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Nine-year experience in Gaucher disease diagnosis at the Spanish reference center Fundación Jiménez Díaz

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ABSTRACT

Background: Fundación Jiménez Díaz (FJD) is a reference center for genetic diagnosis of Gaucher disease (GD) in Spain. Genetic analyses of acid β-glucosidase (*GBA*) gene using different techniques were performed to search for new mutations, in addition to those previously and most frequently found in the Spanish population. Additionally, the study of the chitotriosidase (*CHIT1*) gene was used to assess the inflammatory status of patients in the follow-up of enzyme replacement therapy (ERT). We present the genetic data gathered during the last nine years at FJD.

Methods: Blood samples from patients with suspected GD were collected for enzymatic and genetic analyses. The genetic analysis was performed on DNA from 124 unrelated suspected cases and 57 relatives from 2007 to 2015, starting with a mutational screening kit, followed by Sanger sequencing of the entire gene and other techniques to look for deletions. *CHIT1* was also studied to assess the reliability of this biomarker.

Results: In 46 out of 93 GD patients (49.5%) the two mutant alleles were found. We detected 21 different mutations. The most common mutation was N370S (c.126A > G; p.Asp409Ser current nomenclature) (in 50.5% of patients), followed by L444P (c.1448T > C; p.Leu483Pro current nomenclature) (in 24.7%). The most common heterozygous compound genotype observed (18.3%) was c.1226A > G/c.1448T > C (N370S/L444P). Two novel mutations were found (del. Ex.4–11 and c.1296G > T; pW432C), as well as p.S146L, only once previously reported. Two patients showed the homozygous state for the duplication of *CHIT1*.

Conclusion: N370S and L444P are the most common mutations and other mutations associated to Parkinson's disease have been observed. This should be taken into account in the genetic counseling of GD patients.

1. Introduction

Gaucher disease is an autosomal, recessively inherited, lysosomal storage disorder due to the deficiency of the glucocerebrosidase enzyme, as a consequence of mutations in the acid β-glucosidase (*GBA*) gene (NM_000157; NP_000157) [1,2]. These mutations include multiple pathologic variants in the coding region of the gene, splicing mutations [3], and even recombination events with the *GBA* pseudogene [4]. The *GBA* gene is located at chromosome 1q22 coordinates 1:155,204,238–155,214,652 (from NCBI) and comprises 12 exons [5,6], and the pseudogene, with 96% homology, is localized 16 kb downstream in 3′sense [4].

The deficiency in glucocerebrosidase (UniProt-P04062) results in accumulation of glucosylceramide or glucocerebroside, particularly within the lysosomes of macrophages, which become enlarged, giving rise to the so called "Gaucher cells" [7]. However, the accumulation of glucocerebroside may also occur as a consequence of saposin C deficiency, since saponin C is an activator of glucocerebrosidase essential for its normal function [8]. Therefore, mutations in the Sap C domain of the *PSAP* gene, coding the precursor protein prosaposin, also result in a Gaucher-like phenotype [9].

Classically, Gaucher disease has been classified into the most common non-neuronophatic type 1 (OMIM#230800), and those with neurologic involvement, type 2 (OMIM#230900) and type 3 (OMIM#2301000), which are the neuronopathic acute and subacute forms, respectively [10]. There is a distinct form of type 2 (perinatal lethal) [11], and a distinct form of type 3 (cardiovascular or type 3C) [12].

Type 1 GD presents vast clinical variability [2,13], although common signs and symptoms are cytopenia, hepatosplenomegaly, and bone

Abbreviations: FJD, Fundación Jiménez Díaz; GD, Gaucher disease; GBA, acid β-glucosidase; ERT, enzyme replacement therapy.

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disorders, such as osteoporosis, bone pain, bone lesions and/or pathologic fractures [14]. The form and severity of clinical presentation does not correspond to level of enzyme deficiency, nor involved mutations [2,7,13], although some genotypes are associated with a specific time of presentation and/or severity. For example, the homozygous c.1226A > G (legacy name N370S) genotype is associated with later onset and milder visceral and bone manifestations (this allele protects from neurological involvement) [15], or the homozygous 1342G > C (D409H) genotype is associated to cardiovascular and ocular abnormalities [12].

Types 2 and 3 show neurologic involvement, along with systemic manifestations similar to those of type 1. Patients with type 2 may present early with rapidly progressive encephalopathy, which may cause death at 2–4 years of age. In type 3, progression of neurologic involvement is usually slower than in type 2, and the major neurological manifestation is early development of horizontal supranuclear gaze palsy [16]. Other symptoms include cognitive impairment, myoclonic epilepsy, ataxia, and spasticity, which develop as the illness progresses [17]. The subtype perinatal lethal is associated to non-immune hydrops fetalis and ichthyosis [18]. Cardiovascular subtype is characterized by aortic and mitral calcifications along with mild splenomegaly, supranuclear gaze palsy and corneal opacities [12].

Despite the low overall prevalence, GD is one of the most common of the sphingolipidoses [19,20]. It is well-known that there is a higher prevalence among Ashkenazi Jews, with a carrier frequency of 8.9% and a birth incidence of 1:450 [21]. However, in the Iberian Peninsula, the prevalence of GD is 1 in 149,000 inhabitants, with c.1226A > G (N370S) and c.1448T > C (L444P) as the most frequent mutations [22].

(From this point on we are going to refer to the mutations according to the current nomenclature. We refer the reader to Table 1 where we gather the legacy name and the current name of the mutations present in this paper).

The difference between the legacy name and the current name is that the latter considers the first 39 amino acids of the complete protein before the post-translational modifications.

Fundación Jiménez Díaz (FJD) is a reference center for genetic diagnosis of GD in Spain. Patients suspected of GD based on clinical symptoms, are assessed for glucocerebrosidase activity in total white cells, mononuclear cells, fibroblasts and dried blood on filter paper, and when activity is low, the diagnosis is confirmed by molecular analysis of the *GBA* gene [23].

However, certain particularities apply, since there are specific mutations, which are more commonly found in the Spanish population [24]. In addition, the chitotriosidase gene is studied in the routine diagnostic protocol of GD in order to assess the value of this biomarker on the follow-up of the individual patient.

Therefore, it was of interest to present the data gathered from 2007 until 2015 at the genetics department of FJD, showing the particularities to look for regarding Gaucher disease in the Spanish population, and the modifications introduced in some diagnostic techniques that lead to time and economic savings.

Table 1
Legacy and current name of *GBA* mutations NM_001005741.2.

Legacy name	Current name
N370S	c.1226A > G; p.N409S
L444P	c.1448T > C; p.L483P
R120W	c.475C > T; p.R159W
N188S	c.680A > G; p.N227S
N188 K	c.681T > G; p.N227K
G377S	c.1246G > A; p.G416S
D409H	c.1342G > C; p.D448H
del. Ex.4–11	2 (1)

2. Methods

Samples from 124 unrelated cases of suspected GD and from 57 relatives were referred to the FJD from different localities of Spain, from 2007 to 2015, and assessed at the Department of Genetics, and from 2013 onwards also at the Department of Biochemistry, for diagnosis.

2.1. Enzymatic analyses

The primary screening for acid β-glucosidase (GBA) deficiency was performed on dry-blood spots using the method described by Chamoles et al. [25] with minor modifications.

Blood samples were collected on Whatman paper cards printed with dashed-line circles of 12 mm diameter where blood spots were dispensed according to laboratory instructions.

Assays were performed on 3.2 mm (1/8 in.) punches after treatment with extraction buffer (citrate-phosphate 0.4 M at pH 5.2). Samples were incubated for 20 h with 4-MU-β-Glu using 4-methylumbelliferyl-β-*d*-glucopyranoside 20 mM as substrate in the presence of sodium deoxytaurocholate (0.75 w/v) (Raghavan, Topol, and Kolodny 1980). Samples were run in duplicate. The β-d-glucosidase activity was detected after fluorometric measurement of each determination (355 nm ex/460 nm em).

Normal values for GBA assay range from 0.8 to 5.7 nmol/L^{-1}/h^{-1}. Subjects with activities of <30% of normal average value were re-analyzed on leukocytes using the same protocol; additionally, if the enzymatic defect was confirmed, the diagnosis was completed by genetic analysis of the *GBA* gene.

2.2. Genetic analysis

2.2.1. Screening of eight frequent GBA gene mutations

After extracting DNA from peripheral leukocytes using standard methods (*Bio robot EZ1 Qiagen*), the diagnostic algorithm (Fig.1) starts by analyzing the DNA by mutational screening for the 8 most frequent Gaucher mutations in the European population [22]: *84GG [452 + G],* IVS2 + 1 [484 G > A], *N370S [1226 A > G],* V394L [1297 G > T], *D409H [1342 G > C]*, L444P [1448 T > C], *R463C [1504 C > T]*, R496H [1604 G > A] (Gaucher disease StripAssay® - ViennaLab Diagnostics GmbH, Vienna, Austria).

2.2.2. Direct sequencing of GBA gene

When a single mutation or no mutation was found, PCR amplification of the 12 exons of the *GBA* was performed followed by the Sanger method (*Abiprism 3130*; *Sequencing Analysis v.5.2*) (primers listing available upon request). All new variants were evaluated using the predictor PolyPhen-2 [26].

The presence of the c.(−203)A > G g.1256A > G variant in the non-coding exon 1 of *GBA,* described by Alfonso et al. [27], was also assessed. The PCR reaction was performed using the primers designed by the cited author, followed by Sanger sequencing (Abiprism 3130; Sequencing Analysis v.5.2).

2.2.3. Screening for GBA gene deletions

Multiplex Ligation-dependent Probe Amplification (MLPA) using P338-X1 GBA probemix (MCR-Holland) was used to detect duplications and deletions.

High Resolution Melting (Lightcycler 480; Roche) was performed in order to determine the genetic dose in one family.

2.2.4. Haplotype analysis

Indirect genetic analysis was performed with STR markers (D1S2777, D1S2140 and D1S2721), localized next to the *GBA* gene [28].

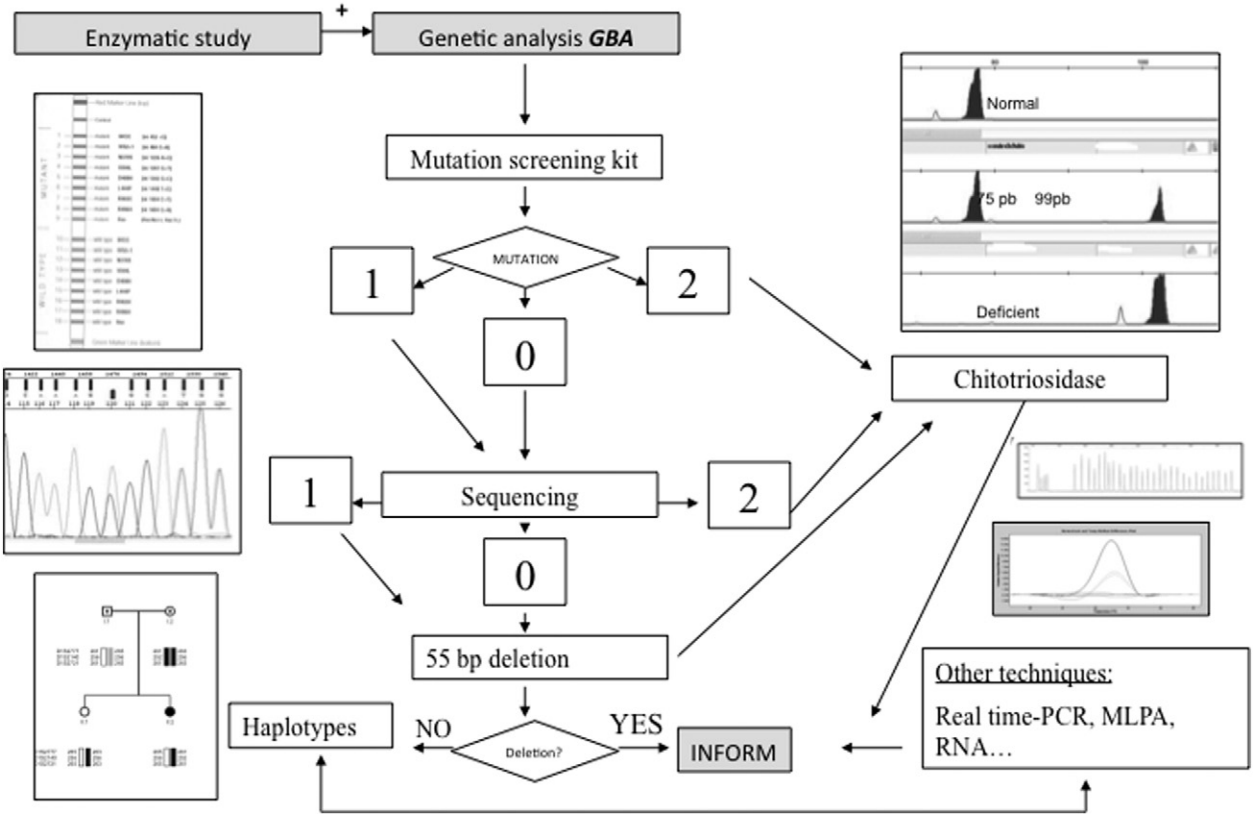

Fig. 1. Genetic diagnostic algorithm for Gaucher disease.

2.2.5. Chitotriosidase gene analysis

To rule out a duplication of 24 bp in exon 10 of *CHIT1*, the following primers were used: CHITO-S-PET: 5′agctatctgaagcagaag 3′ and CHITO-AS: 5′ggagaagccggcaaagtc 3′. Wild type sequence gives rise to a 75 bp fragment while the mutant sequence gives rise to a 99 bp fragment [29].

3. Results

A total of 181 individuals, from 124 non-related families, have been subjected to molecular study for GD from 2007 to 2015. Out of the 181 subjects, 124 were patients with suspected clinical diagnosis and 57 were family members of these patients. Out of the initially suspected 124 patients, 65 had available glucocerebrosidase activity, 31 of whom showed activity of 30% or higher and were ruled out (3 of them were, incidentally, carriers of p.N409S and 1 of p.L375R) (Table 2). The group of index patients included, thus, 93 individuals.

Clinical manifestations of only 14 of the 93 patients were available and are shown in Table 3. The most frequent type of presentation was the type 1. It is worth pointing out that the patient of family 10 was homozygous for the p.L483P, had no history of neurological affectation although he had hepatomegaly, enlarged spleen, anemia, recurrent respiratory infections, and symptomatology that started in childhood.

The mutations found in the patients diagnosed as true GD cases are shown in Table 4.

Twenty-one different mutations were identified. The most common mutation was p. N409S, which was found in 47 patients (50.5%) (3 in homozygosis), followed by p.L483P, which was found in 23 patients (24.7%) (2 in homozygosis). The genotype p.N409S/p.L483P was the most frequent one (17 patients; 18.3%) and the second in frequency was p.N409S/p.R159W (4.3% of patients). In addition, p.N409S was found in heterozygosis with other mutations in 23 patients (in 11 of these later ones, the second mutation has not yet been identified). A total of 47 patients were diagnosed as GD based on enzymatic analysis and/or phenotype but are still lacking genetic diagnosis.

The second genotype in frequency was p.N409S/p.R159W (R120W), this genotype represents 4.3% of our series. The next most prevalent genotype (2.1% each) was formed by the following 3 combinations: homozygous p.L483P, compound heterozygous p.N409S/p.N227S and compound heterozygous p.N409S/c.1263-1317del55.

All homozygotes should be tested for deletions, since they might be a cause of misdiagnosis for these patients, as it has been shown for homozygous p.N409S [30]. One of the p.N409S homozygote patients in our series had a deletion of 55 bp in the exon 9 GBA gene.

Another case (family 74) had a deletion of 8 exons of the gene, not previously described: del. Ex.4–11. This patient was a female (deceased prior to 5 years of age) and initially thought to be homozygous for p.G241R c.721G > A; p.Gly241Arg (p.Gly202Arg legacy name). However, genetic analysis of the parents revealed that only the mother had the p.G241R mutation. A genetic study with markers D1S2777, D1S2140 and D1S2721 was used to identify the paternal haplotype. MLPA technique SALSA (P.338 X.1) showed that both patient and her father had a non-previously described deletion spanning from exon 4 through exon 11 (Fig. 2).

Another p.N409S heterozygote patient had the c.437C > T p.Ser146Leu mutation (dbSNP 758447515). This was a novel missense mutation that causes an amino acid change from serine to leucine, which had been predicted by the Polyphen 2 program as “probably harmful” with a score of 0.99.

We also found a heterozygous change at exon 11, specifically c.1296G > T;p.Trp432Cys (legacy p.Trp393Cys).

Eight of the patients that had one single mutation detected were screened for c.(−203)A > G g.1256A > G but none had this variant form.

Two patients showed the 24 bp duplication in exon 10 of the *CHIT1* in an homozygous state and 20 were heterozygous. The rest were normal.

Table 2
Available enzymatic activity data of suspected patients.

Fam.	Mutations	Enz. activity	Patient status	According to enz. act.
4	?/ex. 8 neg	Undetectable	GD	True +
7	?/p.L375R; c.1124T > G	88%	Ruled out CARRIER	True –
8	N370S/ p.Arg159Trp; c.475C > T	30%	GD	True +
9	N370S/ ex. 3–13 neg ZC	15%	GD	True +
10	L444P/L444P	7%	GD	True +
11	N370S/?	4%	GD	True +
12	NEG	45%	Ruled out	True –
15	N370S/?	80%	Ruled out	True –
16	N370S/?	Undetectable	GD	True +
24	N370S/L444P	10%	GD	True +
26	N370S/?	100%	Ruled out CARRIER	True –
29	N370S/ p.Arg87Trp; c.259C > T	16%	GD	True +
30	N370S/?	40%	Ruled out CARRIER	True –
31	N370S/?	5%	GD	True +
32	N370S/?	20%	GD	True +
34	NEG	54%	Ruled out	True –
35	N370S/L444P	27%	GD	True +
36	NEG	39%	Ruled out	True –
37	N370S/L444P	19%	GD	True +
38	?/?	27%	GD	True +
39	?/?	15%	GD	True +
41	N370S/ p.Arg159Trp; c.475C > T	11%	GD	True +
42	?/?	2%	GD	True +
45	N370S/L444P	13%	GD	True +
46	N370S/L444P	17%	GD	True +
47	NEG	100%	Ruled out	True –
48	NEG	100%	Ruled out	True –
49	?/?	13%	GD	True +
50	D409H/ p.Gly241Arg; c.721G > A	25%	GD	True +
51	NEG	58%	Ruled out	True –
52	NEG	139%	Ruled out	True –
53	NEG	98%	Ruled out	True –
53	NEG	100%	Ruled out	True –
55	NEG	90%	Ruled out	True –
56	NEG	100%	Ruled out	True –
57	Was notperformed	100%	Ruled out	True –
59	NEG	100%	Ruled out	True –
61	?/?	<30%	GD	True +
61	?/?	<30%	GD	True +
64	NEG	100%	Ruled out	True –
69	NEG	77%	Ruled out	True –
70	?/?	46%	Ruled out	True –
72	?/?	12%	GD	True +
73	?/?	14%	GD	True +
75	NEG	100%	Ruled out	True –
76	N370S/?	100%	Ruled out CARRIER	True –
80	N370S/ p.Arg202*; c.604C > T	20%	GD	True +
85	N370S/ p.Trp36*; c.108G > A	33%	GD	False negative
89	NEG	100%	Ruled out	True –
93	NEG	100%	Ruledout	True –
94	NEG	100%	Ruledout	True –
100	L444P/ F252I; c.754T > A	30%	GD	True +
101	N3705S/L444P	30%	GD	True +
105	N3705S/L444P	8%	GD	True +
107	N3705S/L444P	18%	GD	True +
110	NEG	100%	Ruledout	True –
112	NEG	100%	Ruledout	True –
113	NEG	100%	Ruledout	True –
	N3705S/?	18%	GD	True +
115	N3705S/N188K	10%	GD	True +
117	NEG	100%	Ruledout	True –
118	NEG	100%	Ruledout	True –
119	NEG	100%	Ruled out	True –
121	?/?	19%	GD	True +
124	c.680A > G p.Asn227Ser/ c.475C > T p. Arg159Trp	16%	GD	True +

Cutoff value: 30%
Solid grey boxes: GD cases
Striped boxes: Carriers
White boxes: Non GD cases
In bold: False negative

Table 3
Clinical manifestations of Gaucher's disease patients. Available in 14 out of 93 patients.

Family	Genotype	Enzymatic Activity	Neurologic symptoms	Hepatomegaly	Splenomegaly	Anemia	Thrombocytopenia	Leucopenia	Bone involvement	Type Debut
10	c.1448T>C/c.1448 T>C	NA	–*	Present	Present	Present	–	–	–	Type? Childhood
15	c.1226A > G/?	NA	–	Present	–	–	–	Present	Present	Type 1 Childhood
16	c.1226A > G/?	NA	–	–	Present	–	Present	–	Present	Type 1 Adulthood
24	c.1226A > G/c.1448T > C	NA	–	–	Present	–	–	–	–	Type 1 Adulthood
27	c.1226A > G/c.1448T > C	NA	–	–	Present	–	–	–	–	Type 1 Childhood
30	c.1226A > G/?	NA	–	Present	Present	Present	Present	–	Present	Type 1
31	c.1226A > G/?	NA	–	–		Present	–	–	Present	Type 1
32	c.1226A > G/?	20%	–	–	Present	Present	Present	–	Present	Type 1
37	c.1226A > G/c.1448T > C	NA	–	Present	Present	–	Present	–	–	Type 1
41	c.1226A > G/?	NA	–	–	Present	Present	–	Present	–	Type 1
45	c.1226A > G/c.1448T > C	NA	–	–	Present	–	Present	Present	Present	Type 1 Childhood
46	c.1226A > G/c.1448T > C	NA	–	–	Present	–	Present	–	Present	Type 3 Childhood
50	D409H-p.Gly241Arg	NA	Present	Present	Present	Present	Present	–	Present	Type 2 Childhood
100	c.1448T > C/c.754T > A	NA	Present	–	Present	–	Present	–	–	Type 2 Childhood

?: Unidentified; — Absent; *No neurological symptoms at diagnosis at age 12 months; NA: not available.

Table 4
Mutations found in the GD cases.

Mutation 1	Mutation 2	*N* = 93	
		Frequency absolute; n	Frequency relative; %
c.680A > G p.N227S	c.475C > T p. R159W (R120W)	1	1%
c.721G> A p.G241R	c.155C > T p.S52L	1	1%
c.1226A > G p.N409S (N370S)	c.108G > A p.W36*	1	1%
c.1226A > G p.N409S (N370S)	c.259C > T p.R87W	1	1%
c.1226A > G p.N409S (N370S)	c.437C > T p.S146L	1	1%
c.1226A > G p.N409S (N370S)	c.475C > T p.R159W (R120W)	4	4.3%
c.1226A > G p.N409S (N370S)	c.604C > T p.R202*	1	1%
c.1226A > G p.N409S (N370S)	c.680A > G p.N227S	2	2.1%
c.1226A > G p.N409S (N370S)	c.681T > G p.N227 K	1	1%
c.1226A > G p.N409S (N370S)	c.1226A > G p.N409S (N370S)	3	3.2%
c.1226A > G p.N409S (N370S)	c.1246G > A p.G416S	1	1%
c.1226A > G p.N409S (N370S)	c.1263-1317del55	2	2.1%
c.1226A > G p.N409S (N370S)	c.1296G > T p.W432C	1	1%
c.1226A > G p.N409S (N370S)	c.1448T > C p.L483P (L444P)	17	18.3%
c. 1342G > C p. D448H (D409H)	c.721G > A p.G241R	1	1%
c.1448T > C p.L483P (L444P)	c.731A > G p.Y244C	1	1%
c.1448T > C p.L483P (L444P)	c.754T > A p. F252I	1	1%
c.1448T > C p.L483P (L444P)	c.1246G > Ap.G416S	1	1%
c.1448T > C p.L483P (L444P)	c.1342G > C p.D448H (D409H)	1	1%
c.1448T > C p.L483P (L444P)	c.1448T > C p. L483P (L444P)	2	2.1%
c.721G > A p.G241R	del. Ex.4–11	1	1%
c.1226A > G p.N409S (N370S)	24pbc.42_65del	1	1%
c.1207A > G p.S403R	?	1	1%
c.1226A > G p.N409S (N370S)	?	11	11.8%
?	?	35	37.6%

?: Unidentified.

4. Discussion

Fundación Jiménez Díaz is a reference center for GD diagnosis in Spain. All data regarding GD genetic diagnosis in the last nine years were gathered to have a picture of the current situation of Gaucher disease in the Spanish population. This general picture may raise awareness of the national genotype distribution and of the particularities to look for in the Spanish population, and may help the physician during counseling of these patients.

Our results showed that p.N409S was the most frequent allele (26.9%), followed by p.L483P (13.4%), in agreement with previous data reported in Spain. Giraldo et al. [22]. showed an allelic prevalence of 48.5% and 18.5% for p.N409S and p.L483P, respectively, in a series of 436 patients from the Iberian Peninsula. Alfonso et al. showed a prevalence of 55% and 15%, respectively, in 51 Spanish patients [31]. Likewise, the most frequent genotype was p.N409S/p.L483P (18.3%), as in the series presented by Giraldo et al. (29.7%) [22]. The second most frequent genotype in our series was p.N409S/p.R159W (4.3%); this combination appears as the seventh in frequency and represented 1.6% in Giraldo's series published in 2012 [22].

The 55 bp deletion (c.1263del55) in exon 9 of the glucocerebrosidase gene was present as the compound heterozygous p.N409S/c.1263-1317del55 in 2.1% of patients. The *GBA* pseudogene carries a 55 bp deletion in exon 9, that if present in the *GBA* gene turns to be a Gaucher's disease allele [32]. Recombinations between the gene and the pseudogene are the source of this mutation and probably of more of the disease causing mutations [33]. This deletion, c.1263-1317del55, is highly prevalent in the Spanish population and must be suspected in every p.N409S homozygous patient [30].

The group with available phenotype information (14 patients) showed some genotype correlation coherent with previous results observed in other case series of the literature, although variable expression of the disease was observed and different phenotypes were exhibited by patients carrying the same alleles or with equal genotypes (Table 3). None of the patients with at least one allele carrying the p.N409S mutation presented neurological symptoms, in agreement with this mutation being considered a low-severity mutation [15]. The genotypes in the asymptomatic patients of the Giraldo series were p.N409S homozygotes and compound heterozygotes, as well as the p.D448H/p.G416S genotype [22]; and a recent study showed that the pre-treatment mean severity DS3 scores in GD patients were lowest among the p.N409S homozygotes [34]. On the other hand, the presence of p.L483P was associated with more severe or precocious presentation. One of the two patients with neurological manifestations carried the p.L483P mutation in heterozygosis with c.754T > A p.F252I, while the other patient was the compound heterozygote p.G241R/p.D448H. In the Giraldo series, 85.7% of type 2 GD patients were p.L483P compound heterozygotes, and the most frequent genotype for type 3 GD was p.L483P/p.L483P, in addition to p.D448H/p.D448H [22]. However, and according with the phenotype variability in Gaucher disease, the p.L483P homozygous patient did not have neurologic manifestations despite having the genotype classically associated with severe phenotypes. On one hand, this was a 12-month old boy who did not show neurologic manifestations at the time of diagnosis but we do not know his evolution. On the other hand, the phenotypic heterogeneity of Gaucher disease is believed to be due to different modulators of the disease, some of which have already been found, such as the intronic variant c.(−203)A > G g.1256A > G that affects the *GBA* promoter [27,35], or mutations in the *PSAP* gene, that affect Saponin C, an activator of GBA, resulting in a Gaucher-like phenotype [9]. Nonetheless the variant c.(−203)A > G g.1256A > G was not detected in our series.

4.1. Novel mutations

The p.S146L mutation present in a child of our series predicted by the Polyphen 2 program as “probably harmful” was only previously reported by Demina and Bleuter in an infant [36], and has been classified in the HGMD(r) database as disease causing [37], although this variant is still classified by the National Center for Biotechnology Information (NCBI) as being of uncertain significance [38]. Population frequency of this variant is 1%.

Another two novel mutations were found. The deletion spanning exons 4 through 11 (del. Ex.4–11) found in a female patient (deceased before age of 5) and her father, and c.1296G > T; p.W432C. This last mutation codifies the change of tryptophan to cysteine at codon 432 of the RNA, and has not yet been described; however, there are two known

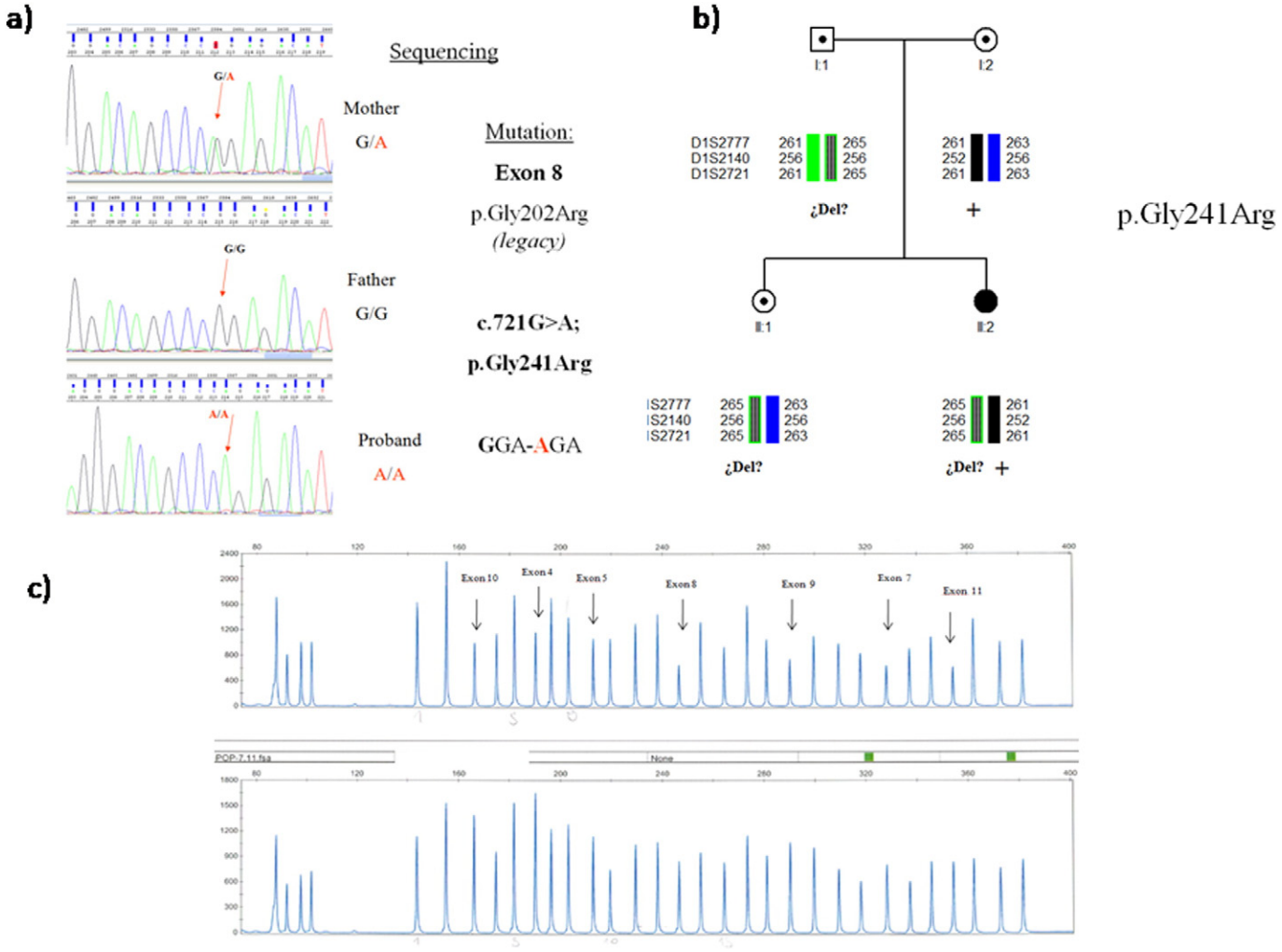

Fig. 2. Results of different techniques for the diagnosis of a patient having the genotype c.721G > A; del Ex.4–11. a) Sanger sequencing: Mother DNA showing the mutation c.721G > A in heterozygosis, and father and proband DNA, which could be misdiagnosed as homozygous for WT and c.721G > A, respectively. b) Haplotypes showing that II:1 and II:2 share the same paternal allele. c) MLPA SALSA P338X1 showing the difference of genetic dose between proband (upper image) and control (lower image), with each peak corresponding to a specific region of the *GBA* gene.

mutations at the same codon that have already been associated with Gaucher disease: c. 1295G > T p. W432L [33], and c.1294T > A p.W432R [39]. Considering the GD causing effect of the previous identified mutations at that same codon and the fact that the amino acid change that occurs with this novel mutation is from tryptophan, an amino acid with an aromatic radical, to cysteine, a neutral amino acid with the potential of create disulphide bonds, it is reasonable to assume that this variant could likely alter the tertiary structure of the protein and have functional consequences that may cause GD.

Regarding the patient carrying the del. Ex.4–11 mutation, it was thanks to using the MRC-Holland® MLPA reagents kit SALSA P.338 X.1, that the rapid identification of the deletion was possible.

4.2. Relationship between GBA mutations and Parkinson disease

The variants p.R159W, p.N227S, p,G416S and p.L483P in heterozygosis have been correlated with familial Parkinson's disease among Asian populations [40,41]. The susceptibility for Parkinson's disease in carriers of mutations in *GBA* was first described by Goker-Alpan in 2004 [42]. Since then, multiple retrospective and prospective studies have been performed on the association of GD and PD [43–46], and evidence has been found for an association between single nucleotide polymorphisms in *GBA* and Parkinson's disease age of onset and progression [43,46]. This knowledge must make us change or at least rethink the genetic counseling that we are providing to our patients and their families.

4.3. Chitotriosidase

As mentioned earlier, chitotriosidase evaluation is fundamental for the patient's follow-up, since it is a reliable biomarker of the disease activity. Chitotriosidase is produced at high levels by Gaucher cells [47], and its level drops when the activity of the disease is low, as in patients receiving enzyme replacement therapy (ERT) [48]. However, about 6% of the population shows a deficiency of the enzyme, due to the homozygous presence of a 24 bp duplication in exon 10 of the *CHIT1* gene that activates a cryptic 3´ splice site, resulting in an in frame deletion of amino acids 344–372 in the open reading frame (ORF) of the spliced mRNA, which renders the enzyme inactive [29]. Twenty patients carried this mutation in the chitotriosidase gene in heterozygosis and two in homozygosis (2%); thus, chitotriosidase could not be used as a marker in these latter two patients.

5. Conclusions

In the last 9 years, the GD Spanish population has shown p.N409S and p.L483P as the most common mutations. Rare *GBA* mutations previously shown as being associated with familial and sporadic Parkinson's

disease have also been observed; thus, physicians should take this association into account in the genetic counseling of the patients, and the carriers identified in the family, as well. Patients and their family carrying these mutations should be made aware of the association to Parkinson disease to be on the lookout for putative symptoms of the disease. The disease presented phenotypic heterogeneity, as it is usual for Gaucher disease, and some of the mutations involved have not yet been identified. Further research with current technologies (New Generation Sequencing) and with the growing knowledge of RNA should be carried out to identify other genes involved and other factors that could modulate the expression of the disease.

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