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Glucocerebrosidase Genotype of Gaucher Patients in The Netherlands: Limitations in Prognostic Value

Rolf G. Boot,[1] Carla E. M. Hollak,[2] Marri Verhoek,[1] Paul Sloof,[1] Ben J.H.M. Poorthuis,[3] Wim J. Kleijer,[4] Ron A. Wevers,[5] Marinus H.J. van Oers,[2] Marcel M.A.M. Mannens,[6] Johannes M.F.G. Aerts,[1*] and Sonja van Weely[1]

[1]*Department of Biochemistry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands*
[2]*Department of Internal Medicine and Hematology, Academic Medical Center, Amsterdam, The Netherlands*
[3]*Clinical Genetics Center, University of Leiden, Leiden, The Netherlands*
[4]*Department of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands*
[5]*Institute of Neurology, University Hospital Nijmegen, Nijmegen, The Netherlands*
[6]*Department of Clinical Genetics, Institute for Anthropogenetics, Academic Medical Center, Amsterdam, The Netherlands*

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Gaucher disease is a recessively inherited lysosomal storage disorder that is caused by a deficiency in glucocerebrosidase activity. The clinical expression is markedly heterogeneous with respect to age of onset, progression, severity, and neurological involvement. The relative incidence of glucocerebrosidase (GC) mutations has been studied extensively for Jewish but not for non-Jewish Caucasian patient populations. The present survey on mutant GC genotypes prevalent in Gaucher disease in The Netherlands was taken of 72 patients from different genetic backgrounds. This number is more than half the total number of affected Gaucher patients to be expected on the basis of the incidence of the disorder in this country. Analysis of nine GC mutations led to the identification of 74% of the mutant GC alleles in patients from 44 unrelated Dutch families (i.e., families that have lived in The Netherlands for at least several generations) and of 44% of the mutant GC alleles in patients from nine unrelated families that recently immigrated from both European and non-European countries. The N370S (cDNA 1226G) GC mutation proved to occur most frequently (41%) in the unrelated Dutch patients and less frequently (6%) in the unrelated immigrant patients and was always associated with the nonneuronopathic (Type 1) form of the disease. Apart from the association of the N370S mutation with Type 1 Gaucher disease, the prognostic value of GC genotyping was limited, since a particular GC genotype did not correlate closely to a specific clinical course, or to a specific relative responsiveness to enzyme-supplementation therapy. Hum Mutat 10:348–358, 1997.

KEY WORDS: glucocerebrosidase; Gaucher disease; glucosylceramide lipidosis; mutation analysis; lysosomal storage disorder

INTRODUCTION: ANALYSING MUTATIONS IN GAUCHER PATIENTS

Gaucher disease is one of the most frequent lysosomal storage disorders in humans. The disease is due to an autosomal recessively inherited deficiency in lysosomal glucocerebrosidase activity (E.C. 3.2.1.45), resulting in accumulation of its substrate glucocerebroside (also named glucosylceramide) in macrophages (Beutler and Grabowski, 1995). Three phenotypes are distinguished on the basis of onset of neurological symptoms: Type 1, the "adult," or nonneuronopathic, form, Type 2, the "infantile," or acute neuronopathic, form, and Type 3, the "juvenile," or subacute neuronopathic, form. Type 1 Gaucher disease is the most prevalent phenotype that varies considerably in age of onset, progression, and severity of clinical manifestation. Common features are hepatosplenomegaly, anemia, thrombocytopenia, and skeletal deterioration (Barranger and Ginns, 1989; Beutler and Grabowski, 1995).

The defective glucocerebrosidase (GC) gene is located at chromosome 1q21 (Barneveld et al., 1983;

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*Correspondence to: J.M.F.G. Aerts, Dept. of Biochemistry, Academic Medical Center, University of Amsterdam, P.O. Box 22700 1100DE Amsterdam, The Netherlands.

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Ginns et al., 1985). A 95% homologous pseudogene is located at the same locus on chromosome 1 (Horowitz et al., 1989). Cloning of the GC gene has allowed the identification of the precise nature of mutations underlying the enzyme deficiency in patients with Gaucher disease. More than 50 GC mutations have been reported (for recent reviews on this topic, see Beutler et al., 1994, 1995; Horowitz and Zimran, 1994). However, it should be realised that for some of the abnormalities in the GC gene, it has not been experimentally proven that they are not polymorphisms, but actually lead to a deficiency of glucocerebrosidase.

In some populations a relatively small number of GC mutations accounts for the majority of the defects in GC alleles of Gaucher patients. In Jewish and non-Jewish Caucasian populations, the N370S (cDNA 1226G) GC mutation is the most prevalent (Amaral et al., 1993; Horowitz and Zimran, 1994; Lacerda et al., 1994a), causing synthesis of a catalytically abnormal enzyme (Grace et al., 1990; Ohashi et al., 1991; van Weely et al., 1993). This mutation has so far not been encountered in the Japanese population (Ida et al., 1995). The L444P (cDNA 1448C) GC mutation is relatively frequent in the Jewish and non-Jewish Caucasian populations and in Japanese populations (Horowitz and Zimran, 1994; Ida et al., 1995) and leads to synthesis of glucocerebrosidase, which is rapidly degraded (Grace et al., 1991; Ohashi et al., 1991; Grace et al., 1994). Another frequent GC mutation in Ashkenazi Jewish populations is the so-called 84GG insertion (cDNA 84GG), resulting in synthesis of a truncated enzyme due to the generation of a frame shift (Beutler et al., 1991). This mutation has not been found in the non-Jewish Caucasian or Japanese populations (Amaral et al., 1994; Horowitz and Zimran, 1994; Ida et al., 1995).

Over the last few years much attention has been given to the analysis of GC mutations in Gaucher patients for a variety of reasons. First, accurate identification of Gaucher disease carriers is not feasible on the basis of residual enzyme activity in extracts of blood cells or cultured fibroblasts (Daniels and Glew, 1982; Wenger and Roth, 1982). In principle, genotyping should allow reliable identification of carriers of (known) mutant GC alleles, and knowledge about the relative frequencies of GC mutations in Gaucher patients should facilitate genetic counseling.

Second, it has been attempted to establish a correlation between GC genotype and severity of Gaucher disease manifestation (see, e.g., Beutler, 1993). Unfortunately, researchers disagree about the prognostic value of GC genotyping for clinical expression of the disorder (see, e.g., Sibille et al., 1993; Sidransky and Ginns, 1994). Although the experience of most investigators concerning the results of genotyping may not in fact be that different, some prefer to stress the actual clinical variability within each GC genotype, whereas others prefer a statistical evaluation of the genotype results. However, consensus does exist about the important fact that hetero-allelic or homo-allelic presence of the N370S GC mutation is not associated with a neuronopathic course of the disease (with the exception of one child) (McCabe et al., 1996).

Third, it has been suggested that GC genotypes of Gaucher patients could be predictive for their response to enzyme-supplementation therapy and, moreover, would allow recommendations to be made for the type of dosing regimen to be used. However, so far, GC genotyping has not rendered such guidelines (Zimran et al., 1994).

Some literature data on GC genotypes of Gaucher patients should be carefully interpreted. In general, proportionally more material from severe Gaucher disease cases (neuronopathic phenotypes, or severe nonneuronopathic phenotypes) has been available for mutation analysis. Thus it cannot be excluded that particular mutations have been over-represented. Furthermore, the most impressive data with respect to GC genotypes of Gaucher patients particularly concern Jewish populations, in which Type 1 Gaucher disease occurs relatively frequently (Barranger and Ginns, 1989, Beutler and Grabowski, 1995).

As in many other countries, Gaucher disease is a rare disorder in The Netherlands. Nevertheless, clinicians and fundamental researchers have long been interested in it so that a relatively accurate estimation can be made for the local incidence of the clinically manifest disorder, being 100–150 patients in the total population of 15,000,000. Both the existence of a national patient society and the collaboration between various clinical genetic centers involved in the biochemical diagnosis of Gaucher disease allowed us to determine GC genotypes for a high percentage (50–70%) of all Gaucher patients in The Netherlands.

Here, we document the frequency of various GC mutations in families with incidence of Gaucher disease that have lived in The Netherlands for several generations and in families that have recently immigrated to The Netherlands. We discuss the prognostic value of the GC genotypes with respect to the phenotypic manifestation and to the response to enzyme-supplementation therapy.

MATERIALS AND METHODS

Gaucher Patients

A diagnosis of Gaucher disease was made in 72 patients from 53 families living in The Netherlands.

The presence of Gaucher disease was either assumed on the basis of characteristic clinical symptoms and/or the presence of Gaucher cells in bone marrow or liver biopsies, or was investigated because of an affected sibling. The clinical diagnosis was biochemically confirmed by the demonstration of a markedly reduced glucocerebrosidase activity in leukocytes and/or urine samples and/or cultured skin fibroblasts (Daniels and Glew, 1982; Aerts et al., 1991).

Patients were classified as Type 1, 2, or 3 according to the criteria described by Barranger and Ginns (1989). Two patients showed an unusual manifestation of the disease. Patient No. 56 (see Table 2) developed characteristic clinical signs of Gaucher disease at a very young age. At the juvenile age, he started to suffer from neurological symptoms that were not identified as signs characteristic for Type 3 Gaucher disease. He died at the age of 21 years due to severe pulmonary involvement. Patient No. 57 so far developed no visceral manifestations of Gaucher disease. At the age of 42, neurological abnormalities resembling Parkinsonian disease developed. Although the glucocerebrosidase activities in blood cells and cultured skin fibroblasts of this patient were markedly reduced, it cannot be excluded that the clinical complications have an unrelated cause.

Only a few of the examined patients were known to have Ashkenazi Jewish ancestry. Such an ethnic background can, however, not be excluded with certainty since there have been various episodes of Jewish immigration in the Low Countries. In our study we distinguish families that have lived in The Netherlands for several generations(so-called native families) and families that have recently emigrated from European and non-European countries to The Netherlands (so-called immigrant patients).

We have also analysed the GC genotype in samples from several unrelated Type 1 Gaucher patients that had been sent to us by other laboratories in Europe. These samples came from Czech Republic (n=4), France (n=5), Germany (n=20), Belgium (n=1), and Portugal (n=1).

Analysis of GC Mutations

Genomic DNA was isolated from leukocytes and/or cultured skin fibroblasts. The analyses of nine GC mutations were performed by restriction-enzyme digestions upon polymerase chain reaction (PCR) amplification of genomic DNA (for details, see Table 1). The primers were manufactured by Pharmacia (Uppsala, Sweden) and chosen in such a way that the pseudogene was not amplified. Restriction-enzymes BsaBI, HphI, StyI, NciI, and BanI were from New England Biolabs (Beverly, MA) and XhoI, MspI, and HincII from Pharmacia. The restriction-enzyme reactions were performed according to the instructions of the manufacturers. The resulting fragments were analysed on polyacrylamide gels or agarose gels as described by Sambrook et al. (1989).

The mutations resulting in the amino acid substitutions N370S and V394L (cDNA 1297T) were analysed using mismatch PCR, as described by Beutler et al. (1990) for the N370S mutation and by Beutler et al. (1993a) for the V394L mutation, with modifications in the primers used. Also, the insertion mutation 84GG was analysed using mismatch PCR, as described by Beutler et al. (1991).

The L444P, D409H, (cDNA 1342C), and R463C (cDNA 1504T) mutations were analysed using the same upstream and downstream primers (see Table 1). The products were digested with different restriction enzymes.

We did not investigate the presence of the R456G (cDNA 1483C) and V460V (cDNA 1497C) mutations, which are present in the complex alleles RecNcil and RecTL due to recombinations with the pseudogene (for a review see Horowitz and Zimran, 1994). Thus we cannot exclude that in some cases the L444P mutation was in fact a RecNcil mutation.

Southern Blot Analysis

Genomic DNA was isolated and digested with restriction enzyme SspI (New England Biolabs) according to a previously described procedure (Zimran et al., 1990). After separation of the fragments on a 0.5% agarose gel and transfer to Hybond (Amersham, Buckinghamshire, UK), hybridisation was performed with radioactively labelled full-length glucocerebrosidase cDNA.

RESULTS

Incidence of Glucocerebrosidase Genotypes of Gaucher patients

We analysed nine GC mutations, i.e., the N370S, L444P, 84GG, IVS2+1 (genomic DNA 1067A), V394L, D409H, R463C, R496H (cDNA 1604A), and a 55-bp deletion at cDNA position 1263-1317, in 72 Gaucher patients who live or have lived in The Netherlands. An overview of the GC genotypes of the patients is presented in Table 2, including their country of origin. The relative incidence of GC mutations in unrelated Gaucher patients is shown in Figure 1.

The most common mutation in the so-called native Type 1 Gaucher patients, whose families have lived in The Netherlands for several generations, proved to be the N370S GC mutation. In fact, > 90% of these patients carried the N370S GC muta-

TABLE 1. Analysis of Nine GC Mutations in Gaucher Patients Living in The Netherlands[a]

Mutation									
Name	cDNA	Sequence upstream primer	Genomic sequence	Sequence downstream primer	Genomic sequence	Restriction enzyme used	Control fragments (bp)[b]	Mutant fragments (bp)[b]	Reference of primers used
N370S	1226G	5′ CTTTGCCTTTGTCCTTACCCTcGA 3′	5817-5840	5′ GTTACGCACCCAATTGGGTCCTCC 3′	5897-5920	XhoI	104	84, 20	Beutler et al., 1990; this study
V394L	1297T	5′ GGACCGACTGGAACCTTGCCC 3′	5865-5885	5′ GACTGTCGACAAAGTTAgGC 3′	5914-5933	BanI	47, 22	69	Beutler et al., 1993a
D409H	1342C	5′ GGAGGACCCAATTGGGTGCGTAAC 3′	5897-5920	5′ GAGGCACATCCTTAGAGGAGCTAGGG 3′	6579-6604	StyI	396, 156 63, 56, 37	396, 153, 93, 63	This study
L444P	1448C	5′ GGAGGACCCAATTGGGTGCGTAAC 3′	5897-5920	5′ GAGGCACATCCTTAGAGGAGCTAGGG 3′	6579-6604	NciI	708	536, 172	This study
R463C	1504T	5′ GGAGGACCCAATTGGGTGCGTAAC 3′	5897-5920	5′ GAGGCACATCCTTAGAGGAGCTAGGG 3′	6579-6604	MspI	592, 116	708	This study
R496H	1604A	5′ GCTCTGCTGTTGTGGTCGTG 3′	6463-6482	5′ GCCCAGTGCCTCCTTGAGTA 3′	6700-6719	HphI	124, 81, 40, 12	124, 46, 40, 35, 12	Beutler et al., 1993b
84GG	84GG	5′ GAATGTCCCAAGCCTTTGA 3′	979-997	5′ CACTGCCTGAAGTAGAtGC 3′	1035-1053	BsaBI	75	57, 18	Beutler et al., 1991
IVS2+1	—	5′ GAATGTCCCAAGCCTTTGA 3′	979-997	5′ AGCTGAAGCAAGAGAATCG 3′	1317-1335	HphI	141, 116 100	241, 116	This study
55del	1263-1317del	5′ TGTGTGCAAGGTCCAGGATCAGTTGC 3′	5177-5202	5′ GAGGCACATCCTTAGAGGAGCTAGGG 3′	6579-6604	HincII	750, 678	1373	This study

[a]Genomic DNA was amplified using the PCR technique and products were digested with several restriction enzymes as indicated. Numbering of sequences of the primers corresponds to the genomic sequence numbering according to Horowitz et al. (1989). Sequences were derived from Horowitz et al. (1989) with corrections described by Beutler et al. (1992). Mismatch nucleotides are given in lower case.

TABLE 2. GC Genotypes of Individual Patients Living in The Netherlands[a]

A. Native Gaucher patients of families living in The Netherlands for several generations

Patient no.	Family no.	Genotype
Type 1 patients		
1	1	N370S/?
2	2	N370S/L444P
3	3	N370S/IVS2 + 1
4	4	N370S/84GG
5	5	N370S/L444P
6	5	N370S/L444P
7	5	N370S/L444P
8	5	N370S/L444P
9	5	N370S/L444P
10	6	N370S/?
11	6	N370S/?
12	6	N370S/?
13	7	N370S/N370S
14	7	N370S/N370S
15	8	N370S/L444P
16	9	N370S/L444P
17	10	N370S/IVS2+1
18	10	N370S/IVS2+1
19	11	N370S/?
20	12	N370S/?
21	13	N370S/L444P
22	14	N370S/?
23	14	N370S/?
24	15	N370S/L444P
25	15	N370S/L444P
26	15	N370S/L444P
27	16	N370S/?
28	17	N370S/?
29	18	N370S/?
30	19	N370S/?
31	20	?/?
32	21	N370S/84GG
33	22	N370S/?
34	23	N370S/L444P
35	23	N370S/L444P
36	23	N370S/L444P
37	24	?/?
38	25	?/?
39	26	N370S/L444P
40	27	N370S/L444P
41	27	N370S/L444P
42	28	N370S/?
43	29	N370S/?
44	30	N370S/L444P
45	31	N370S/L444P
46	32	N370S/?
47	33	N370S/L444P
48	33	N370S/L444P
49	34	N370S/L444P
50	35	N370S/L444P
51	36	N370S/?
52	37	N370S/L444P
53	38	N370S/L444P
54	38	N370S/L444P
55	38	N370S/L444P
Adult patients with unusual clinical presentation		
56	39	L444P/L444P
57	40	?/?
Type 2 and 3 patients		
58	41	L444P/L444P
59	41	L444P/L444P
60	42	L444P/L444P
61	43	L444P/L444P
62	44	L444P/?[b]

B. Gaucher patients of families recently immigrated to The Netherlands

Patient no.	Family no.	Genotype	Country ancestry
Type 1 patients			
63	45	R463C/?	Surinam
64	46	R463C/?	Great Britain
65	47	N370S/?	Former Yugoslavia
66	48	?/?	Morocco
67	48	?/?	Morocco
Type 2 and 3 patients			
68	49	L444P/L444P	Antilles
69	50	L444P/L444P	Morocco
70	51	L444P/?	Poland
71	52	?/?	Poland
72	53	?/?	Turkey

[a]Genomic DNA from leukocytes or cultured fibroblasts was isolated and the presence of nine GC mutations was analysed using restriction enzyme analyses of PCR products as described in Materials and Methods. The mutations analysed were the N370S (cDNA 1226G), L444P (cDNA 1448C), 84GG (cDNA 84GG), IVS2+1 (genomic DNA 1067A), V394L (cDNA 1297T), D409H (cDNA 1342C), R463C (cDNA 1504T), R496H (cDNA 1604A), and a 55 bp deletion at cDNA position 1263–1317. In some cases, the L444P mutation might be a RecNcil mutation (see Materials and Methods). Patients No. 62 and Nos. 70–72 were diagnosed as presumably Type 2 Gaucher patients; patients Nos. 58–61, 68, and 69 as presumably Type 3 Gaucher patients.

[b]Patient identified on the basis of DNA analysis of parents and relatives.

?Mutation unknown (no. N370S, L444P, 84GG, IVS2+1, V394L, D409H, R463C, R496H, or a 55-bp deletion at cDNA-position 1263–1317).

tion at least in the hetero-allelic form (Table 2A). The second most common mutation in these patients was the L444P GC mutation, which occurred, however, only in the hetero-allelic form, except for one case (Patient No. 56). All the other mutations tested were not or were rarely detected in these patients. The two unrelated Type 1 patients showing the 84GG insertion (Patients No. 4, No. 32) had Ashkenazi Jewish ancestors, substantiating the claim that this mutation is exclusively found within this ethnic group (Beutler et al., 1991).

In the neuronopathic Type 2 as well as Type 3 Gaucher patients, homozygosity for the L444P mutation is commonly encountered, and this was also the case for the native Types 2 and 3 Gaucher patients. None of the investigated neuronopathic patients carried the N370S GC mutation.

The analysis of four GC mutations (N370S,

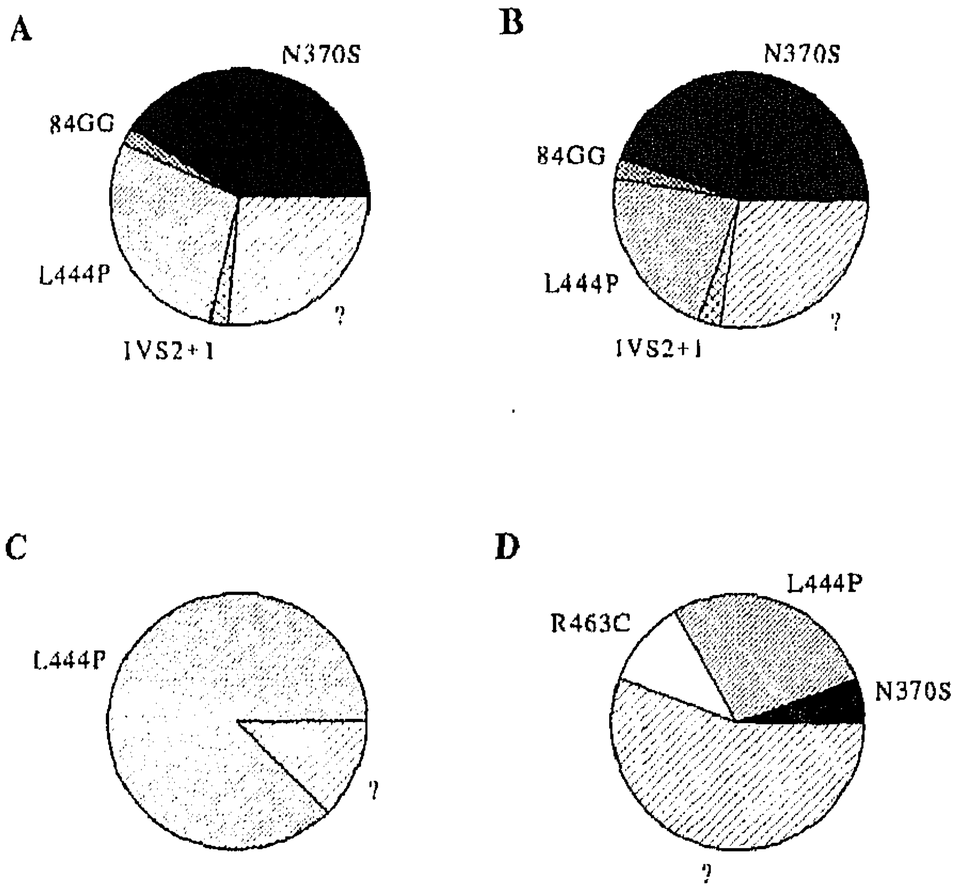

FIGURE 1. Relative frequencies of GC mutations in unrelated Gaucher patients in The Netherlands. **A.** Native Gaucher patients; **B.** native Type 1 Gaucher patients; **C.** native Types 2 and 3 Gaucher patients; **D.** immigrant Gaucher patients. The isolation of genomic DNA and analyses of nine mutations are described in the legend to Table 1.

L444P, 84GG, and IVS2+1) led to the identification of 79% of the mutant GC alleles of all native Dutch Gaucher patients.

The relative incidence of GC mutations in the investigated Gaucher patients who had recently immigrated from both European and non-European countries was different (Table 2B). Most importantly, the N370S GC mutation occurred less frequently, i.e., in 20% of the immigrant Type 1 Gaucher patients (cf. Fig. 1D). However, the small number of immigrant Gaucher patients, their diverse ethnic background, and the fact that in some cases the parents were consanguineous do not allow a statement about the statistical significance of the difference in frequency of mutant alleles among native and immigrant Gaucher patients.

In Table 3, a comparison is made of the incidence of the GC genotypes of native Type 1 Gaucher patients in The Netherlands with those of patients in other populations. It can be concluded that the present findings for the Dutch Type 1 Gaucher patients resemble those reported for patients living in other European countries (including Type 1 Gaucher patients in France) (Caillaud et al., 1995), as well as our own data for 34 patients living in other European countries. Furthermore, Table 3 shows that among Jewish Type 1 Gaucher patients, the N370S GC mutation is particularly prevalent: > 65% of the GC alleles carried a N370S mutation. It was initially reported that among non-Jewish Gaucher patients, the N370S GC mutation is far less prevalent, constituting only 23% of the total mutant GC alleles (Horowitz et al., 1993). However, more recent data indicate that the difference in frequency of this mutation among Jewish and non-Jewish Caucasian Type 1 Gaucher patients is less spectacular. Among the Type 1 Gaucher patients in the European countries (summarised in Table 3), the N370S mutation was present in 47% of the mutated GC alleles.

Value of GC Genotype With Respect to Manifestation of Gaucher Disease

Correlation between GC genotype and severity of Type 1 Gaucher disease

A score taking into account the severity of a variety of clinical symptoms is commonly used to assess the severity of disease manifestation in Gaucher patients (Zimran et al., 1992). The severity scoring index (SSI) was calculated for 41 Type 1 Gaucher patients. No significant differences in disease severity were noted between male and female Gaucher patients with similar genotypes. It can be seen in Figure 2 that the SSI did not correlate with specific GC genotypes. Moreover, differences in SSI were observed between siblings carrying the same GC mutations. Patients 5–9 from family 5 (Table 2A), e.g., had an SSI of 7, 5, 12, 5, and 8, respectively, and patients 24–26 from family 15 showed an SSI of 16, 5, and 3, respectively. When GC genotypes of patients were related to individual parameters of disease expression, such as splenomegaly, hepatomegaly, hematological symptoms, or bone deterioration, a comparable variability was noted (not shown).

In our experience, homozygosity for the L444P GC mutation was not always absolutely linked to a specific clinical course. Patient No. 56 (cf. Table 2A), who developed lethal pulmonary complications due to excessive infiltration of the lungs by storage cells, was found to be homozygous for the L444P GC mutation. At the age of death (21 years), no neurological abnormalities characteristic for Type 3 Gaucher disease had yet developed. Table 3 shows that this mutant genotype was present in several Type 1 Gaucher patients from European and non-European countries.

Patient No. 57 (cf. Table 2A) showed an unusual clinical presentation of Gaucher disease. He developed Parkinson-like neurological symptoms and no visceral Gaucher disease symptoms. The genotype of this patient is still under investigation, since he carried none of the nine GC mutations tested for.

TABLE 3. Comparison of Incidence of GC Genotypes of Native Dutch Type 1 Gaucher Patients With Literature Data

Origin	No. of unrelated patients[a]	Genotype												References[c]
		N370S/ N370S	N370S/ L444P	N370S/ complex[b]	N370S/ 84GG	N370S/ IVS2+1	N370S/ R463C	N370S/ ?	L444P/ L444P	L444P/ D409H	L444P/ ?	D409H/ ?	?/ ?	
The Netherlands	40	1	16		2	2	0	14	1	0	0	0	4	This study
Several European countries[d]	34	5	7		1	2	1	11	1	1	1	1	2	This study
Spain	26	6	10	1	0	0	0	8	0	0	0	1	0	e
Portugal	23	5	4	2		1	2	6	0		1		2	f,g
Italy	48	6	15	3			0	14	1	0	4		3	h,i
Greece	6	0	3		0	0	1	2	0	0	0	0	0	j
Great-Britain	21	1	6		0	0	0	6	1		0		4	k,l
Europe	198	24	61	6	3	5	4	61	4	1	6	2	15	
Non-Jewish (USA and Israel)	102	10	22	9	0	0	1	25	7	0	4	0	4	m,n,o
Jewish (USA and Israel)	545	245	48	10	118	17[†]	0	41[†]	0	0	0	0	2[†]	m–p
Austral(as)ian	18	2	2	1	2		0	5	3		1		1	q,r
Japan	20	0	0		0	0	0	0	6	0	4	0	6	s

[a]Not indicated for the non-Jewish patients noted in Refs. i, l, m, o.

[b]RecNciI or RecTL mutation.

[c]In this overview, the genotypes of 40 unrelated Dutch Gaucher patients and 34 Gaucher patients from other European countries are indicated. In all studies, the N370S and L444P mutations were analysed. The other mutations analysed were: This study: D409H, R463C, V394L, 84GG, IVS2+1, R496H, 55bp deletion; (e) Cormand et al., (1995): D409H, R463C, 84GG, IVS2+1, RecTL and RecNciI; (f) Amaral et al. (1994): 17 mutations; (g) Sá Miranda et al. (1995): exons 5, 9, 10, R463C, IVS2+1, RecNciI, P415R, R120Q; (h) Tuteja et al. (1993): exons 9 and 10; (i) Filocamo et al. (1995): exons 8–11; (j) Michelakakis et al. (1995): D409H, R463C, 84GG, IVS2+1, R496H, 55bp deletion; (k) Mistry et al. (1992): R463C, 84GG, P415R, R120Q; (l) Walley et al. (1993): R463C, 84GG, IVS2+1; (m) He and Grabowski (1992): 84GG, IVS2+1; (n) Horowitz et al. (1993): D409H, R463C, R496H, 84GG, IVS2+1, RecTL and RecNciI; (o) Beutler and Gelbart (1993): 27 mutations; (p) Sibille et al. (1993): 84GG, IVS2+1; (q) Lewis et al. (1994): R463C, 84GG; (r) Nelson et al. (1995): RecNciI and RecTL; (s) Ida et al. (1995): D409H, R463C, 84GG, IVS2+1, F213I. Several rare GC genotypes comprising one or a few Type 1 Gaucher patients are not included here; see Refs. i, k–o, q, s, and this study (R463C/?, Table 2B).

[d]Czech Republic (n=4), France (n=5), Germany (n=20), Belgium (n=1), Turkey (n=1), Portugal (n=1), Great Britain (n=1) and former Yugoslavia (n=1).

[†]The minimal number of patients with the particular genotype is indicated (in Ref. p, it was noted that 48 patients had genotype N370S/IVS2+1, N370S/? or ?/?).

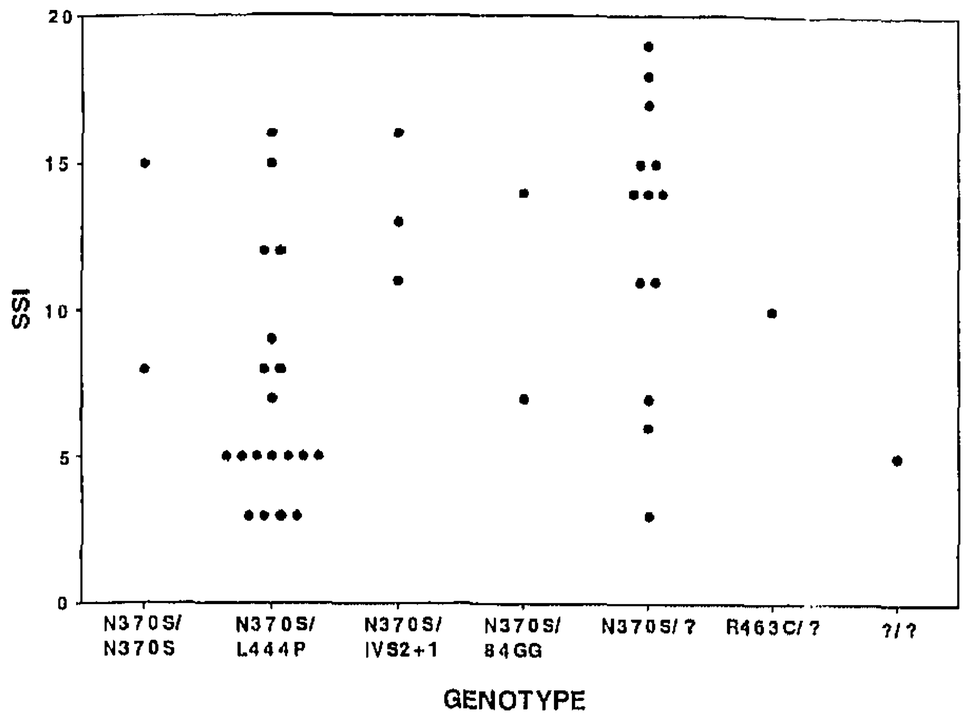

FIGURE 2. Correlation between GC genotypes of 41 Type 1 Gaucher patients in The Netherlands and the severity scoring index. The isolation of genomic DNA and analyses of nine mutations are described in the legend to Table 1. The severity scoring index (SSI) was determined using the criteria described by Zimran et al. (1992).

Asymptomatic individuals

During our studies we noted in at least three unrelated families the occurrence of individuals with low glucocerebrosidase activities compared with those in control subjects having no clear clinical symptoms related to Gaucher disease, despite the fact that they were older than 65 years. An example is presented in Figure 3, showing the GC genotypes of members of a family (family 5 in Table 2) with Type 1 Gaucher disease. In the second generation, five out of nine siblings were heterogeneously affected patients. All five patients were compounds for the L444P and N370S GC mutations. Interestingly, the asymptomatic, 80-year-old mother proved to be homozygous for the N370S mutation.

Prognostic Value of GC Genotypes of Type 1 Gaucher Patients With Respect to Response to Enzyme-supplementation Therapy

In The Netherlands, 34 Type 1 Gaucher patients are presently treated by enzyme-supplementation therapy, i.e., chronic intravenous supplementation with a modified glucocerebrosidase (alglucerase; marketed by Genzyme, Boston, MA as Ceredase). All patients participate in a co-ordinated study that aims to establish the minimal dose required for a satisfactory clinical response for each individual (Hollak et al., 1995). Briefly, patients initially receive a low dose of enzyme (1.15 IU/kg body weight) three times a week. On the basis of defined response criteria, the dose is adjusted every half year (doubling, maintenance, or tapering). As previously described (Hollak et al., 1995), the response to enzyme-supplementation therapy is variable among the patients. No relationship between the various GC genotypes and responsiveness to therapeutic intervention was noted (data not shown), as also described by Zimran et al. (1994).

DISCUSSION

The GC genotypes of Gaucher patients in The Netherlands have been analysed in this study. Because of the large number of analysed patients (n=72 from 53 unrelated families), the data are probably representative for this population.

The data obtained in The Netherlands are consistent with findings (made by several researchers and ourselves) for Gaucher patient populations living in other countries (Table 3). The most commonly encountered mutation is the N370S GC mutation, which in the homo- and hetero-allelic form is associated with a nonneuronopathic clinical course. Another common defect is the L444P GC mutation. A large proportion of the native Dutch Type 1 Gaucher patients carries both the N370S and the L444P GC mutations. Homozygosity for the L444P GC mutation was noted for Type 2 as well as for Type 3 Gaucher patients and for one adult patient with an unusual manifestation of Gaucher disease. The observed distribution of homozygotes and heterozygotes for the L444P GC mutation is almost exactly identical to that predicted by the Hardy-Weinberg equilibrium.

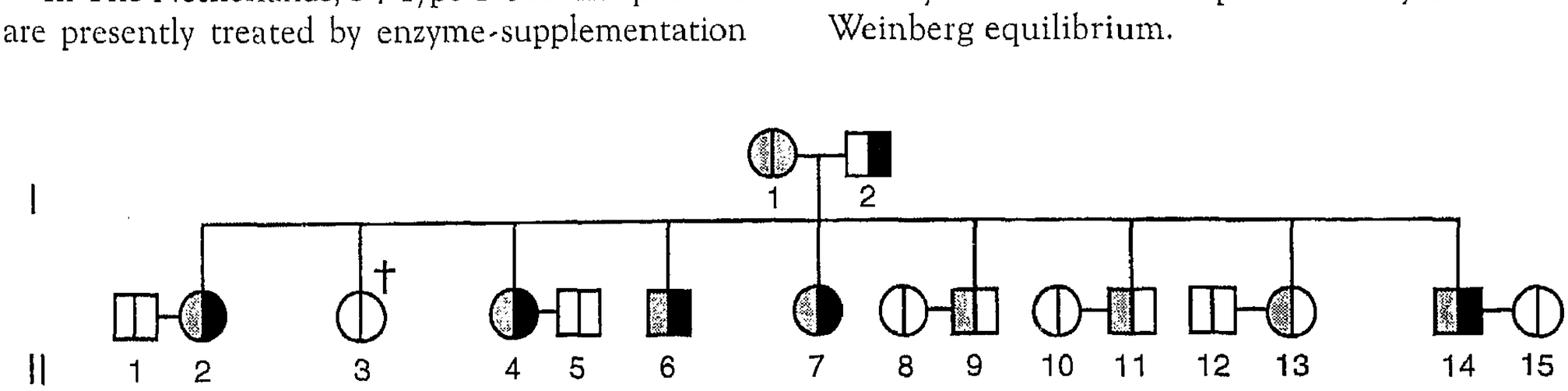

FIGURE 3. GC genotypes in a Dutch family with incidence of Type 1 Gaucher disease. It was noted that the glucocerebrosidase activity in urine samples and leukocyte extracts from the mother (I.1) was not higher than that in the affected siblings. The SSI of the symptomatic Gaucher patients was as follows: 7 (II.2), 8 (II.4), 5 (II.6) 12 (II.7), and 5 (II.14). A N370S mutated allele is indicated in grey, a L444P mutated allele in black and a wild-type allele in white.

The most important conclusion to be drawn from our investigation is that the (hetero-allelic) presence of the N370S GC allele allows prediction of a nonneuronopathic clinical course, as proposed earlier by McCabe et al. (1996). This conclusion is particularly relevant for those ethnic groups in which the N370S mutation occurs frequently (e.g., the Jewish and non-Jewish Caucasian populations). Our findings for immigrant Gaucher patients and those described by Ida et al. (1995) for Japanese patients suggest that the significance of GC genotyping for genetic counseling may be less for other ethnic populations.

Despite the high frequency of the N370S mutation, relatively few homozygotes for this mutation were identified among the Gaucher patients investigated by us. The observed distribution of homozygotes and heterozygotes for the N370S mutation among native Dutch Gaucher patients, 2% and 77%, respectively, differs significantly for that predicted by the Hardy-Weinberg equilibrium, 17% and 48% respectively. Only the patients in a single Dutch family were found to be homozygous for this mutation. However, in family studies we detected an 80-year-old asymptomatic N370S homozygote. These results suggest that the N370S/N370S GC genotype may usually result in no (or an extremely mild) manifestation of Gaucher disease. This was also suggested after analysis of the N370S GC gene frequency in the Portuguese population and in the American Ashkenazi Jewish population (Beutler et al., 1993c; Lacerda et al., 1994b). Interestingly, a relatively large number of symptomatic, although usually mildly affected, N370S homozygotes have been noted in Ashkenazi Jewish populations (see Table 3).

The origin of the N370S GC gene mutation in the Dutch population is unclear. As previously noted for Portuguese Gaucher patients (Lacerda et al., 1994a), the N370S GC mutation in Dutch Gaucher patients is linked to the same Pv1.1 haplotype as in Ashkenazi Jewish Gaucher patients. This neither proves nor excludes the likely possibility that the N370S mutation was introduced by Ashkenazi Jewish individuals in The Netherlands.

During our genotype analysis, we observed a pitfall in the assignment of homozygosity for the N370S mutated GC allele. Using a common procedure for the detection of the N370S mutation, we initially assigned the N370S/N370S GC genotype to several Type 1 Gaucher patients from two unrelated families (families 6 and 17 in Table 2). However, we observed that one of the parents in each family did not carry a N370S mutated GC allele. In the case of one family, false paternity can be excluded and in the case of the other family, it is extremely unlikely on the basis of the transmission of another inherited abnormality. It was examined by us whether an intragenic deletion underlay the problems with PCR-based analysis for the N370S mutation. Southern blot analysis of genomic DNA digested with the restriction enzyme Ssp1 (Zimran et al., 1990) rendered no indications for the presence of a deletion of 100 bp or more. Comparison of the ratios of radioactivity of the bands for the glucocerebrosidase gene and pseudogene did not point to the occurrence of a deletion of the entire coding region of the glucocerebrosidase gene (Beutler and Gelbart, 1994). We are studying this further. It is evident that special care should be taken to verify apparent homozygosity for a particular mutation as established with PCR techniques. Ideally, the genotype of patients should be confirmed by those of their parents.

The prognostic value of GC genotypes of Type 1 Gaucher patients with respect to the severity of the manifestation of the disease, or with respect to responsiveness to enzyme-supplementation therapy proved to be limited in our experience. Among patients with an identical mutant GC genotype, considerable variability existed with respect to severity of clinical expression and to responses to therapeutic intervention. This was, in fact, even noted among siblings. We feel that the value of GC mutation analysis in countries such as The Netherlands is, therefore, to be found in the exclusion of a neuronopathic course of the disease by identification of the presence of the N370S GC allele, as well as accurate identification of carriers and presymptomatic patients in families with incidence of Gaucher disease.

It will be of interest to determine the N370S gene frequency in The Netherlands in order to obtain insight in the incidence of N370S GC homozygosity and its association with actual disease manifestation. At present, random screening for carriers in The Netherlands should not be advocated, since the general outcome of homozygosity for the N370S GC mutation, theoretically the most frequent mutant genotype, is still not known. Consequently, no solid basis is present for adequate genetic counseling that should be associated with carrier testing in the general population.

In conclusion, the results of our investigation indicate once more that other factors besides the nature of mutant glucocerebrosidase in Gaucher patients modulate the clinical expression of the disease (for a more detailed discussion, see Aerts et al., 1993). Although GC genotyping of Gaucher patients is recommendable, its prognostic value for disease manifestation is limited.

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REFERENCES

Aerts JMFG, Sa Miranda MC, Wanzeller de Lacerda L, van Weely S, Donker-Koopman W, Brouwer-Kelder B, Jansen DC, van Leeuwen M, Schram AW, Tsiapara A, Tager JM (1991) The identification of type I Gaucher disease patients, asymptomatic cases and carriers in The Netherlands using urine samples: an evaluation. Clin Chim Acta 203:349–362.

Aerts JMFG, van Weely S, Boot R, Hollak CEM, Tager JM (1993) Pathogenesis of lysosomal storage disorders as illustrated by Gaucher disease. J Inher Metab Dis 16:288–291.

Amaral O, Lacerda L, Santos R, Pinto RA, Aerts H, Sa Miranda MC (1993) Type 1 Gaucher disease: Molecular, biochemical, and clinical characterization of patients from Northern Portugal. Biochem. Med Metab Biol 49:97–107.

Amaral O, Fortuna AM, Lacerda L, Pinto RA, Sa Miranda MC (1994) Molecular characterization of type 1 Gaucher disease families and patients: Intrafamilial heterogeneity at the clinical level. J Med Genet 31:401–404.

Barneveld RA, Keijzer W, Tegelaers FPW, Ginns EI, Geurts van Kessel A, Brady RO, Barranger JA, Tager JM, Galjaard H, Westerveld A, Reuser AJJ (1983) Assignment of the gene coding for human β-glucocerebrosidase to the region q21-q31 of chromosome 1 using monoclonal antibodies. Hum Genet 64:227–231.

Barranger JA, Ginns EI (1989) Glucosylceramide lipidoses: Gaucher disease. In Scriver CR, Beaudet AL, Sly WS, Valle D (eds): The Metabolic Basis of Inherited Disease. New York: McGraw-Hill, pp 1677–1698.

Beutler E (1993) Modern diagnosis and treatment of Gaucher's disease. Am J Dis Child 147:1175–1183.

Beutler E, Demina A, Gelbart T (1994) Glucocerebrosidase mutations in Gaucher disease. Mole Med 1:82–92.

Beutler E, Gelbart T (1993) Gaucher disease mutations in non-Jewish patients. Br J Haematology 85:401–405.

Beutler E, Gelbart T (1994) Erroneous assignment of Gaucher disease genotype as a consequence of a complete gene deletion. Hum Mutat 4:212–216.

Beutler E, Gelbart T, Demina A, Zimran A, LeCoultre P (1995) Five new Gaucher disease mutations. Blood Cells, Molecules, Diseases 21:20–24.

Beutler E, Gelbart T, Kuhl W, Sorge J, West C (1991) Identification of the second common Jewish Gaucher disease mutation makes possible population-based screening for the heterozygous state. Proc Natl Acad Sci USA 88:10544–10547.

Beutler E, Gelbart T, Kuhl W, Zimran A, West C (1993a) Mutations in Jewish patients with Gaucher disease. Blood 79:1662–1666.

Beutler E, Gelbart T, West C (1990) The facile detection of the nt 1226 mutation of glucocerebrosidase by 'mismatched' PCR. Clin Chim Acta 194:161–166.

Beutler E, Gelbart T, West C (1993b) Identification of six new Gaucher disease mutations. Genomics 15:203–205.

Beutler E, Grabowski GA (1995) Gaucher disease. In Scriver CR, Beaudet AL, Sly WS, Valle D (eds): The Metabolic and Molecular Bases of Inherited Disease. New York: McGraw-Hill, pp 2641–2670.

Beutler E, Nguyen NJ, Henneberger MW, Smolec JM, McPherson RA, West C, Gelbart T (1993c) Gaucher disease: Gene frequencies in the Ashkenazi Jewish Population. Am J Hum Genet 52:85–88.

Beutler E, West C, Gelbart T (1992) Polymorphisms in the human glucocerebrosidase gene. Genomics 12:795–800.

Caillaud C, Puech J-P, Crosnier JM, Castelnau L, Manicom J, Poenaru L (1995) Molecular epidemiology of Gaucher disease in France. In Aerts JM, Goudsmit R, Tager JM (eds): Proc. Of the First Workshop of the European Working Group on Gaucher Disease, October 13-16, 1994. Drukkerij Universiteit Amsterdam, p 54.

Cormand B, Vilageliu L, Burguera JM, Balcells S, Gonzàlez-Duarte R, Grinberg D, Chabás A (1995) Gaucher disease in Spanish patients: Analysis of eight mutations. Hum Mutat 5:303–309.

Daniels LB, Glew RH (1982) β-Glucosidase assays in the diagnosis of Gaucher's disease. Clin Chem 28:569–577.

Filocamo M, Seri M, Corsolini F, Stroppiano M, Carozzo R, Gatti R (1995) Prevalence of mutations in Italian patients. In Aerts JM, Goudsmit R, Tager JM (eds): Proc. Of the First Workshop of the European Working Group on Gaucher Disease, October 13-16, 1994. Drukkerij Universiteit Amsterdam, p 49.

Ginne EI, Choudary PV, Tsuji S, Martin B, Stubblefield B, Sawyer J, Hozier J, Barranger JA (1985) Gene mapping and leader polypeptide sequence of human glucocerebrosidase: Implications for Gaucher disease. Proc Natl Acad Sci USA 82:7101–7105.

Grace ME, Berg A, He G-S, Goldberg L, Horowitz M, Grabowski G (1991) Gaucher disease: Heterologous expression of two alleles associated with neuronopathic phenotypes. Am J Hum Genet 49:646–655.

Grace ME, Graves PN, Smith FI, Grabowski GA (1990) Analyses of catalytic activity and inhibitor binding of human acid β-glucosidase by site-directed mutagenesis. J Biol Chem 265:6827–6835.

Grace ME, Newman KM, Scheinker V, Berg-Fussman A, Grabowski GA (1994) Analysis of human acid β-glucosidase by site-directed mutagenesis and heterologous expression. J Biol Chem 269:2283–2291.

He G-S, Grabowski GA (1992) Gaucher disease: A $G^{+1} \rightarrow A^{+1}$ IVS2 splice donor site mutation causing exon 2 skipping in the acid β-glucosidase mRNA. Am J Hum Genet 51:810–820.

Hollak CEM, Aerts JMFG, Goudsmit R, Phoa SSKS, Ek M, van Weely S, von dem Borne AEGKr, van Oers MHJ (1995) Individualised low-dose alglucerase therapy for type 1 Gaucher's disease. Lancet 345:1474–1478.

Horowitz M, Tzuri G, Eyal N, Berebi A, Kolodny EH, Brady RO, Barton NW, Abrahamov A, Zimran A (1993) Prevalence of nine mutations among Jewish and non-Jewish Gaucher disease patients. Am J Hum Genet 53:921–930.

Horowitz M, Wilder S, Horowitz Z, Reiner O, Gelbart T, Beutler E (1989) The human glucocerebrosidase gene and pseudogene: Structure and evolution. Genomics 4:87–96.

Horowitz M, Zimran A (1994) Mutations causing Gaucher disease. Hum Mutation 3:1–11.

Ida H, Iwasawa K, Kawame H, Rennert OM, Maekawa K, Eto Y (1995) Characteristics of gene mutations among 32 unrelated Japanese Gaucher disease patients: Absence of the common Jewish 84GG and 1226G mutations. Hum Genet 95:717–720.

Lacerda L, Amaral O, Pinto R, Aerts J, Sá Miranda MC (1994a) The N370S mutation in the glucocerebrosidase gene of Portuguese Type 1 Gaucher patients: Linkage to the PvuII polymorphism. J Inher Metab Dis 17:85–88.

Lacerda L, Amaral O, Pinto R, Oliveira P, Aerts J, Sá Miranda MC

(1994b) Gaucher disease: N370S glucocerebrosidase gene frequency in the Portuguese population. Clin Genet 45:298–300.

Lewis BD, Nelson PV, Robertson EF, Morris CP (1994) Mutation analysis of 28 Gaucher disease patients: The Australasian experience. Am J Med Genet 49:218–223.

McCabe ERB, Fine BA, Golbus MS, Greenhouse JB, McGrath GL, New M, O'Brien WE, Rowley PT, Sly WS, Spence MA, Stockman JA, Whyte M, Wilson W, Wolf B, Aerts JMFG, Barranger JA, Barton NW, Beutler E, Brady RO, Cohen IJ, Cox TM, Ekstein J, Eng CM, Erikson A, Findling DM, Garber AM, Ginns EI, Grabowski GA, Hill SC, Hollak CEM, Kaback MM, Lee RE, Mankin HJ, Mistry PK, Moscicki RA, Murray GJ, Natowicz M, Pastores GM, Sá Miranda MC, Sidransky E, Ware Jr JE, Willemsen R, Zaizov R, Zimran A et al. (1996) Gaucher disease: Current issues in diagnosis and treatment. NIH Technology Assessment Panel on Gaucher disease. JAMA 275:548–553.

Michelakakis H, Dimitriou E, van Weely S, Boot RG, Mavridou I, Verhoek M, Aerts JMFG (1995) Characterization of glucocerebrosidase in Greek Gaucher disease patients: Mutation analysis and biochemical studies. J Inher Met Dis 18:609–615.

Mistry PK, Smith SJ, Ali M, Hatton CSR, McIntyre N, Cox TM (1992) Genetic diagnosis of Gaucher's disease. Lancet 339:889–892.

Nelson PV, Carey WF, Morris CP, Lewis BD (1995) Mutation analysis of Australasian Gaucher disease patients. Am J Med Genet 58:382.

Ohashi T, Hong CM, Weiler S, Tomich JM, Aerts JMFG, Tager JM, Barranger JA (1991) Characterization of human glucocerebrosidase from different mutant alleles. J Biol Chem 266:3661–3667.

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning, a Laboratory Manual, 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1,659 pp.

Sá Miranda MC, Amaral O, Fortuna AM, Lacerda L, Pinto R (1995) Inter- and intrafamilial genotype/phenotype correlation in Portuguese Type 1 Gaucher disease patients. In Aerts JM, Goudsmit R, Tager JM (eds): Proc of the First Workshop of the European Working Group on Gaucher Disease, October 13–16, 1994. Drukkerij Universiteit Amsterdam, pp 59–60.

Sibille A, Eng CM, Kim S-J, Pastores G, Grabowski GA (1993) Phenotype/genotype correlations in Gaucher disease Type 1: Clinical and therapeutic implications. Am J Hum Genet 52:1094–1101.

Sidransky E, Ginns EI (1994) Phenotypic and genotypic heterogeneity in Gaucher disease: Implications for genetic counseling. J Genet Counsel 3:13–22.

Tuteja R, Bembi B, Agosti E, Baralle FE (1993) 1448C mutation linked to the Pv1.1 genotype in Italian patients with Gaucher disease. Hum Molec Genet 2:781–784.

Van Weely S, Van den Berg M, Barranger JA, Sá Miranda MC, Tager JM, Aerts JMFG (1993) Role of pH in determining the cell-type-specific residual activity of glucocerebrosidase in Type 1 Gaucher disease. J Clin Invest 91:1167–1175.

Walley AJ, Barth ML, Ellis I, Fenson AH, Harris A (1993) Gaucher's disease in the United Kingdom: Screening non-Jewish patients for the two common mutations. J Med Genet 30:280–283.

Wenger DA, Roth S (1982) Homozygote and heterozygote identification. In Desnick RJ, Gatt S, Grabowski GA (eds): Gaucher Disease: A Century of Delineation and Research. New York, Alan R. Liss, pp 551–572.

Zimran A, Elstein D, Kannai R, Zevin S, Hadas-Halpern I, Levy-Lahad E, Cohen Y, Horowitz M, Abrahamov A (1994) Low-dose enzyme replacement therapy for Gaucher's disease: Effects of age, sex, genotype, and clinical features on response to treatment. Am J Medicine 97:3–13.

Zimran A, Kay A, Gelbart T, Garver P, Thurston D, Saven A, Beutler E (1992) Gaucher disease: Clinical, laboratory, radiologic, and genetic features of 53 patients. Medicine 71:337–353.

Zimran A, Sorge J, Gross E, Kubitz M, West C, Beutler E (1990) A glucocerebrosidase fusion gene in Gaucher disease. Implications for the molecular anatomy, pathogenesis, and diagnosis of this disorder. J Clin Invest 85:219–222.