Journal of Genetics, Vol. 96, No. 4, September 2017, pp. 583–589
DOI 10.1007/s12041-017-0821-8

RESEARCH ARTICLE

GBA mutations in Gaucher type I Venezuelan patients: ethnic origins and frequencies

GILBERTO GÓMEZ[1], SERGIO ARIAS[1], LEONOR CÁRDENAS[2], DALAL ZOGHBI[2] and IRENE PARADISI[1]*

[1] *Laboratory of Human Genetics, Venezuelan Institute for Scientific Research (IVIC), Caracas 10200-A, Venezuela*
[2] *Hematology Service, Miguel Pérez Carreño Hospital, Caracas 10200-A, Venezuela*
*For correspondence. E-mail: iparadis@ivic.gob.ve, ireneparadisi@hotmail.com.

Received 21 June 2016; revised 23 September 2016; accepted 5 December 2016; published online 8 September 2017

Abstract. Gaucher disease (GD), the most frequent lysosomal storage disease, is caused by heterogeneous mutations in the locus coding for glucocerebrosidase (GBA). It is an autosomal recessive disorder with different phenotypes of which the most frequent is the nonneuronopathic or type 1, prevalent worldwide. To date, more than 430 mutations have been described, but their frequency distribution varies in different populations with four, N370S, L444P, IVS2 + 1G > A and 84insG, being the most frequent ones. In Venezuela, 20 unrelated index cases with GD type I were assessed for *GBA* mutation detection and for their in-phase haplotype identification, to gather genetic epidemiological data on the disease in the country and of its eventual ethnic origin. Ten missense mutations and two complex alleles were identified. The most frequent were N370S (42.5%), L444P (20%), IVS2 + 1G > A (10%) and R48W (5%); mutations R120W, P245H, H311R, R496H, W36X and R433G which were carried by a single chromosome each one. Three geographical foci were identified, displaying mutation heterogeneity. N370S had multiple genetic origins, different from the Ashkenazi's; a single common remote ancestor for this mutation in the country was dismissed, according to the haplotype analysis. All mutations have a likely European Caucasoid descent.

Keywords. Gaucher disease; glucocerebrosidase mutations; ethnic origins; haplotype analysis.

Introduction

Gaucher disease (GD) (OMIM: 230800), the most common of lysosomal storage diseases, is an autosomal recessive disorder resulting from deficit or lack of β-glucosidase activity. The glucocerebrosidase (acid-β glucosidase, glucosyl-ceramidase, EC.3.2.1.45), a lysosomal glycoside hydrolase, cleaves the β-glucosidic linkage of glucosylceramide, a normal intermediate of glycolipid catabolism (Beutler and Grabowski 1995). The abnormal function leads to accumulation of the substrate (glucocerebroside) in cells of the macrophage–monocyte system.

This disease is highly heterogeneous and has been classified into three types based on the presence and rate of progression of neurologic manifestations. The most common (95% of cases) nonneuronopathic GD type 1 (OMIM: 230800) is characterized by the presence of hepatosplenomegaly, pancytopenia and skeletal involvement, without neurological manifestations, and a variable age of onset of symptoms; bone marrow infiltration by 'Gaucher cells' is typical. In the type 2 disease (OMIM: 230900) or acute neuronopathic, the patients present with a severe hepatosplenomegaly and progressive neurologic deterioration being usually fatal within the first two years of life. Type 3 (OMIM: 231000) or chronic neuronopathic disease has a heterogeneous presentation with variable neurological and visceral involvement, onset in childhood or in adulthood; almost all patients survive until adulthood (Vellodi *et al.* 2001).

The gene encoding GBA (1q21) encompasses 7 kb with 11 exons, producing a cDNA of ~2.5 kb; the mature protein has 39 amino acids signal peptide. *GBA* has a highly homologous pseudogene (*psGBA* with 96% identity), located 16-kb downstream (Horowitz *et al.* 1989) with the same organization of exons and introns as the functional gene but carrying large deletions in introns 2, 4, 6 and 7, correspond to *Alu* elements. These highly homologous sequence and physical proximity leads to gene conversion and crossing over events between *GBA* and *psGBA*, producing mutated complex alleles.

To date, in *GBA* gene, around 437 different mutations have been registered in the human mutation database at Cardiff, UK (www.hgmd.cf.ac.uk/ac/). Nevertheless, four mutations (N370S, L444P, 84insG, IVS2 + 1G > A) account for 90% of Ashkenazim patients worldwide but for only 50–60% of cases in different populations, many of them being compound heterozygotes.

In this study, 20 Venezuelan independent families with GD type I ascertained for 20 years were assessed to identify *GBA* mutations and to study genetic epidemiological features of the disease in the country. The most frequently found mutation N370S had a heterogeneous origin, different from the Ashkenazim's according to the haplotype analysis.

Materials and methods

Sample

A total of 20 genetically unrelated index cases with biochemical and clinical diagnosis of GD, who were referred to the Human Genetics Laboratory (HGL) at the Venezuelan Institute for Scientific Research (IVIC) and to the Hematology Service at the Miguel Pérez Carreño Hospital (Caracas) were included in this study. Two additionally affected family members (a sister and an aunt) of one of the families (patient number 13, table 1) were also included in the study.

The diagnosis of GD was established by the clinical manifestations and a diminished β-glucosidase activity. The enzymatic activities were measured either at the HGL, according to protocols by Peters *et al.* (1977) and Daniels *et al.* (1981), or at Centogene, Germany, using mass spectrometry. The geographical origin of each family was established by recording the precise place of birth of the remote ancestors (grandparents and great-grandparents).

For the molecular analyses, 5 mL blood sample was collected in EDTA and DNA was extracted by saline method (Lahiri and Nurnberger 1991). Written voluntary informed consent was obtained from all family members according to the bioethical institutional guidelines.

DNA analyses

Mutation detection: A two stage polymerase chain reaction (PCR) method was used to selectively amplify the glucocerebrosidase functional gene but not the pseudogene. In the first stage, only the functional gene was amplified in three large amplicons (between 1681 and 2971 bp). In the second stage, the first round PCR products were used as templates for the amplification of each and all the exons of the *GBA* gene (nested PCR). Primers were previously reported by Stone *et al.* (2000). The GenBank reference sequence accession number was NM_000157.3. Single strand conformation polymorphism (SSCP) analysis of each exon was performed as previously reported (Paradisi and Arias 2010), and those showing any abnormal migration pattern were sequenced at Macrogen, Seoul, Korea.

Polymorphisms used to construct haplotypes

Two intragenic single-nucleotide polymorphisms (SNP) at introns 6 and 7, g.4813G > A [rs762488; NM_000157.3: c.762 − 180G > A] and g.5470G > A [NM_000157.3: c.999 + 240G > A], and two microsatellites (5GC3.2 and ITG6.2) located in the flanking regions of the gene were used to construct the haplotypes in phase with the mutations. To establish the phases, genotypes of each marker in carriers and noncarriers family members were established, and segregation analysis of the allele transmission from parents to descendants was assessed in each polymorphic site. Primers were previously reported by Lau *et al.* (1999) and Rodríguez-Marí *et al.* (2001). Alleles at the SNPs were detected by the restriction enzymes (*Pvu*II for g.4813G > A and *Bsu*36I for g.5470G > A); microsatellites 5GC3.2 (dinucleotide repeats CT) and ITG6.2 (tetranucleotide repeats AAAT) were analysed in a 10% and 8% polyacrylamide (acrylamide:bisacrylamide, 39:1) gel electrophoresis, respectively. Some samples were Sanger sequenced to confirm the actual allele sizes.

Results

The main phenotypic and genotypic data of the studied index cases are shown in table 1. All patients had a type I form of the disease (nonneuronopathic), with a highly variable age of onset and common clinical manifestations.

Almost all patients had splenomegaly (94.7%) and hepatomegaly (84.2%); more than half of them had thrombocytopenia (52.6%) and 42.1% were anaemic. Bone crisis occurred in 10.5% of cases. In 45% of patients, the diagnosis was made in the first decade of life, in 20% the diagnosis was made in the second decade, and in 35% during the third, fourth or fifth decades; 14 of 20 were female. All the patients are receiving enzymatic replacement therapy.

GBA analysis

Complete coding region and intron-exon boundaries of the *GBA* gene were screened; 10 different mutations and two complex alleles in 40 studied chromosomes were identified, all of them previously reported in different populations.

The most frequent mutation was N370S, carried by 17 chromosomes (42.5%), followed by L444P (20%), presenting as a complex allele or as a single allele (5%); IVS2+1G > A was present in 10% of chromosomes, while the R48W mutation was found in 5%.

Mutations R120W, P245H, H311R, R496H, W36X and R433G showed low frequencies, being each one carried by only one chromosome out of 40. All these mutations cause deleterious effects on the protein structure, with Polyphen scores of 1.0 (P245H, H311R and R433G), 0.999 for R120W and 0.488 for R496H. Mutation W36X causes a severely truncated protein. Ninety-five per cent of index cases were compound heterozygotes. Mutated amino acid positions in all mutation names refer to the processed protein, which does not include the 39-residue signal peptide.

Haplotype analysis and geographic distribution

Only seven of 20 families (35%) had geographic aggregation, with remote ancestors from three different geographic foci (Arias 1994) in Zulia state (three independent families), in Lara state (three independent families) and in Yaracuy state (one family); the remaining 65% families' ancestors were scattered across the country (table 1). Within the geographic foci there was mutation heterogeneity, with two different mutations in the Zulia state focus (centre in La Cañada), five mutations in the Lara state focus (centre in Barquisimeto) and two mutations in the Yaracuy state focus (centre in San Felipe), suggesting different genetic origins for GD in each one.

In the Zulia state focus, the three unrelated index cases (numbers 8, 9 and 13, table 1) were compound heterozygotes for mutations IVS2 + 1G > A and N370S, which were both in phase with the same 314; G; 222; G haplotype in the three families.

Family of index case number 13 contained two compound heterozygous sisters (N370S/IVS2 + 1G > A) and an affected maternal aunt carrying N370S plus another nonidentified change; the paternal one (IVS2 + 1G > A) had a recent central European Ashkenazi origin but the maternal N370S frequent in the focus, is not a Jewish mutation according to its in-phase haplotype, being apparently only identical by nature (IBN).

Index case number 7 also carried IVS2+1G > A/N370S mutations with the same in-phase haplotypes, but her remote ancestors origins were different from two far apart states (Sucre state and Falcón state, at the oriental and occidental regions of the country).

This finding strongly suggests that in most cases, those mutations both in nonJewish and in Ashkenazi carriers might be likely also identical by descent (IBD), going back in their ancestry much earlier than one thousand years.

In the Lara state focus, three different mutations and two complex alleles were present; the three unrelated index cases (numbers 3, 4 and 6, table 1) were compound heterozygotes [R48W]/[R120W], [N370S]/[L444P+A456P +V460V], and [N370S]/[E326K+L444P]. Interestingly, the N370S mutation had the same in-phase 314; G; 222; A haplotype, shared by the two independent families, and different from that found in the Zulia state focus, thus supporting different origins between foci for N370S. The R48W, a very infrequent mutation worldwide, was found in two foci (Yaracuy and Lara states, 90 km apart: index case numbers 2 and 3, table 1) with the same in-phase haplotype 318; G; 222; A, suggesting a common but very remote ancestor.

The haplotypes in phase with the 12 detected mutations are shown in table 2. The most frequent mutation N370S was in phase with four different haplotypes, as well as the second most frequent L444P (alone and in complex alleles); in three instances, N370S and L444P had the same haplotypes, which were also very frequent in patients: 314; G; 222; G (37.5%), 314; G; 222; A (22.5%) and 318; G; 224; G (7.5%) but not so in controls. Infrequent mutations had a unique in-phase haplotype, except R48W, as already mentioned.

Discussion

GD is the most frequent disorder among lysosomal storage diseases, although it is infrequent in populations worldwide. In Venezuela, the 'Venezuelan association of patients with lysosomal diseases' (AVEPEL) has 81 registered independent index cases from the country at large, all receiving enzymatic replacement therapy provided by the Governmental Social Security Agency. Thus, the disease prevalence in Venezuela can be estimated as 1:77000 families if all existing cases had been detected, calculated as previously reported (Paradisi *et al.* 2015), which is similar to the figure quoted in general worldwide populations, around 1.3:100000 inhabitants, excluding the Ashkenazi Jewry of eastern and central European ancestry, in which the prevalence is 1:500 to 1:1000 (Orphanet Reports Series, http://www.orpha.net).

In 20 independent index cases, the mutation detection rate was 92.5%. A genotype–phenotype correlation could not be established, since there were 13 different genotypes, all but one index case was compound heterozygotes for different mutations. The exception were the IVS2+1G>A/N370S carriers, which accounted for 20% of the sample; between them, however there was no clear genotype–phenotype correlation (table 1).

Mutations N370S and L444P are the most common mutations worldwide; jointly they represent between 50 and 62% of the *GBA* mutations in all populations, except among the Ashkenazi, which accounts for 93% of the mutant alleles (Koprivica *et al.* 2000); in Venezuelan patients, its joint frequency was 62.5%. N370S is virtually absent in Mongoloid populations.

Mutation N370S has been reported to cause relatively minor changes in the glucocerebrosidase structure and therefore, in its catalytic activity (Dvir *et al.* 2003). It is located at the interface of domains II and III, too far from the active site to participle directly in catalysis. Thus,

Table 1. Clinical and genetic epidemiological features of Gaucher disease index cases.

Patient	Sex	Age at diagnosis (years) (years)	Clinical manifestation	Genotype[a]	In-phase haplotype[b]	Geographic origin
1	♀	4	A; S; T	H311R N370S	314; G; ?; ? 318; G; ?; ?	HGO
2	♀	2	H; S; T; Hy	R48W ?	318; G; 222; A 322; G; 222; G	YSF
3	♀	2	A; H; S; T	R48W R120W	318; G; 222; A 326; G;222, G	LSF
4	♂	9	A; H; T	N370S Rec*Nci*I	314; G; 222; A 314; G; 222; A	LSF
5	♂	33	NA	P245H N370S	314; G; 222; G 314; G; 222; G	HGO
6	♀	27	A; H; S; T; Bc	N370S [E326K+L444P]	314; G; 222; A 318; G; 222, G	LSF
7	♀	2	H; S; T	IVS2 + 1G > A N370S	314; G; 222; G 314; G; 222; G	HGO
8	♂	24	H; S	IVS2 + 1G > A N370S	314; G; 222; G 314; G; 222; G	ZSF
9	♀	8	A; H; S; T	IVS2 + 1G > A N370S	314; G; 222; G 314; G; 222; G	ZSF
10	♂	2	A; H; S; T	R433G L444P	318; G; 224; A 318; G; 224; G	HGO
11	♀	4	H; S; T	N370S L444P	314; G, 222; G 318; G; 222;G	HGO
12	♀	11	A; H; S	N370S ?	318; G; 224; G 318; G; 224; G	HGO
13	♀	8	S; T	IVS2 + 1G > A N370S	314; G; 222; G 314; G; 222; G	ZSF
14	♀	56	H; S	N370S ?	314; G; 222; A 314; G; 222; A	HGO
15	♀	50	H; S; Bc	Rec*Nci*I R496H	314; G; 222; A 318; G; 222; A	HGO
16	♂	9	A; H; S	N370S Rec*Nci*I	314; G; 222; A 318; G; 222; G	HGO
17	♀	21	H; S	W36X N370S	314; G; 222; G 314; G; 222; G	HGO
18	♂	44	S	N370S N370S	314; G; 222; G 314; G; 222; G	HGO
19	♀	13	H; S	N370S Rec*Nci*I	314; G; 222; A 314; G; 222; G	HGO
20	♂	18	H; S	N370S Rec*Nci*I	314; G, 222; A 318; G; 222; G	HGO

A, anemia; H, hepatomegaly; S, splenomegaly; T, thrombocytopenia; Bc, bone crisis; Hy, hypotonia; NA, data not available; Rec*Nci*I: complex allele [L444P+A456P+V460V]; '?' unidentified genotype; HGO, heterogeneous geographic origins; ZSF, Zulia state focus; YSF, Yaracuy state focus; LSF, Lara state focus.

[a]Mutation nomenclature refers to the processed protein, not including the 39 residues of the signal peptide.

[b]Haplotype markers from left to right: ITG6.2; g.5470G > A (c.999 + 240G > A); 5GC3.2; g.4813G > A (c.762 − 180G > A).

its phenotypic effect tends to be moderate, producing always type I disease with mild clinical manifestations and an older age at diagnosis, even when in heterozygosity (Charrow *et al.* 2000), as was observed in our patients (table 1). Polyphen analysis classifies its pathogenicity as possibly damaging, with a score of 0.607.

As mentioned, mutation N370S is the most frequent in Ashkenazi due to a founder effect and a genetic drift phenomena. In such populations, it shows a strong allelic disequilibrium with the 222 bp and 318 bp alleles of microsatellite markers 5GC3.2 and ITG6.2 (Zimran *et al.* 1990; Amaral *et al.* 1997; Cormand *et al.* 1998; Rockah *et al.* 1998), as well as with the A allele at intron 6 (g.4813G > A, which suppress the restriction site for the *Pvu*II enzyme known as the Pvu1.1^{-} haplotype); and the G allele at g.5470G > A (c.999 + 240G > A). Thus, haplotype 318; G; 222; A (ITG6.2; g.5470G > A; 5GC3.2; g.4813G > A) is almost always in phase with the Ashkenazi N370S mutation. In Venezuelan patients, the N370S had at least three different in-phase haplotypes: 314; G; 222; G (47.1%), 314; G; 222; A (35.3%), 318; G; 224; G (11.8%) and 318; G; (-); (-) (5.8%) (table 2). The

Table 2. *GBA* mutations, in-phase haplotypes and its frequencies in patients and in a control sample.

GBA mutation	In-phase haplotype	Haplotype frequencies (%)	
		Patients ($n = 40$)	Controls ($n = 56$)
N370S Rec*Nci*I*	314; G; 222; A	22.5	14.3
R48W R496H L444P [E326K/L444P]* Rec*Nci*I*	318; G; 222; G	12.5	41.1
P245H W36X Rec*Nci*I* IVS2 + 1G > A N370S	314; G; 222; G	37.5	16.1
Unidentified	322; G; 222; G	2.5	1.8
N370S L444P	318; G; 224; G	7.5	0
R433G	318; G; 224; A	2.5	0
R120W	326; G; 222; G	2.5	0
N370S	318; G; (-); (-)	2.5	0
H311R	314; G; (-); (-)	2.5	0

Some haplotypes were found to be in phase with more than one mutation type, and some mutations had more than one in-phase haplotypes (N370S, L444P). Haplotype frequencies were different between patients and control individuals; the most frequent in controls (318; G; 222; G) were not in patients chromosomes, suggesting that some mutations arose in a different genetic background. Some in-phase haplotypes were not found in the control sample. *n*, Number of chromosomes; '-' uninformative ($n = 2$ chromosomes); '*' complex alleles; Rec*Nci*I, L444P+A456P+V460V.
Haplotype markers from left to right: ITG6.2; g.5470G > A (c.999 + 240G > A); 5GC3.2; g.4813G > A (c.762 − 180G > A).

in-phase haplotype with the highest frequency (314; G, 222; G), has also been found by Wilches *et al.* (2006) in Colombian N370S carriers; Colombian and Venezuelan populations share similar demographic history, suggesting a plausible common remote origin for the mutation carrying this haplotype in phase. On the other hand, haplotypes in phase with this N370S 'Venezuelan' mutation suggest that the origin is different from the Ashkenazi's in most cases, and that there is not a single common ancestor for the mutation in the country. In Venezuela, most of the N370S alleles are distributed in the northwestern region, showing geographic aggregation in two foci: La Cañada (Zulia state) and Barquisimeto (Lara state). Nevertheless, in-phase haplotypes were different between foci (314; G; 222; G and 314; G; 222; A) respectively, suggesting separate origins for the N370S in each focus, but a common source within each one.

The L444P occurs in the hydrophobic core of the Ig-like domain (domain II), causing protein instability due to disruption of the hydrophobic core and altered folding of the domain (Beutler and Kuhl 1986; Dvir *et al.* 2003); its Polyphen score is 0.938, suggesting a strong mutation effect on the protein structure. This mutation in homozygous condition produces the chronic neuronopathic form of GD. It is the second most frequent mutation (after N370S) in Ashkenazi, Caucasoids (Stinermann *et al.* 2012) and also in the Venezuelan studied patients, but not in east Asians, Caucasoids or sub-Saharan Africans.

Two haplotypes in phase with the L444P mutation as a single allele (318; G; 222; G and 318; G; 224; G), and two other in complex alleles (table 2) were found in the index cases. Tuteja *et al.* (1993) demonstrated that several haplotypes are in phase with this mutation and suggested multiple ancestral origins for it, since codon 444 could be a hot spot for mutations. The mutation has been associated with the Pv1.1^{-} haplotype in Jewish populations (Rodríguez-Marí *et al.* 2001). In Venezuelan patients, all chromosomes carrying L444P were in phase with allele G (haplotype Pv1.1^{+}), thus discarding a possible remote Jewish origin for the mutation. Further, haplotype diversity suggests multiple origins of L444P in the country.

Mutation IVS2 + 1G > A was identified in four patients (four chromosomes, 10%). The change produces aberrantly spliced mRNAs, missing exon 2, and severely impaired catalytic activity (He and Grabowski 1992). This mutation represents 1–4% of patients with GD, in the 'general' population, ordinarily associated to the N370S mutation, as was seen in our patients. In all instances, IVS2 + 1G>A was in phase with the same haplotype

314; G; 222; G. Three of the four alleles were found in the same geographic focus (La Cañada, Zulia state); thus haplotype sharing and geographic aggregation strongly suggest a common remote ancestor and a founder effect as well, for these families in the country.

Mutation R48W had only one in-phase haplotype found in two unrelated patients from different, although contiguous geographic foci (in Lara and Yaracuy states). The mutation is very infrequent worldwide (Choy *et al.* 2007), thus haplotype sharing might suggest a common and very remote origin for it in the northern occidental area of the country. R48W has a severe effect on the protein, as is suggested by its Polyphen score of 1.0.

Patients with GD in Venezuela are geographically widespread, and their parents typically are not closely related, but three geographic foci were detected for 35% of the families. This suggests both remote kinship and a relatively high frequency of diverse mutant alleles, beside a founder phenomenon. There was great genetic heterogeneity of mutations, with most of the patients being compound heterozygotes as is the usual finding everywhere, except among the Ashkenazi. However three mutations account for 72.5% of the chromosomes: N370S (42.5%), L444P (20%), and IVS2+1G>A (10%). Thus, in any new patient, these mutations should be the first tested in our populations, being they also the most common in European Caucasoids from diverse geographic regions, but not so in other ethnic groups.

Acknowledgements

To all the patients who generously gave their consent to participate in the study. To Mrs Joelkys Romero and Mrs Nereyda Guédez, president and vice-president of the Venezuelan Association of patients with lysosomal diseases (AVEPEL) for their support. To Lic. Neida González for the biochemical processing of the samples at the Human Genetics Laboratory, IVIC.

References

Arias S. 1994 Selective detection among the high risk populations, instead of mass screening, proposed for countries with various homogeneous origins and a stratified distribution of abnormal genes. In *New horizons in neonatal screening* (ed. J. P. Farriaux and J. L. Dhondt), Int. congr ser, vol. **1041**, pp. 97–180. Excerpta media, Amsterdam.

Amaral O., Marcao A., Pinto E., Zimran A. and Sa Miranda M. 1997 Distinct haplotype in non-Ashkenazi Gaucher disease patients with N370S mutation. *Blood Cells Mol. Dis.* **23**, 416–425.

Beutler E. and Kuhl W. 1986 Glucocerebrosidase processing in normal fibroblasts and in fibroblasts from patients with type I, type II and type III Gaucher disease. *Proc. Natl. Acad. Sci. USA* **83**, 7472–7474.

Beutler E. and Grabowski G. 1995 Gaucher disease. In *The metabolic and molecular bases of inherited disease* (ed. C. R. Scriver and A. Beaudet), 7th edition, pp. 2641–2670. Mc Graw-Hill, New York, USA.

Cormand B., Harboe T., Gort L., Campoy C., Blanco M. and Chamoles N. 1998 Mutation analysis of Gaucher disease patients from Argentina: High prevalence of the Rec*NciI* mutation. *Am. J. Med. Genet*. **80**, 343–351.

Charrow J., Andersson H., Kaplan P., Kolodny E., Mistry P., Pastores G. *et al.* 2000 The Gaucher registry: demographics and disease characteristics of 1698 patients with Gaucher disease. *Arch. Intern. Med*. **160**, 2835–2843.

Choy F., Zhang W., Hui-Ping S., Zay A., Campbell T., Tang N. and Ferreira P. 2007 Gaucher disease among Chinese patients: review on genotype/phenotype correlation from 29 patients and identification of novel rare alleles. *Blood Cells Mol. Dis.* **38**, 287–293.

Daniels L., Glew W., Diven R., Lee E. and Radin N. 1981 An improved fluorometric leucocyte β-glucosidase assay for Gaucher's disease. *Clin. Chim. Acta* **115**, 369–375.

Dvir H., Harel M., Mc Carthy A., Toker L., Silman I., Futerman H. and Sussman J. 2003 X-ray structure of human acid-β-glucosidase, the defective enzyme in Gaucher disease. *EMBO Rep.* **4**, 704–710.

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

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

Koprivica V., Stone D., Park J., Callahan M., Frisch A. and Cohen I. 2000 Analysis and classification of 304 mutant alleles in patients with type 1 and type 3 Gaucher disease. *Am. J. Hum. Genet*. **66**, 1777–1786.

Lahiri D. and Nurnberger J. 1991 A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res.***19**, 5444.

Lau E., Tayebi N., Ingraham L., Winfield S., Koprivica V., Stone D. *et al*. 1999 Two novel polymorphic sequences in the glucocerebrosidase gene region enhance mutational screening and founder effect studies of patients with Gaucher disease. *Hum. Genet.* **104**, 293–300.

Paradisi I. and Arias S. 2010 Marked geographic aggregation of acute intermittent porphyria families carrying mutation Q180X in Venezuelan populations, with description of further mutations. *J. Inherit. Metab. Dis*. **33**, suppl 3, S455–S463.

Paradisi I., De Freitas L. and Arias S. 2015 Most frequent mutation c.3402delC (p.Ala1135GlnfsX13) among Wilson disease patients in Venezuela has a wide distribution and two old origins. *Eur. J. Med. Genet*. **58**, 59–65.

Peters S., Coffee R., Glew R., Lee D., Wenger S. and Li Y. 1977 Isolation of heat-stable glucocerebrosidase activators from the spleens of three variants of Gaucher disease. *Arch. Biochem. Biophys*. **183**, 290–297.

Rockah R., Narinsky R., Frydman M., Cohen I., Zaizov R., Weizman A. and Frisch A. 1998 Linkage disequilibrium of common Gaucher disease mutations with a polymorphic site in the *Pyruvate Kinase (PKLR)* gene. *Am. J. Med. Genet*. **78**, 233–236.

Rodríguez-Marí A., Díaz-Font A., Chabás A., Pastores G., Grinberg D. and Vilageliu L. 2001 New insights into the origin of the Gaucher disease-causing mutation N370S: extended haplotype analysis using the 5GC3.2, 5470G/A, and ITG6.2 polymorphism. *Blood Cells Mol. Dis*. **27**, 950–959.

Stirnemann J., Vigan M., Hamroun D., Heraoui D., Rossi-Semerano L., Berger M. *et al*. 2012 The French Gaucher's

disease registry: clinical characteristics, complications and treatment of 562 patients. *Orphanet. J. Rare Dis*. **7**, 1172–1177.
Stone D., Tayebi N., Orvisky E., Stubblefield B., Madike V. and Sidransky E. 2000 Glucocerebrosidase gene mutations in patients with type 2 Gaucher disease. *Hum. Mutat*. **15**, 181–188.
Tuteja R., Bembi B., Agosti E. and Baralle F. 1993 1448C mutation linked to the Pv1.1^{-} genotype in Italian patients with Gaucher disease. *Hum. Mol. Genet*. **2**, 781–784.
Vellodi A., Bembi B., de Villemeur T., Collin-Histed T., Erickson A., Mengel E. *et al*. 2001 Neuronopathic Gaucher disease task force of the European working group on Gaucher diseases. Management of neuronopathic Gaucher disease: a European consensus. *J. Inherit. Metab. Dis.* **24**, 319–327.
Wilches R., Vega H., Echeverri O. and Barrera L. 2006 Los haplotipos colombianos de la mutación N370S causante de la enfermedad de Gaucher pueden provenir de un haplotipo ancestral común. *Biomédica* **26**, 434–441.
Zimran A., Gelbart T. and Beutler E. 1990 Linkage of the PvulI polymorphism with the common Jewish mutation for Gaucher disease. *Am. J. Hum. Genet*. **46**, 902–905.

Corresponding editor: KUNAL RAY