

## Gaucher's disease in the United Kingdom: screening non-Jewish patients for the two common mutations

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### Abstract

**Twenty-six patients with Gaucher's disease diagnosed in the United Kingdom and two obligate carriers, all of non-Jewish origin, were screened for the two common disease causing mutations and two rarer mutations in the glucocerebrosidase gene. These mutations are referred to as N370S, L444P, Ins84G, and 1066 + 1G → A, respectively. The results showed that out of 54 alleles screened, 26% were N370S, 35% were L444P, and the remaining 39% were rare or undefined. The results also showed a clear correlation between the presence of at least one N370S allele and mild disease.**

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Gaucher's disease is the most common lysosomal storage disorder and is characterised by accumulation of the sphingolipid glucocerebroside (glucosyl ceramide). This is the result of a functional deficiency of the enzyme glucocerebrosidase<sup>1</sup> (D-glucosyl acylsphingosine glucohydrolase, E.C. 3.2.1.45) or, in rare cases, of the glucocerebrosidase activator protein saposin C.<sup>2</sup> Glucocerebrosidase is a glycoprotein of 58 to 66 kDa which catalyses the hydrolysis of glucocerebroside to ceramide and glucose.

The disease is inherited as an autosomal recessive trait, the gene for glucocerebrosidase mapping to chromosome 1q21.<sup>3</sup> Gaucher's disease is the most common genetic disorder among Ashkenazi Jews where the mutant gene frequency has been estimated as 0.047.<sup>4</sup> The mutant gene frequency in the general population in the United Kingdom has been reported as being 30 times lower than that among Ashkenazi Jews<sup>1</sup> which gives a frequency estimate of approximately 0.0016.

There are two clinical subtypes of Gaucher's disease: non-neuronopathic and neuronopathic. Non-neuronopathic, or type 1, disease is seen in patients from the first to the eighth decade of life and varies widely in severity. Primary manifestations of the disease are thought to be a consequence of the build up of glycolipid in macrophages; typically there is hepatosplenomegaly, secondary hepatic dysfunction, thrombocytopenia, and bone involvement including necrosis of the femoral head. Neuronopathic disease is further subdivided into two types: type 2, or infantile, and type 3, juvenile or Norrbottnian. Differing only in the age of onset, both types of neuronopathic disease are characterised by neurological symptoms such as psychomotor retardation,

seizures, and pyramidal tract and bulbar dysfunction in addition to the symptoms associated with type 1 disease.

Gaucher's disease is confirmed at a biochemical level by assay of glucocerebrosidase enzyme activity. This assay is carried out in leucocytes or fibroblasts and uses the fluorescent substrate 4-methylumbelliferyl-β-D-glucoside or radiolabelled glucocerebroside.<sup>1</sup> Typically, levels of enzyme activity that are well below those of both carrier and unaffected subjects are detected in patient's leucocytes and fibroblasts.

The wide degree of overlap of enzyme activities between Gaucher's disease carrier and unaffected populations renders carrier screening by enzyme activity impractical in most cases. Following the cloning and sequencing of the glucocerebrosidase gene and its associated pseudogene,<sup>5</sup> it is now possible to analyse mutant glucocerebrosidase genes at the DNA level. To date 30 independent disease associated mutations have been reported.<sup>6</sup> The majority of these are point mutations affecting the coding sequence but insertions, splice site mutations, and crossover events involving the pseudogene have also been reported.

In Ashkenazi Jews, four mutations account for 96% of all cases of Gaucher's disease. These are commonly known as 1226G (or 370),<sup>7</sup> 1448C (or *NciI*),<sup>8</sup> 84GG,<sup>9</sup> and IVS2 + 1.<sup>10</sup> In patients of non-Jewish origin, however, only the 1226G and 1448C mutations occur frequently. In accordance with notation in common usage for other genetic disorders these mutations will hereafter be referred to as N370S (1226G), L444P (1448C), Ins84G, and 1066 + 1G → A, respectively. One previous study on a small number of UK Gaucher's disease patients (five Jewish and seven non-Jewish),<sup>11</sup> detecting mutations by the amplification refractory mutation system (ARMS), also used this nomenclature.

We have examined the incidence of the two common non-Jewish mutations together with the Ins84G and 1066 + 1G → A mutations in a total of 54 disease associated alleles obtained from 26 non-Jewish patients with Gaucher's disease, both clinically and enzymatically proven, and two unrelated non-Jewish carriers all diagnosed in the United Kingdom and mainly of UK origin.

### Materials and methods

#### GENOMIC DNA

Genomic DNA was prepared by standard methods from blood samples or skin fibroblasts obtained from patients.<sup>12</sup>

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#### AMPLIFICATION AND PURIFICATION OF FUNCTIONAL GENE DNA SEQUENCES

Polymerase chain reaction (PCR) primers for amplification of exon 9 were GD9A (5'-CC-AGTGTGAGCCTTTGTC-3') and GD9B (5'-TCCGATGTAGGAGATGATAG-3'), for exon 10, GDMID9A (5'-GCGTAACTTT-GTCGACAGTC-3') and GD10B (5'-GAGGAGAAGCTGAGAGTGTG-3'), and for exon 2, GD2A (5'-CTGCCCAGGAGAG-TAGTTGAG-3') and GD2B (5'-CCCAGG-CAACAGAGTAAGAC-3'). Exon 9 PCR conditions were as follows: five minutes at 94°C, followed by 30 cycles of one minute at 94°C, one minute at 65°C, two minutes at 72°C, followed by a final five minutes at 72°C. Exon 10 PCR conditions were identical except that the annealing temperature was 63°C and not 65°C. Exon 2 conditions were similar to exon 9 except that the annealing temperature was 69°C and the extension time at 72°C was one minute.

The exon 9 PCR product was purified away from the pseudogene product by electrophoresis in a 2% (w/v) agarose gel followed by excision of the band and Gene Clean II (BIO 101, La Jolla, CA) purification. The GDMID9A PCR primer, for amplification of exon 10, was designed to anneal to part of a 55 base pair insert in exon 9 of the functional gene which is absent in the pseudogene and so eliminate the possibility of amplification of the pseudogene sequence. The specific PCR product was then purified by the Gene Clean II technique.

The exon 2 product was purified away from low levels of non-specific PCR products by electrophoresis in a 1.5% (w/v) agarose gel followed by excision of the band and Gene Clean II purification.

#### SCREENING FOR THE N370S MUTATION

This was achieved by direct sequencing of the PCR product<sup>13</sup> using only the C and T termination mixes and the reverse primer GDMID9B (5'-GACTGTCGACAAAGT-TACGC-3'). The reactions were analysed by denaturing polyacrylamide gel electrophoresis followed by autoradiography.

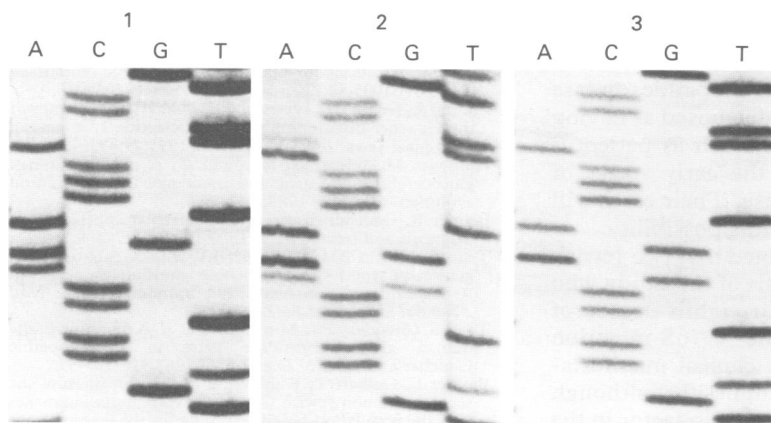


Figure 1 Direct PCR screening for the N370S mutation using the reverse primer GDMID9B (see text). (1) Unaffected control, (2) known Gaucher's disease N370S carrier, (3) Gaucher's disease patient homozygous for N370S. The sequence is read from the top as GTCCTTACCCTAGAA/GCCTCCTGTA where the bases shown in bold are the normal/mutant bases.

#### SCREENING FOR THE L444P MUTATION

The L444P mutation creates an *Nci*I site so the population was screened for this mutation by *Nci*I restriction enzyme digest of the exon 10 PCR product. The digested DNA was then analysed by electrophoresis on a 1.8% (w/v) agarose gel. If the mutation was present, the 658 base pair PCR product was digested to give two bands of 139 and 519 base pairs.

#### SCREENING FOR THE INS84G AND 1066 + 1G → A MUTATIONS

This was done by direct sequencing, as for the N370S mutation, using the GD2A primer and the G and A termination mixes only. Reactions were analysed by denaturing polyacrylamide electrophoresis followed by autoradiography.

Confirmation of the presence of a mutation was obtained in all cases by direct sequencing using all four termination mixes.

#### Results

Twenty-six patients and two unrelated carriers, constituting 54 alleles, were screened for N370S and L444P by the methods described. The presence of the N370S mutation as determined by sequence analysis is shown in fig 1. The mutation from A to G can be easily seen in panels 2 and 3. Fig 2 shows the result of the *Nci*I restriction enzyme digest used for screening for the L444P mutation. The 658 bp band is the full size PCR product while the 519 bp band is the restriction digest fragment. On this percentage of acrylamide gel the other product of the restriction enzyme digest is not visible. The screening results are detailed in the table. Out of 54 alleles screened, 14 were N370S, 19 were L444P, one was identified as a rare mutation (R463C, previously known as 1504),<sup>14</sup> one was identified as Ins84G, and the other 19 alleles remained undefined by these screening procedures.

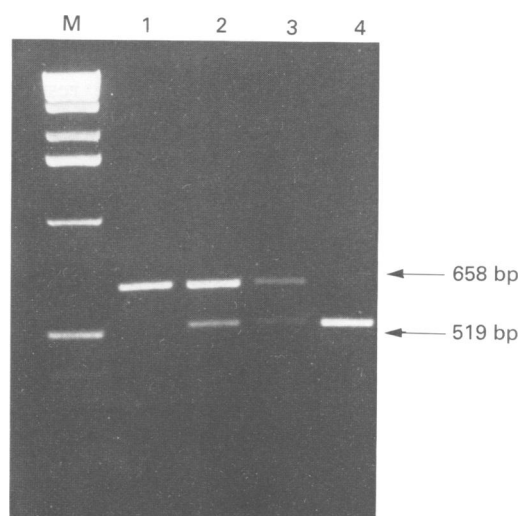


Figure 2 *Nci*I restriction enzyme digest screening for the L444P mutation. (M) Lambda DNA HindIII digest size markers, (1) unaffected control, (2) known Gaucher's disease carrier, (3) Gaucher's disease patient with L444P/? genotype, (4) Gaucher's disease patient homozygous for L444P.

## Patient screening data.

Clinical diagnosis	Patient	Enzyme activity (nmol/h/mg protein)	N370S	L444P	Other
Type 1 (Severe) (? Type 3)	1	1.90 *L	-	-	-
	2	1.00 *L	+	+	-
	3	1.50 *L	+	+	-
	4	0.70 *L	+	+	-
	5	NA	-	+	+
	6	1.70 *L	+	-	-
	7	2.20 *L	-	+ / +	-
	8	0.58 *L	+	-	-
	9	NA	-	-	-
	10	NA	+	-	-
	11	0.65 *L	+	-	-
	12	25.8 *F	+	-	-
	13	2.90 *L	-	-	-
	14	4.20 †F	+ / +	-	-
	15	7.20 †F	+	+	-
	16	1.20 †F	+	+	-
	Type 2	17	1.02 *L	+	+
18		NA	+	-	-
19		110.0 *F	-	+	-
20		70.0 *F	-	+	+
21		16.0 *F	-	+	-
22		6.00 †F	-	+	-
23		45.9 †F	-	+ / +	-
Type 3	24	59.4 *F	-	+ / +	-
	25	68.0 *F	-	-	-
	26	12.00 †F	-	+ / +	-
Carriers	27	NA	-	-	-
	28	NA	-	-	-
Allele frequencies		N370S	0.26	0.35	0.04
		L444P			
		Other			
		[Undefined		0.35]	

- indicates allele not present, + indicates allele present in heterozygous form, +/+ indicates allele present in homozygous form. Other mutations were: patient 5 = R463C, patient 20 = Ins84G.

## Enzyme activity.

\*Determined at Guy's Hospital, London. Normal ranges were: 8.4-32.8 nmol/h/mg protein in leucocytes (L), 350-1100 nmol/h/mg protein in fibroblasts (F).

†Determined at Royal Hospital For Sick Children, Edinburgh. Normal ranges were: 180-420 nmol/h/mg protein in fibroblasts (F). NA = not available.

## Discussion

The table is essentially similar to data presented elsewhere in a recent report on the incidence of Gaucher's disease associated alleles in a North American non-Jewish population.<sup>6</sup> The N370S and L444P mutant alleles make up just less than two-thirds of the mutant alleles and the rest consist of rare and undefined mutations. From the data the correlation between mutation and clinical type of disease is striking. In all cases where at least one copy of the N370S mutation is present, the clinical diagnosis is invariably non-neuronopathic, type 1 disease. In the absence of the N370S allele, the L444P mutation correlates with severe disease of type 1, 2, or 3 and patients 23, 24, and 26 who are homozygous for the L444P mutation have neuronopathic disease. These results support previous reports that the L444P mutation is associated predominantly with type 2 or 3 disease.<sup>15,16</sup> It is possible that the L444P mutation is exclusively associated with neuronopathic disease and the patients presently diagnosed as having non-neuronopathic disease, such as patient 7 in our study, may be in the early stages of neuronopathic type 3 disease. Their cases will be followed to investigate this possibility.

However, it should be noted that two Jewish patients also screened as part of this study who are identical twins, in their eighth decade of life, are homozygous for the N370S mutation but only one of them has clinical manifestations of the disease. This implies that although genotype is undoubtedly the main factor in the development of Gaucher's disease there appears to be a contributory role for non-genetic factors.

Having established the frequency of com-

mon mutations in our population we are now investigating the undefined disease associated mutations.

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