patients included in the study originated, to a large extent, from Western Europe, most prominently from France and the United Kingdom, but also from Belgium, Germany, Switzerland, Italy, Spain, and Portugal. Excluding families with the neurological infantile phenotype in which the I1061T mutation is apparently never present, our results indicate that in France (9/24) and in the United Kingdom (9/14), the I1061T mutation is frequent among families affected by NPC, with at least one allele found in 40%–60% of the families. Although a lesser frequency was observed in other European countries, one should be very careful not to overinterpret the data, considering the smaller number of families tested. The mutation was not observed in a study of 10 Japanese patients but constituted one mutant allele of the GM03123 cell line (Yamamoto et al. 1999). The hypothesis that it probably originates from Western Europe is strengthened by the observation that the mutation occurs in countries with a strong historical European immigration. We have indications that this applies to Quebec and Australia and, definitely, to the United States.

In the United States, we demonstrated that the I1061T substitution is highly prevalent in Hispanic patients from the Upper Rio Grande valley. Two additional patients with roots in this region were recently diagnosed with NPC, and both were homozygous for the mutation (D. A. Wenger and M. A. Rafi, unpublished data). The only two heterozygous patients (including 20.1) currently known to be living in this area proved to have a non-Hispanic parent or grandparent. The mutation is naturally not limited to this population, and an additional study of 26 U.S. patients with Anglo-Saxon or Asian surnames showed that 7 carried one mutant allele (D. A. Wenger and M. A. Rafi, unpublished). No copy of the mutant allele was found in the four Hispanic patients from California and Florida studied so far. Consistent with our previous statement, no patient with one copy of this mutation had a severe infantile phenotype.

Most of the families in the Upper Rio Grande valley can trace their ancestors to Spanish settlers who came up from Mexico in the late 17th and early 18th centuries.

It seems obvious that one or more carriers of this mutation were present in this group of settlers. A number of Hispanic patients homozygous for this mutation recently diagnosed in Colorado and New Mexico live in the larger cities, such as Denver or Albuquerque. However, all have parents or grandparents originating from the smaller towns between Santa Fe, New Mexico and Alamosa, Colorado. The carrier rate in that population may be as high as 1 in 30, similar to that of Tay-Sachs disease (MIM 272800) in the Ashkenazi Jewish population. A similar founder effect has previously been demonstrated (Winsor and Welch 1978) in the Acadian population of Nova Scotia, Canada, where a different common mutation, G992W, was identified (Greer et al. 1998). Screening programs to identify carriers in these populations may be useful to those seeking genetic information. More generally, considering the frequency of the I1061T substitution found in the present study, we advocate testing all white patients with NPC for this mutation, since every positive allele will allow reliable heterozygote screening for family members with this mutation.

The I1061T substitution, resulting from to a T→C transition located in exon 21 of the *NPC1* gene, affects the predicted transmembrane domain 10 of NPC1 not homologous to Patched1. Antibodies against NPC1, now available in several laboratories, including ours, should allow cytochemical and biochemical studies of the mutated protein. Considering the very "classic" clinical and biochemical phenotypes of patients homozygous for the mutation, such studies may provide interesting information for functional characterization of NPC1.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for NPC1 cDNA [accession number AF002020])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for NPC [257220] and Tay-Sachs disease [272800]

References

Bowler LM, Shankaran R, Das I, Callahan JW (1990) Cholesterol esterification and Niemann-Pick disease: an approach to identifying the defect in fibroblasts. J Neurosci Res 27:505–511

Carstea ED, Morris JA, Coleman KG, Loftus SK, Zhang D, Cummings C, Gu J, et al (1997) Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis. Science 277:228–231

Ceuterick C, Martin JJ, Foulard M (1986) Niemann-Pick disease type C: skin biopsies in parents. Neuropediatrics 17: 111–112

Fensom AH, Grant AR, Steinberg SJ, Ward CP, Lake BD, Logan EC, Hulman G (1999) An adult with a non-neuronopathic form of Niemann-Pick C disease. J Inher Metab Dis 22:84–86

Greer WL, Riddell DC, Gillan TL, Girouard GS, Sparrow SM, Byers DM, Dobson MJ, et al (1998) The Nova Scotia (type D) form of Niemann-Pick disease is caused by a G3097→T transversion in NPC1. Am J Hum Genet 63:52–54

Kobayashi T, Beuchat MH, Lindsay M, Frias S, Palmiter RD, Sakuraba H, Parton RG, et al (1999) Late endosomal membranes rich in lysobisphosphatidic acid regulate cholesterol transport. Nat Cell Biol 1:113–118

Lange Y, Steck T (1998) Four cholesterol-sensing proteins. Curr Opin Struct Biol 8:435–439

Maconochie IK, Chong S, Mieli Vergani G, Lake BD, Mowat AP (1989) Fetal ascites: an unusual presentation of Niemann-Pick disease type C. Arch Dis Child 64:1391–1393

Morris JA, Zhang D, Coleman KG, Nagle J, Penchev PG, Carstea ED (1999) The genomic organization and polymorphism analysis of the human Niemann-Pick C1 gene. Biochem Biophys Res Commun 261:493–498

Neufeld EB, Wastney M, Patel S, Suresh S, Cooney AM, Dwyer NK, Roff CF, et al (1999) The Niemann-Pick C1 protein resides in a novel organelle linked to retrograde transport of multiple lysosomal cargo. J Biol Chem 274:9627–9635

Patterson MC, Vanier MT, Suzuki K, Morris JA, Carstea ED, Neufeld EB, Blanchette-Mackie EJ, et al. Niemann-Pick disease type C: a lipid trafficking disorder. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 8th ed. McGraw-Hill, New York (in press)

Pentchev PG, Vanier MT, Suzuki K, Patterson M (1995) Niemann-Pick disease type C: a cellular cholesterol lipidosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 7th ed. McGraw-Hill, New York, pp 2625–2639

Pons G, Ponsot G, Leveque B, Lyon G (1976) Maladie de surcharge neuro-viscérale avec ophtalmoplégie supra-nucléaire de la verticalité et présence dans la moelle d'histiocytes bleutés ou maladie de Neville. Ann Pediat 23: 503–508

Steinberg SJ, Ward CP, Fensom AH (1994) Complementation studies in Niemann-Pick disease type C indicate the existence of a second group. J Med Genet 31:317–320

Vanier MT, Duthel S, Rodriguez-Lafrasse C, Pentchev P, Carstea ED (1996) Genetic heterogeneity in Niemann-Pick C

disease: a study using somatic cell hybridization and linkage analysis. Am J Hum Genet 58:118–125

Vanier MT, Rodriguez-Lafrasse C, Rousson R, Duthel S, Harzer K, Pentchev PG, Revol A, et al (1991*a*) Type C Niemann-Pick disease: biochemical aspects and phenotypic heterogeneity. Dev Neurosci 13:307–314

Vanier MT, Rodriguez-Lafrasse C, Rousson R, Gazzah N, Juge MC, Pentchev PG, Revol A, et al (1991*b*) Type C Niemann-Pick disease: spectrum of phenotypic variation in disruption of intracellular LDL-derived cholesterol processing. Biochim Biophys Acta 1096:328–337

Vanier MT, Suzuki K (1996) Niemann-Pick diseases. In: Moser HW (ed) Neurodystrophies and neurolipidoses. Elsevier Science B.V., Amsterdam, pp 133–162

——— (1998) Recent advances in elucidating Niemann-Pick C disease. Brain Pathol 8:163–174

Vanier MT, Wenger DA, Comly ME, Rousson R, Brady RO, Pentchev PG (1988) Niemann-Pick disease group C: clinical variability and diagnosis based on defective cholesterol esterification. A collaborative study on 70 patients. Clin Genet 33:331–348

Watari H, Blanchette-Mackie EJ, Dwyer NK, Glick JM, Patel S, Neufeld EB, Brady RO, et al (1999) Niemann-Pick C1 protein: obligatory roles for N-terminal domains and lysosomal targeting in cholesterol mobilization. Proc Natl Acad Sci USA 96:805–810

Wenger DA, Barth G, Githens JH (1977) Nine cases of sphingomyelin lipidosis, a new variant in Spanish-American children: juvenile variant of Niemann-Pick Disease with foamy and sea-blue histiocytes. Am J Dis Child 131:955–961

Winsor EJT, Welch JP (1978) Genetic and demographic aspects of Nova Scotia Nieman-Pick disease (type D). Am J Hum Genet 30:530–538

Yamamoto T, Nanba E, Ninomiya H, Higaki K, Taniguchi M, Zhang H, Akaboshi S, et al (1999) NPC1 gene mutations in Japanese patients with Niemann-Pick disease type C. Hum Genet 105:10–16