

# Glucocerebrosidase Mutations in Gaucher Disease

Ernest Beutler, Anna Demina, and Terri Gelbart

The Scripps Research Institute, La Jolla, California, U.S.A.

## Abstract

**Background:** Thirty-six mutations that cause Gaucher disease, the most common glycolipid storage disorder, are known. Although both alleles of most patients with the disease contain one of these mutations, in a few patients one or both disease-producing alleles have remained unidentified. Identification of mutations in these patients is useful for genetic counseling.

**Materials and Methods:** The DNA from 23 Gaucher disease patients in whom at least one glucocerebrosidase allele did not contain any of the 36 previously described mutations has been examined by single strand conformation polymorphism (SSCP) analysis, followed by sequencing of regions in which abnormalities were detected.

**Results:** Eight previously undescribed mutations were detected. In exon 3, a deletion of a cytosine at cDNA nt 203 was found. In exon 6, three missense mutations were identified: a C→A transversion at cDNA nt 644

(Ala<sup>176</sup>→Asp), a C→A transversion at cDNA nt 661 that resulted in a (Pro<sup>182</sup>→Thr), and a G→A transition at cDNA nt 721 (Gly<sup>202</sup>→Arg). Two missense mutations were found in exon 7: a G→A transition at cDNA nt 887 (Arg<sup>257</sup>→Gln) and a C→T at cDNA nt 970 (Arg<sup>285</sup>→Cys). Two missense mutations were found in exon 9: a T→G at cDNA nt 1249 (Trp<sup>378</sup>→Gly) and a G→A at cDNA nt 1255 (Asp<sup>380</sup>→Asn). In addition to these disease-producing mutations, a silent C→G transversion at cDNA nt 1431, occurring in a gene that already contained the 1226G mutation, was found in one family.

**Conclusions:** The mutations described here and previously known can be classified as mild, severe, or lethal, on the basis of their effect on enzyme production and on clinical phenotype, and as polymorphic or sporadic, on the basis of the haplotype in which they are found. Rare mutations such as the new ones described here are sporadic in nature.

## INTRODUCTION

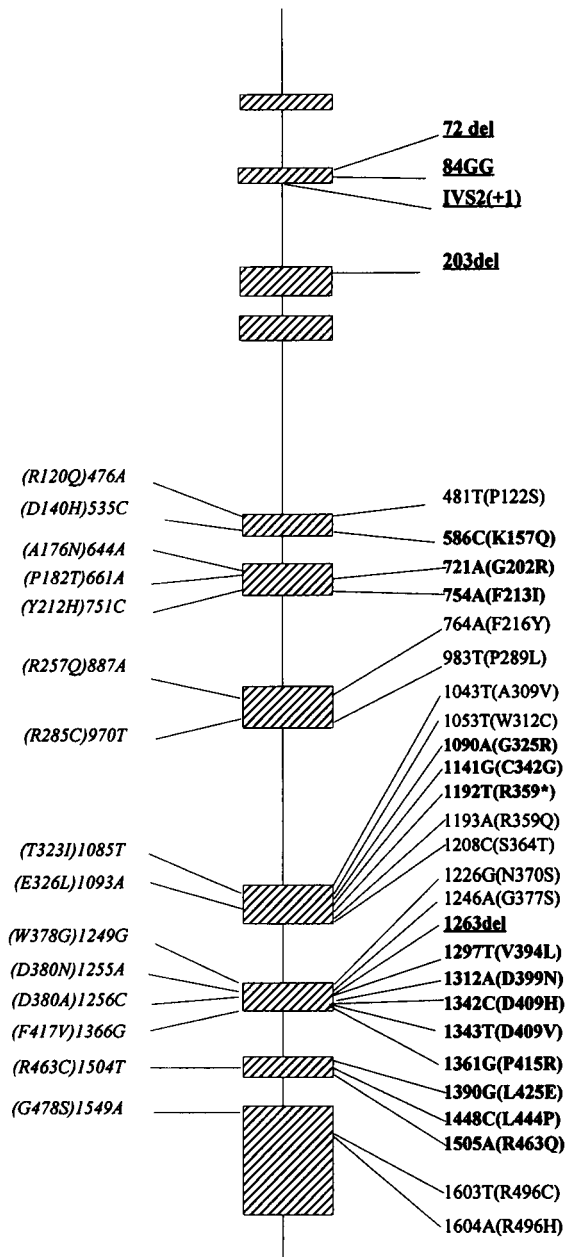
Glucocerebrosidase is a lysosomal  $\beta$ -glucosidase that hydrolyzes glucosylceramide (glucocerebroside). Deficiency of the enzyme leads to accumulation of insoluble glucocerebrosides in the tissues, resulting in the clinical manifestations of Gaucher disease (1). Gaucher disease is a heterogeneous disorder even within its three subtypes. The majority of patients, those without neurologic manifestations of the disease, are classified as type I. Types II and III are neuronopathic forms, classified with respect to severity and to the time of onset of neurologic disease, type II being the most severe, with symptoms at or near the time of birth, and type III with later onset.

Over 30 point mutations, deletions, and gene

fusions and conversions with the homologous pseudogene have been previously reported in the gene coding for glucocerebrosidase (*GBA*) (2). These mutations and the new ones described here are summarized in Table 1 and in Fig. 1. In patients of Jewish ancestry we have found that 97% of the mutations can be attributed to five of these mutations in the following order of frequency: 1226G, 84GG, Ivs2(+1), 1448C, and 1297T (3). In non-Jewish patients these five mutations account for 75% of the disease-producing alleles (4).

When both mutations causing this autosomal recessive disease are not identified, further investigation may be warranted to identify the responsible nucleotide change. This information may be useful in screening other family members for carrier status of the mutations as well as prenatal counseling. Genotype/phenotype correlations are important, since association of a mu-

Address correspondence and reprint requests to: Ernest Beutler, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, U.S.A.



**FIG. 1. The mutations of the glucocerebrosidase gene**

At the right the deduced severity of each mutation is designated by the use of different type styles: lethal = underline; severe = bold; mild = normal. At the left, mutations of unknown severity are shown in italics. The cDNA number followed by the base that is found at that position in the mutant are shown next to the line. Within parentheses the amino acid change is shown, where applicable. cDNA numbers are assigned with the upstream ATG beginning with 1. Amino acid numbers are numbered beginning with the putative cleavage site of the leader sequence.

tation with neurologic disease may have important implications in genetic counseling.

## MATERIALS AND METHODS

DNA from leukocytes of 215 unrelated Gaucher disease patients (130 Jewish and 85 non-Jewish) was studied. Each sample was examined by the methods indicated in Table 1 for as many of the 36 mutations shown until the mutations on both alleles had been identified (Table 2). Four hundred and four of the 430 putative mutations were identified as indicated in previous publications from this laboratory (3–6).

DNA from 23 individuals (22 non-Jewish and 1 Jewish) with 26 unidentified alleles was subjected to single-stranded conformation polymorphism analysis (SSCP) as reported previously (5). With the following exceptions, all exons of all samples were examined, and in addition genomic nt 201–396 (7) containing the putative promotor was examined in all samples. The amount of DNA available was insufficient for us to be able to examine two exons in Patient 6, 1 exon in Patient 4, and 1 exon in each of four other patients, each with a single remaining unidentified allele.

When abnormal bands were encountered in SSCP analysis, the DNA from that region of the glucocerebrosidase gene was amplified by the polymerase chain reaction (PCR) and sequenced (6) to determine the exact site of the mutation. To confirm the mutation, the opposite strand was also sequenced. In addition, if the mutation created or destroyed a restriction endonuclease site, this was used to further confirm the mutation. In some cases allele-specific oligonucleotide hybridization (ASOH) was used to confirm the mutations (Table 3).

Whenever a new mutation was found, all of the samples with unidentified Gaucher disease mutations were screened for the new mutation using either restriction endonuclease analysis or ASOH as indicated in Table 3.

## RESULTS

When SSCP was carried out on exons 1 through 11 and the promotor region of the glucocerebrosidase gene, eight new mutations were detected in the 26 alleles with unidentified mutations that were examined (Table 4). The 644A mutation was found in one additional unrelated patient,

TABLE 1. Mutations known to cause Gaucher disease

cDNA Number from Upstream ATG	Amino Acid Number from Cleavage Site	Genomic Number (7)	Exon	Base Substitution	Amino Acid Substitution	Rapid Detection Method	Disease Type	Reference
72	N/A	1023	2	C→Del	FS <sup>a</sup>	+AluI	I	(5)
84	N/A	1035	2	G→GG	FS <sup>a</sup>	(+BsaBI) <sup>b</sup>	I	(10,18)
Ivs2	N/A	1067	Ivs2(+1)	g→a <sup>c</sup>	Splice	-HphI	I	(3)
476	120	3060	5	G→A	Arg→Gln	+BstNI	I	(19)
481	122	3065	5	C→T	Pro→Ser	-KpnI	I	(3,5)
535 <sup>d</sup>	140	3119	5	G→C	Asp→His	+BspHI	I	(20)
586	157	3170	5	A→C	Lys→Gln	+ScrFI	II	(20,21)
751	212	3545	6	T→C	Tyr→His	+DraIII	I	(3,5)
754	213	3548	6	T→A <sup>c</sup>	Phe→Ile	(+Nsi) <sup>b</sup>	I,III	(22)
764	216	4113	7	T→A	Phe→Tyr	+KpnI	I	(23)
983	289	4332	7	C→T	Pro→Leu	(-BglI) <sup>b</sup>	I	(24)
1043	309	5259	8	C→T	Ala→Val	-BamI	I	(21)
1053	312	5269	8	G→T	Trp→Cys	-KpnI	I	(21)
1085	323	5301	8	C→T	Thr→Ile	+FokI	I	(24)
1090	325	5306	8	G→A <sup>c</sup>	Gly→Arg	+Bsu36I	II	(25)
1093 <sup>d</sup>	326	5309	8	G→A	Glu→Lys	-BsmI	I	(20)
1141	342	5357	8	T→G	Cys→Gly	-StuI	II	(25)

1192	359	5408	8	C→T	Arg→Stop	-Sau3AI	I,II	(6)
1193	359	5409	8	G→A	Arg→Gln	-TaqI	I	(26)
1208	364	5424	8	G→C	Ser→Thr	(+AlwNI) <sup>b</sup>	I	(21)
1226	370	5841	9	A→G	Asn→Ser	(+XhoI) <sup>b</sup>	I	(27)
1246	377	5861	9	G→A	Gly→Ser	+PvuII	I	(28)
1256	380	5871	9	A→C	Asp→Ala	+ScrFI	I	(29)
1263	N/A	5878	9	55Del	FS <sup>a</sup>	-SalI	I	(3,5)
1297	394	5912	9	G→T	Val→Leu	(-BamI) <sup>b</sup>	I,III	(30)
1312	399	5927	9	G→A	Asp→Asn	-TaqI	II	(6)
1342	409	5957	9	G→C <sup>c</sup>	Asp→His	-StyI	I,III	(25,30)
1343	409	5958	9	A→T	Asp→Val	-AflIII	III	(30)
1361	415	5976	9	C→G	Pro→Arg	+HhaI	II	(31)
1390	425	6375	10	A→G	Lys→Glu	(+SacI) <sup>b</sup>	III	(26)
1448	444	6433	10	T→C <sup>c</sup>	Leu→Pro	+NciI	I,II,III	(16)
1504	463	6489	10	C→T	Arg→Cys	+BsrI	I,III	(32)
1505	463	6490	10	G→A	Arg→Gln <sup>c</sup>	-MspI	III	(33)
1549	478	6628	11	G→A	Gly→Ser	+AluI	I	(5)
1603	496	6682	11	C→T	Arg→Cys	-BsaHI	I	(26)
1604	496	6683	11	G→A	Arg→His	+HphI	I	(3,5)

<sup>a</sup>Frame shift with early termination.

<sup>b</sup>Restriction site created artificially by mismatching a PCR primer.

<sup>c</sup>Mutation creates the sequence found in the pseudogene.

<sup>d</sup>Both mutations found on the same chromosome in one report (20), but we have encountered the 1093A mutation alone.

<sup>e</sup>Splicing defect with early termination.

TABLE 2. Number of identified and unidentified Gaucher disease alleles in the subjects of this study

	Jewish			Non-Jewish		
	Type I	Type II	Type III	Type I	Type II	Type III
Total patients	130	0	0	73	7	5
Known mutations (# of alleles)	259	0	0	124	12	9
Unidentified alleles	1	0	0	22	2	1

and the 721A mutation was found in two additional unrelated patients, one of whom had type II Gaucher disease. Thus, 11 of the 26 mutations were detected.

The mutation in exon 3 was a deletion of a cytosine at nt 203. Instead of the normal six cytosines at this position, only five were found. This patient is a 34-year-old female with moderately severe type I Gaucher disease (severity score = 19) (8). She has recently developed Hodgkin's disease. Her other allele carries the 1226G mutation (Table 4). The existence of the deletion was confirmed by allele-specific oligonucleotide hybridization (ASOH).

Two patients were found to carry a C→A transversion in exon 6 at cDNA nt 644 (Ala<sup>176</sup>→Asp). They were unrelated individuals from Germany and Austria, who had been diagnosed at ages 11 and 22 and had mild to moderate disease. This mutation in exon 6 destroys a PflMI site.

The C→A transversion at cDNA nt 661 (<sup>182</sup>Pro→Thr) mutation in exon 6 was identified in a 47-year-old male diagnosed at age 12 who now has moderate disease (severity score = 8). This mutation was confirmed by the loss of an HphI restriction site.

The third mutation in exon 6 was a G→A transition at nt 721 of the cDNA (<sup>202</sup>Gly→Arg). This is also the normal sequence of the glucocerebrosidase pseudogene at the corresponding position. The surrounding sequence was not that of the pseudogene and therefore not the product of a crossover or an extensive gene conversion event. An NciI restriction site is destroyed by this mutation. Three patients were found to have this mutation. One of these died at age 3 of type II disease.

The 887 G→A mutation (<sup>257</sup>Arg→Gln) was found in a 49-year-old non-Jewish Gaucher disease patient with extensive skeletal involvement

(severity score = 14). The mutation was confirmed by sequencing the opposite strand and by restriction analysis.

The other mutation found in exon 7, a C→T transition at cDNA nt 970 (<sup>285</sup>Arg→Cys) was identified in a 39-year-old male of Italian, non-Jewish ancestry with moderate disease (severity score = 10). To verify this mutation it was necessary to create an NsiI restriction site by PCR with a mismatched primer (Table 3).

In exon 9 a T→G mutation at nt 1249 (<sup>378</sup>Trp→Gly) was found in a patient diagnosed at the age of 20, now 59 years old, with a severity score of 11. Since the mutation neither created nor destroyed a restriction site, ASOH was used to confirm this mutation. No other patients were found to have this substitution.

A 1255 G→A mutation (<sup>380</sup>Asp→Asn) was found in exon 9 of a non-Jewish male with type I Gaucher disease. We were able to create an XcmI restriction site for this mutation by performing PCR with a mismatched primer (Table 3). This patient's other allele has the 1226G mutation also located in exon 9. This patient also had an abnormal SSCP pattern when exon 10 was examined. Sequencing determined that this was due to a C→G transversion at cDNA nt 1431, a silent mutation <sup>438</sup>Ala→Ala. Two daughters did not carry either the 1226G mutation or the 1431G silent mutation but both were demonstrated to carry the 1255A mutation. Thus, the 1431G mutation segregates with the 1226G mutation and appears to represent a second mutational event in this allele. This silent mutation is apparently rare. The DNA from 36 Caucasians, 13 of whom were Hispanic, 12 Ashkenazi Jews, and 12 normal Orientals, representing a total of 116 alleles, were normal at this site as determined by testing an appropriately amplified fragment for cleavage with HinFI.

**TABLE 3. Methods of mutation analysis**

<b>cDNA Number</b>	<b>Substitution</b>	<b>Sense Primer</b>	<b>Antisense Primer</b>	<b>Rapid Detection Method</b>	<b>ASOH Primers</b>
203	C→Del	1361-GCCACCACACTACGCCAGCTA-1380	1914-TAAGGGTATCAGTACCCAGC-1895	ASOH	1695-CCTTTGA C CCCCCGACCT-1702 1695-CCTTTGACCCCCCGACCT1701
644	C→A	3389-CTGATTCACCCAGCCCTGCA-3408	3504-TTGAGTGACCCCTTCCCATT-3485	-PflMI	-
661	C→A	3389-CTGATTCACCCAGCCCTGCA-3408	3504-TTGAGTGACCCCTTCCCATT-3485	-HphI	-
721	G→A	3052-CATCATCCGGGTACCCATGG-3071	3916-GCGCCTATAATCCCAGCT-3899	-NciI	-
887	G→A	3465-TCAAGACCAATGGAGCGGTG-3484	4259-CTAGGTCAGGCAATGAAG-4240	-BsmAI	-
970	C→T	4063-TCCTGGTCCACTTCTTTGGCC-4082	4340-CCCAGTGGGCGCAGCATG-4321	(+NsiI)	-
1249	T→G	5183-CAAGGTCCAGGATCAGTTGC-5202	6487-TTTAGCACGACCAACACAGC-6468	ASOH	5854-TGTGGTCTGGACCG-5870 5854-TGTGGTCTGGGACCG-5870
1255	G→A	5797-CCAGTGTGAGCCTTTGTCT-5816	5892-GGGTTCAGGGCCAGGTTCCA-5873	(+XcmI)	

Underlining indicates that the nucleotide is mismatched to the normal sequence to produce a restriction site for the enzyme that is enclosed in parentheses.

TABLE 4. New mutations found in the present study

Patient #	cDNA Number	Genomic Number	Base Substitution	Exon	Amino Acid Number	Amino Acid Substitution	Rapid Detection Method	Other Mutation	Age at Dx/ Current Age	Type	Severity Score (8)	Pvl.1.
1	203	1707	C→del	3	29	Pro→Arg <sup>d</sup>	ASOH <sup>d</sup>	1226G	9/34	I	19	-/-
2	644	3438	C→A	6	176	Ala→Asp	-PflMI	?	11/31	I	7	+/+
2A	—	—	—	—	—	—	—	1226G	22/38	I	4	+/-
3	661	3455	C→A	6	182	Pro→Thr	-HphI	1226G	12/47	I	8	-/-
4	721	3515	G→A <sup>b</sup>	6	202	Gly→Arg	-NciI	1226G	19/53	I	6	-/-
4A	—	—	—	—	—	—	—	1226G	29/38	I	6	+/-
4B	—	—	—	—	—	—	—	1448C	0.5/died at 3	II	24	+/-
5	887	4236	G→A	7	257	Arg→Gln	-BsmAI	1226G	51/8	I	14	-/-
6	970	4319	C→T	7	285	Arg→Cys	(+Nsi) <sup>c</sup>	1226G	39/20	I	10	-/-
7	1249	5864	T→G	9	378	Trp→Gly	ASOH <sup>d</sup>	764A	20/59	I	11	+/+
8	1255	5870	G→A	9	380	Asp→Asn	(+XcmI) <sup>c</sup>	1226G	71/40	I	8	+/-

All patients were unrelated non-Jewish individuals with Gaucher disease. Patient designations without letter represent the index case.

<sup>a</sup>Frame shift with early termination.

<sup>b</sup>Mutation creates the sequence found in the pseudogene.

<sup>c</sup>Restriction site created artificially by mismatching a PCR primer.

<sup>d</sup>Allele-specific oligonucleotide hybridization.

**TABLE 5.** The expected clinical phenotype when mutations classified as lethal, severe, and mild are combined

		One Allele		
		Lethal	Severe	Mild
Other Allele	Lethal	Non-viable	Type II/III	Type I
	Severe	Type II/III	Type II/III	Type I
	Mild	Type I	Type I	Type I

## DISCUSSION

### Severity of Mutations

Mutations of the glucocerebrosidase gene may be classified on the basis of the severity of the phenotypic effect that they produce. Three types of mutations may be distinguished: lethal, severe, and mild. The expected phenotypic effects of combinations of these types of mutations are summarized in Table 5.

Mutations that prevent the formation of any enzyme at all are considered to be lethal. These include the complete deletion of the gene (9), the 84GG mutation, which causes early termination (10), and the *Ivs2* mutation, which prevents splicing of the transcript (3). We regard these as being lethal because they have never been identified either in the homozygous (e.g., 84GG/84GG) or the combined heterozygous (e.g. 84GG/*Ivs2*(+1)) form. The fact that targeted disruption of the murine glucocerebrosidase gene is lethal at or before birth (11) is consistent with this interpretation of such mutations.

Severe mutations include all of those that have been associated with neuronopathic disease, except those that are regarded as being lethal. The prototype of this type of mutation is 1448C, which is often found in the homozygous state, almost invariably with neuropathic disease.

Mild mutations are defined as those that are not associated with neuronopathic disease, even when inherited together with lethal or severe mutations. The 1226G mutation is the most common of these.

Of the new mutations we have found, the deletion at nt 203 may be considered a lethal variant. It produces a frame shift in amino acid 29 with termination at amino acid 51. The 721A mutation can be classified as severe, since it was found in a patient with type II disease. As would

be expected, the other mutation in this patient, viz. 1448C, is known to be severe.

In type I patients' classification of mutations by severity is not possible when the new mutation is inherited together with a mild or unidentified mutation; unfortunately, all of the other mutations we have described here are in this category. Both the 1226G and the 764A mutations are known to be mild, and even a severe or lethal mutation inherited with them results in type I disease. The 644A, 661A, 887A, 970T, 1249G, and 1255A mutations could all be mild or severe. Only if they were found together with a severe mutation would it be possible to make a definitive classification. If type II or III disease were found in such a patient they would be classified as severe; if such a patient had type I disease, they would be classified as mild.

### Population Genetics

The study of the population genetics of Gaucher disease is facilitated by polymorphic markers that encompass not only the *GBA* but also the adjacent *PKLR* gene (12). Although a considerable number of haplotypes exist (13), only two of these are common and are referred to as *Pv1.1*<sup>+</sup> and *Pv1.1*<sup>-</sup> (14). From the point of view of population genetics, the mutations that cause Gaucher disease may be considered to belong to one of two broad classes. There are those that reach polymorphic frequencies in the Jewish population, the 1226G, 84GG, *Ivs2*(+1), mutations and at somewhat lower frequencies, the 1297T, 1604A, 1504T mutations. Each of these mutations are always found in the context of a single haplotype (2) and it is a reasonable assumption that each of these mutations only arose once, and that the mutations were amplified in the Jewish population because of some as yet uni-



identified selective advantage. Such polymorphic mutations are superimposed upon a background of mutational noise consisting of sporadic mutations that have occurred panethnically. The only one of the panethnic mutations that is relatively common is that at nt 1448. It is significant that this mutation is found in both common glucocerebrosidase haplotypes, suggesting that its relatively high prevalence is not due to selection, but rather to a mutational hot spot, in this case based, in all likelihood, on the fact that the same sequence is present in the glucocerebrosidase pseudogene. An exception is the Norbottnian population of Northern Sweden, where the 1448C mutation has reached a very high frequency, presumably as the result of a founder effect (15).

### The New Mutations

In the present study we have identified 8 point mutations, in addition to the 36 that had already been described in the 7 years since the first such mutation was identified (16). The assumption that a cause-and-effect relationship exists between these mutations and the patients' Gaucher disease is a reasonable one, given that in most cases the entire coding region was examined for other mutations and none were found. It is to be expected that mutations found in such a survey will be rare, sporadic mutations and will be found predominantly in the non-Jewish population. This expectation has been largely borne out in the present study. Eight new mutations were found, and only two of these were detected in more than one unrelated person. Thus far there are too few cases of these mutations to be able to determine whether they, like the polymorphic mutations, had a common origin.

In the case of the 721A mutation, the other allele in two cases carried the common 1226G mutation. Pv1.1 haplotypes were not the same. Haplotype analysis showed that one patient with this mutation was Pv1.1<sup>-</sup>/Pv1.1<sup>-</sup> and the other Pv1.1<sup>+</sup>/Pv1.1<sup>-</sup>. This indicates that the new mutation was found in context of both Pv1.1<sup>-</sup> and Pv1.1<sup>+</sup> haplotypes, since the other allele carrying the 1226G is always in the Pv1.1<sup>-</sup> context (2,3,17). Thus it is reasonable to conclude that this mutation was the result of two independent mutational events. The mutant sequence matches the normal pseudogene sequence at this location; it could well be a mutational hot spot.

In contrast, the 644A mutation was found in context of the Pv1.1<sup>+</sup> haplotype in both cases in which it was detected. The first patient had the Pv1.1<sup>+</sup>/Pv1.1<sup>+</sup> genotype; the second patient a Pv1.1<sup>+</sup>/Pv1.1<sup>-</sup> genotype. The Pv1.1<sup>-</sup> allele is always associated with the 1226G mutation. This establishes that the 644A mutation was in the Pv1.1<sup>+</sup> context in the second patient. However, the population frequency of the Pv1.1<sup>+</sup> gene is 30% in European populations (12,14) and thus there is a 30% probability that, given a Pv1.1<sup>+</sup> haplotype for the first patient, the second mutation would be in the Pv1.1<sup>+</sup> context also.

Fifteen mutant alleles examined by SSCP in this series have not yet been identified. In a few cases there was insufficient DNA available to examine all exons. SSCP is a powerful but not infallible method for the detection of mutations. Complete sequencing of the coding region may reveal mutations missed by SSCP analysis.

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