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Gaucher disease: Biochemical and molecular findings in 141 patients diagnosed in Greece

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ABSTRACT

Gaucher disease (GD) is characterized by a marked phenotypic and genetic diversity. It is caused by the functional deficiency of the lysosomal enzyme β-glucocerebrosidase (GCase), which in most instances results from mutations in the *GBA1* gene and over 500 different disease causing mutations have been described. We present the biochemical and molecular findings in 141 GD cases (14 were siblings) with the three types of the disorder diagnosed in Greece over the last 35 years. 111/141 (78%) GD patients were of Greek origin. The remaining patients were Albanian (24/141; 17%), Syrian (2/141; 1.4%), Egyptian (2/141; 1.4%), Italian (1/141; 0.7%) and Polish (1/141; 0.7%). Mutation analysis identified 28 different mutations and 37 different genotypes. Seven of the mutations were not previously reported (T231I, D283N, N462Y, LI75P, F81L, Y135S and T482K). The most frequent mutations were N370S, D409H;H255Q and L444P. Mutation D409H;H255Q was only identified in Greek and Albanian patients. Sixteen mutations, including the novel ones, were identified only in one allele. Although the N370S mutation was identified only in type 1 patients, not all of type 1 patients carried this mutation. Our results highlight the heterogeneity of Gaucher disease and support the Balkan origin of the double mutant allele D409H;H255Q.

1. Introduction

Gaucher disease (GD) is a rare autosomal recessive disorder that belongs to the group of lysosomal storage diseases. It results from the functional deficiency of the lysosomal enzyme β-glucocerebrosidase (GCase; also called glucosylceramidase or acid β-glucosidase, E.C. 3.2.1.45) which, in most instances, is associated with mutations in the *GBA1* gene located on chromosome 1 (1q21) (MIM#606463, Gen Bank accession no J030591). The deficient enzyme activity leads to the accumulation of its substrate glucosylceramide, mainly in tissue macrophages, transforming them into the characteristic Gaucher cells. On the basis of the presence and rate of progression of neurological involvement, GD has traditionally been subdivided into three phenotypes. GD type 1 is the non-neuronopathic (MIM#230800), type 2 is the acute neuronopathic with early onset and rapid neurological deterioration (MIM#230900), whereas type 3 is the chronic neuronopathic phenotype with slower progressing neurological features [1]. However, it has become evident that this distinction is often blurred and the disease would be more correctly characterized as a continuum of phenotypes ranging from asymptomatic adult patients at the mild end to perinatal lethal disease at the severe end of the spectrum [2–5]. The *GBA1* gene is located on the long arm of chromosome 1 (1q21) with a highly homologous pseudogene (*GBAP*) located 16 kb downstream. The high degree of sequence identity and proximity of *GBA1* and *GBAP* contributes to recombination events between the two loci [6,7].

Up to date more than 500 mutations in the *GBA1* gene have been described in GD patients (HGMD professional 2019.4) highlighting the molecular heterogeneity of the disorder.

Some of these mutations, such as N370S, L444P, c.84dup, IVS2 + 1 G > A and RecNciI, are more common. Differences in the frequency of different mutations is observed in different populations. For instance, mutations N370S, L444P, c.84dupG and c.115 + 1G > A account for 90% of the mutant alleles in Ashkenazi Jewish patients, whereas they account for < 75% of the mutant alleles in other populations [7–9].

Although genotype - phenotype correlations are not straightforward, it is possible to draw some predictions. For example, the N370S mutation is only found in association with type 1 GD whereas

Abbreviations: GBA1, Glucocerebrosidase gene; GD, Gaucher disease; GCase, β-Glucocerebrosidase

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Table 1
Age of diagnosis and clinical characteristics of the Gaucher disease patients diagnosed in Greece.

GD patients	Age of diagnosis	Reasons for referral
Type 1 *n* = 120 (59F-61M)	3–77 years Median 28 years	Splenomegaly Hepatosplenomegaly Bone Involvement Chololithiasis Thrombocytopenia Pancytopenia Leucopenia Anemia Member of patient family
Type 2 *n* = 13 (9F-4M)	Birth – 8 months Median 1 month	Hepatosplenomegaly Hydrops Fetalis, Collodion Baby Ascites Bilirubinemia, ↑ transaminases Seizures, Hypertonia, Opisthotonus Irritability, Oculomotor Apraxia
Type 3 n = 8 (3F-5M) F: Female,	10 months – 8 years Median 2.5 years M: Male	Hepatosplenomegaly Seizures, Opisthotonus, horizontal supranuclear paralysis, Pshychomotor retardation

homozygosity for mutation L444P is generally associated with some neurological involvement. Yet, considerable heterogeneity in disease manifestations and severity can be observed in patients homozygous for the same mutation and even in identical twins [10].

We present the biochemical and molecular findings in 141 (14 were siblings) cases of GD diagnosed in Greece the last 35 years. 111/141 (11 were siblings) were of Greek origin and 30/141 (3 were siblings) of other ethnic origins, mainly Albanians, living in Greece. We also describe 7 novel mutations identified in our cohort of GD patients.

2. Patients and methods

2.1. Patients

The present report includes 141, 111 of Greek origin and 30 of other ethnic origins, GD patients of which 14 were siblings. They were referred for diagnosis to the Institute of Child Health on the basis of their clinical evaluation and/or laboratory studies.

A brief description of the patients is shown in Table 1.

The laboratory investigations performed include assaying of chitotriosidase activity in plasma, assaying of GCase in white blood cells and/or skin fibroblast cultures and DNA analysis. The study was approved by the Ethics Committee of the Institute of Child Health, Athens.

2.2. Methods

2.2.1. Enzyme studies

Chitotriosidase activity was assayed in plasma using the 4-methylumbelliferyl β-D-N, N′, N»– triacetylchitotrioside substrate [11].

GCase activity was assayed in white blood cells isolated from heparinized blood and/or cultured fibroblast homogenate using the fluorogenic substrate 4-methylumbelliferyl β-D-glucopyranoside [12].

For mutation F81L, site-directed mutagenesis was performed in a pcDNA3 plasmid bearing the WT *GBA1* cDNA. The plasmid was transfected in COS7 cells and enzyme activity was measured in comparison to that of cells transfected with the WT cDNA.

2.2.2. Molecular analysis

Mutations N370S, D409H; H255Q, L444P, R120W, IVS10-1G > A, IVS6-2A > G and Y108C were investigated by PCR and restriction enzyme analysis. Automated sequencing of all exons and flanking regions of the *GBA1* gene was applied for the identification of the rest of the mutations. Automated sequencing was also used, when appropriate, to study the presence of Rec alleles and discriminate between L444P and L444R.

The prediction tools used for investigating the pathogenicity of novel mutations were PolyPhen-2 HumDiv and Hum Var; SIFT Human Protein and SIFT Blink; Mutation Taster.

Table 2
Biochemical findings in the Gaucher disease patients diagnosed in Greece.

Patients	Chitotriosidase Activity (nmoles/ml/h)	β-Glucosidase Activity WBC (nmoles/mg Protein/h)	β-Glucosidase Activity Fibroblasts (nmoles/mg Protein/h)
Type 1	426–35,825[a] mean: 12545 *n* = 113	0.25–4.6 mean: 1.89 *n* = 97	0–8.0 Mean: 3.47 *n* = 23
Type 2	720–7236 mean: 2948 n = 11	0.25–3.5 mean: 1.3 *n* = 12	0.34–4.0 Mean: 2.0 *n* = 8
Type 3	8332–35,000 mean: 15232 n = 8	1.9–2.5 mean: 2.18 *n* = 6	0–1.6 mean: 0.8 n = 2
Normal Range	0–150	6–23	19–113

[a] Two patients had zero chitotriosidase activity being homozygotes for the 24 bp duplication.

3. Results

The demographics of all the patients diagnosed, 111 of Greek origin and 30 of other ethnic origins, are shown in Table 1. Depending on the presence and severity of neurological findings they were classified as Type 1 (*n* = 120), Type 2 (*n* = 13) and Type 3 (*n* = 8). Age of diagnosis for the type 1 patients ranged from 3 to 77 years, for the type 2 cases from birth to 8 months and for type 3 from 10 months – 8 years.

The diagnosis of GD patients involved assaying of the activity of chitotriosidase and GCase and was completed with DNA molecular analysis. All, but two patients homozygous for the 24 bp duplication in the *CHIT1* gene, showed increased chitotriosidase activity in plasma. Assaying of GCase activity in white blood cell and/or in cultured skin fibroblast homogenates was diagnostic for all patients (Table 2).

DNA was available from 125 patients that were not siblings. 98/125 of these patients were of Greek origin and 27/125 were of other ethnic origins, mainly Albanians, living in Greece.

Mutation analysis resulted in the identification of 246/250 mutant alleles,

the unknown alleles belonging to Greek patients. Overall 28 different mutations and 37 different genotypes were identified (Tables 3 and 4).

In particular, in the Greek patients, 26 different mutations (192/196 identified alleles; 98%) and 35 genotypes were identified (Table 5), compared to 7 different mutations (54/54 identified alleles; 100%) and 10 different genotypes identified in patients that were not of Greek origin (Table 6).

N370S was the most frequent mutation accounting for 121 of the 246 identified alleles (96/192 alleles identified in the Greek patients; 50% and 25/54 alleles identified in non - Greek patients; 46.3%). The mutation was only identified in type 1 patients, however not all type 1 patients carried this mutation.

The second most frequently identified allele was the double mutant allele D409H;H255Q, with an overall frequency of 46/246 identified alleles (18.7%). It was identified only in Greek and Albanian patients. It accounted for 29/192 alleles identified in Greek patients (15%) and for 17/46 alleles identified in Albanian patients (36.9%). It was identified in heterozygosity in patients of all the types of GD but in homozygosity only in patients with type 2 disease.

Table 3
Allele distribution in our cohort of patients (n = 125).

Mutations			
cDNA[a]	Protein (Traditional in GD[b])	Protein (as recommended by HGVS[c])	No. of alleles
c.1226A > G	N370S	p.N409S	121 (49.2%)
c.1342G > C;c.882 T > G	D409H;H255Q	p.D448H;H294Q	46 (18.7%)
c.1448 T > G	L444P	p.L483P	25 (10.2%)
c.475C > T	R120W	p.R159Q	8 (3.3%)
c.1505G > A (IVS10-1G-A)			7 (2.8%)
c.762-2A > G (IVS6-2A-G)			6 (2.4%)
exons 7–11	RecNciI		5 (2.0%)
c.1043C > T	A309V	p.A348V	3 (1.2%)
c.721G > A	G202R	p.G241R	3 (1.2%)
c.886C > T	R257X	p.R296*	2 (0.8%)
c.256C > T	R47X	p.R86*	2 (0.8%)
c.260G > A	R48Q	p.R87Q	2 (0.8%)
c.440A > G	Y108C	p.Y147C	1(0.4%)
c.115 + 1 G > A (IVS2 + 1 G > A)			1 (0.4%)
c.1603C > T	R496C	p.R535C	1 (0.4%)
c.754 T > A	F213L	p.F252I	1 (0.4%)
c.1049A > G	H311R	p.H350R	1 (0.4%)
c.667 T > C	W184R	p.W223R	1 (0.4%)
c.463 T > C	Y116H	p.Y155H	1 (0.4%)
c.1342G > C	D409H	p.D448H	1 (0.4%)
c.1192C > T	R359X	p.R398*	1 (0.4%)
[d]c.809C > T	T231I	p.T270I	1 (0.4%)
[d]c.1501A > T	N462Y	p.N501Y	1 (0.4%)
[d]c.641 T > C	L175P	p.L214P	1 (0.4%)
[d]c.358 T > C	F81L	p.F120L	1 (0.4%)
[d]c.521A > C	Y135S	p.Y174S	1 (0.4%)
[d]c.964G > A	D283N	p.D322N	1 (0.4%)
[d]c.1562C > A	T482K	p.T521K	1 (0.4%)

[a] Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the first ATG translation initiation codon in the reference cDNA sequence (M16328.1)

[b] Traditional codon numbering begins 39 codons downstream from the first ATG.

[c] HGVS recommended nomenclature numbers codons beginning with the first ATG as codon 1.

[d] Mutation first described in this study which overall accounted for 1.6% of the identified alleles.

The third most frequent mutation was L444P, found in 25/246 identified alleles (10.2%). It accounted for 17/192 (8.9%) and 8/54 (14.8%) of the alleles identified in Greek and non-Greek patients, respectively. The mutation was identified in heterozygosity in type 1 and type 3, and in homozygosity in type 3 patients.

Of the remaining mutations, 16 (T231I, D283N, N462Y, L175P, R496C, F213L, H311R, W184R, R359X, F81L, Y135S, T482K, Y108C, Y116H, D409H, IVS2 + 1G → A) were only identified in single alleles in both Greek and non-Greek patients. Mutations IVS10-1G > A, IVS6-2A > G and G202R were only identified in Greek patients, accounting for 7/192 (3.6%), 6/192 (3.1%) and 3/192 (1.6%), respectively, of the identified alleles. IVS10-1G > A was identified in heterozygosity in type 1 and type 2 patients whereas IVS6-2A > G was identified in heterozygosity only in type 1 patients. G202R was identified in heterozygosity in type 1 and in homozygosity in type 2 patients. R120W was identified in heterozygosity in both type1 and type 2 Greek patients 7/192, 3.6% of the identified alleles) and in heterozygosity in one type 1 Albanian patient (1/54,1.9% of the identified alleles).

Along with the previously published mutations, 7 novel mutations were identified all in heterozygosity in our cohort of patients. Six of them (T231I, p.T270I; D283N (p.D322N); N462Y, p.N501Y; F81L, p.F120L; Y135S, p.Y174S; T482K, p.T521K) were identified in Greek type 1 patients and one (L175P, p.L214P) in an Albanian type 1 patient. Mutations in the same codon have been described for three of the above. These were mutations T231R [13], N462K [14], N462S [15], Y135C [16], Y135X [7] and were identified in GD patients and/or patients with Parkinson's disease.

Table 4
Genotypes and clinical subtypes of all diagnosed Gaucher disease patients.

Genotypes	TYPE 1 N = 106	TYPE 2 N = 12	TYPE 3 N = 7	TOTAL N = 125
N370S/D409H;H255Q	29			29 (23.4%)
N370S/N370S	17			17 (13.7%)
N370S/L444P	14			14 (11.3%)
N370S/IVS6-2A → G	6			6 (4.8%)
N370S/R120W	6			6 (4.8%)
N370S/IVS10-1G → A	5			5 (4%)
N370S/?	3			3 (2.4%)
N370S/A309V	3			3 (2.4%)
N370S/RecNciI	3			3 (2.4%)
N370S/R257X	2			2 (1.6%)
N370S/R47X	2			2 (1.6%)
N370S/R48Q	2			2 (1.6%)
N370S/T231I	1			1 (0.8%)
N370S/N462Y	1			1 (0.8%)
N370S/L175P	1			1 (0.8%)
N370S/R496C	1			1 (0.8%)
N370/F213L	1			1 (0.8%)
N370S/H311R	1			1 (0.8%)
N370S/W184R	1			1 (0.8%)
N370S/R359X	1			1 (0.8%)
N370S/D283N	1			1 (0.8%)
L444P/F81L	1			1 (0.8%)
N370S/Y135S	1			1 (0.8%)
N370S/ G202R	1			1 (0.8%)
N370S/ T482K	1			1 (0.8%)
L444P/ Y116H	1			1 (0.8%)
D409H;H255Q/D409H; H255Q		5		5 (4%)
D409H;H255Q/R120W		2		2 (1.6%)
IVS10-1G → A/D409H;H255Q		1		1 (0.8%)
D409H;H255Q/RecNciI		1		1 (0.8%)
G202R/G202R		1		1 (0.8%)
IVS2 + 1G → A/?		1		1 (0.8%)
RecNcil/IVS10-1G → A		1		1 (0.8%)
L444P/L444P			3	3 (2.4%)
L444P/D409H;H255Q			2	2 (1.6%)
D409H;H255Q/Y108C			1	1 (0.8%)
L444P/D409H			1	1 (0.8%)

Their pathogenicity was evaluated using different prediction tools. The results are shown in (Table 7). Overall, mutations T231I, N462Y, L175P, F81L, D283N and Y135S were classified as disease causing whereas T482K was evaluated as damaging with low confidence with the SIFT Human Protein and SIFT Blink tool and as benign with the other tools used. The fact that some of the novel mutations affect the same codons of previously described mutations confirms the relevance of these codons and are data in favor of the pathogenicity of these novel mutations. The effect of mutation F81L on the enzyme activity was evaluated by in vitro expression. According to our findings, the mutation reduces the enzyme activity to 12% of wild type.

PolyPhen: PolyPhen-2 HumDiv and Hum Var; SIFT Human Protein and SIFT Blink; MUT Taster: Mutation Taster.

Pr da: probably damaging; da: damaging (* damaging with low confidence); dis causing: disease causing; be: benign. [a] Nucleotide numbering reflects cDNA numbering with + 1corresponding to the A of the first ATG translation initiation codon in the reference cDNA sequence (M16328.1).[b] Traditional codon numbering begins 39 codons downstream from the first ATG.[c] HGVS-recommended nomenclature number codons beginning with the first ATG as codon 1.

Table 5
Genotypes and clinical subtypes of Gaucher disease patients of Greek origin.

Genotypes	TYPE 1 *N* = 84	TYPE 2 N = 9	TYPE 3 *N* = 5	TOTAL *N* = 98
N370S/D409H;H255Q	19			19 (19.4%)
N370S/N370S	14			14 (14.3%)
N370S/L444P	9			9 (9.2%)
N370S/IVS6-2A → G	6			6 (6.1%)
N370S/R120W	5			5 (5.1%)
N370S/IVS10-1G → A	5			5 (5.1%)
N370S/?	3			3 (3.1%)
N370S/A309V	3			3 (3.1%)
N370S/RecNciI	3			3 (3.1%)
N370S/R257X	2			2 (2.0%)
N370S/R48Q	2			2 (2.0%)
N370S/R47X	1			1 (1.0%)
N370S/T231I	1			1 (1.0%)
N370S/N462Y	1			1 (1.0%)
N370/F213L	1			1 (1.0%)
N370S/H311R	1			1 (1.0%)
N370S/W184R	1			1 (1.0%)
N370S/R359X	1			1 (1.0%)
L444P/F81L	1			1 (1.0%)
N370S/Y135S	1			1 (1.0%)
N370S/ G202R	1			1 (1.0%)
N370S/ D283N	1			1 (1.0%)
N370S/ T482K	1			1 (1.0%)
L444P/ Y116H	1			1 (1.0%)
D409H;H255Q/D409H;H255Q		2		2 (2.0%)
D409H;H255Q/R120W		2		2 (2.0%)
IVS101G → A/ D409H;H255Q		1		1 (1.0%)
D409H;H255Q/RecNciI		1		1 (1.0%)
G202R/G202R		1		1 (1.0%)
IVS2 + 1G → A/?		1		1 (1.0%)
RecNcil/IVS10-1G → A		1		1 (1.0%)
L444P/L444P			2	2 (20%)
L444P/D409H;H255Q			1	1 (1.0%)
D409H;H255Q/Y108C			1	1 (1.0%)
L444P/D409H			1	1 (1.0%)

4. Discussion

GD, the most prevalent lysosomal storage disease, is a rare panethnic disorder and this is illustrated in our cohort of 141 patients, which includes patients of six different national origins. Its estimated prevalence in the general population ranges from 1/40,000 to 1/60,000 births but it can be as high as 1/800 births in the Ashkenazi Jewish population [17–19]. Assuming a birth rate of 100,000/year, a rough incidence estimate for GD in the Greek population would be 2.8/100,000 births. However, this is most likely an underestimate since the vast clinical variability of the disease along with the existence of patients which lack overt clinical symptoms can lead to its underdiagnosis. In fact, in a previous study of the genomic DNA of 1933 Guthrie cards, we showed that the frequency of the N370S mutation in our population was 0.0046 with 95% limits between 0.0025 and 0.0068 and thus the expected number of type 1 GD patients homozygous for the N370S mutation would be 238 [20]. However, up to date only 17 Greek GD type 1 patients with this genotype have been diagnosed in our lab, which is the only center in Greece providing the diagnosis of lysosomal storage diseases. Similar findings were reported in studies of the Ashkenazi and Portuguese populations [21,22]. Despite its apparent underdiagnosis, GD is still the most frequently diagnosed lysosomal storage disease in Greece. The cases presented here account for 27.4% (141/514) of the total number of patients diagnosed with a lysosomal storage disorder in our center. The Greek patients of our cohort originated from all parts of Greece, however clusters of the disease were identified in Central and Northern Greece, the Peloponnese and the greater area of Athens where more than half of the population resides. The majority of the patients from countries other than Greece were Albanians that immigrated to Greece since 1990 when the borders between the two countries opened. The disorder was also diagnosed in refugees that arrived in Greece in recent years.

GD is a highly heterogeneous disorder both clinically and genetically and this is also observed in our cohort of patients. Clinically our patients covered the whole spectrum of phenotypes associated with GD. Overall and on the basis of their clinical picture, 85.1% of the patients were classified as type 1, 9.2% as type 2 and 5.7% as type 3. At the severe end of the spectrum we identified patients with severe perinatal disease, one of whom had the collodion phenotype and was previously described in detail [23]. At the mild end of the spectrum we identified two asymptomatic patients that were diagnosed at the age of 8 and 19 years following the diagnosis of GD in their mothers. The genotype of the former was N370S/R120W, whereas the latter was homozygous for the N370S, carrying a genotype that is often associated with very mild disease.

Over 500 different mutations have been described in the *GBA1* gene including point mutations, deletions, insertions, splicing aberrations and various rearrangements. 425 are classified as disease-causing mutations, 79 as probable/possible pathological mutations and 1 as functional polymorphism (HGMD professional 2019.4). A notable variation in the distribution of different mutations has been observed in different populations [7–9,24–27].

The N370S mutation is particularly common in the Ashkenazi Jewish population where it accounts for 75%–80% of the alleles, its frequency is much lower in other populations and is totally absent in populations like the Japanese [7,8,13,18,24,25]. In our study, it was the most frequently identified mutation accounting for 49.2% of the alleles in our cohort of GD patients and showing similar frequencies

Table 6
Genotypes, Clinical subtypes and origin of non-Greek Gaucher disease patients.

GENOTYPES	Origin	TYPE 1 *N* = 22	TYPE 2 *N* = 3	TYPE 3 *N* = 2	TOTAL *N* = 27
N370S/D409H;H255Q	Albanian (10)	10			10 (37%)
N370S/L444P	Syrian (1) Polish (1) Albanian (2) Italian (1)	5			5 (18.5%)
N370S/N370S	Albanian (3)	3			3 (11.1%)
N370S/R47X	Albanian (1)	1			1 (3.7%)
N370S/L175P	Albanian (1)	1			1 (3.7%)
N370S/R496C	Albanian (1)	1			1 (3.7%)
N370S/R120W	Albanian (1)	1			1 (3.7%)
D409H;H255Q/D409H; H255Q	Albanian (3)		3		3 (11.1%)
L444P/L444P	Egyptian (1)			1	1 (3.7%)
L444P/D409H;H255Q	Albanian (1)			1	1 (3.7%)

Table 7
In silico prediction of the effect of the novel GBA mutations identified in this study.

cDNA[a]	Protein (Traditional in GD[b])	Protein (as recommended by HGVS[c])	Pathogenicity Poly Phen SIFT MUT TASTER		
c.809C > T	Thr231Ile	p.T270I	pr da	da	dis causing
c.1501 A > T	Asn462Tyr	p.N501Y	pr da	da	dis causing
c.641 T > C	Leu175Pro	p.L214P	pr da	da	dis causing
c.358 T > C	Phe81Leu	p.F120L	pr da	da	dis causing
c.521 A > C	Tyr135Ser	p.Y174S	pr da	da	dis causing
c.1562C > A	Thr482Lys	p.T521K	be	da*	be
c.964G > A	Asp283Asn	p.D322N	pr da	da	dis causing

between the Greek and non-Greek patients (50.0% and 46.3% respectively). The mutation is known as the 'neuroprotective mutation' since it is exclusively found in type 1 patients. However, not all type 1 GD patients carry this mutation [7,13,17,18,25]. In our cohort of patients, the mutation was not identified in two patients classified as type 1. The first patient was diagnosed at the age of 10 with massive hepatosplenomegaly, which had manifested 2 months earlier. In her medical history, however, it is reported that six years earlier, following a respiratory infection, she developed splenomegaly, which regressed after six months. Her growth was normal. The second, diagnosed by enzyme studies and DNA analysis at the age of 48 years, had been splenectomised in early childhood and had severe bone involvement. They both remain free of any neurological symptoms at the age 17 and 60 years (Dr Garoufi and Dr. Marinakis, personal communication) and their respective genotypes are L444P/Y116H and L444P/F81L. L444P is the major mutation in the Norbottnian type 3 GD and in homozygosity is generally associated with some neurological involvement, whereas in heterozygosity it is identified in all types of GD [28]. The Y116H (c.463 T > C, p.Y155H) mutation was identified in homozygosity in a 10 year old Mexican GD patient along with the variant A448T (c.1459G > A, p.A487T) which was identified in heterozygosity. The patient had failure to thrive, massive splenomegaly, anemia, low platelet count but was free of neurological findings. Using different prediction tools Y116H was classified as disease causing mutation, whereas A448Twas classified as likely to be disease causing. The impact of the coexistence of the two different mutations on the phenotype of the patient is not clear [29]. F81L identified in the second patient is a novel mutation. It is predicted to be disease causing and in vitro expression studies showed it results in severe reduction of enzyme activity. Although the patient bearing the genotype L444P/F81L did not exhibit any neurological signs, she had an early onset disease that required splenectomy at a very young age supporting the association of this genotype with severe type 1 disease.

In a previous study, in which 17 patients included in the present report were investigated, we presented results that suggested that the double mutant allele D409H; H255Q has a single origin in a region around Albania, comprising the Balkans and the Adriatic area of Italy [30]. In the larger cohort of patients presented here, we found this double mutant allele to be the second most frequent mutation in Greek and Albanian patients. Furthermore, the majority of Greek patients bearing the mutation came either from areas near the border with Albania or areas in which Albanians have settled since the medieval times. These observations further support our pervious findings.

A common origin for the A309V mutation was identified since it was found in three Greek families originating from Asia Minor, that came to Greece following the destruction of Smyrna in 1922. The mutation was first described by Latham et al. [31] in a type 1 GD Greek patient but no details regarding his/her origin are available.

In conclusion, we present our findings regarding GD patients in Greece. Our cohort includes 141patients of six different national origins who covered the whole spectrum of phenotypes associated with the disease.

Mutation analysis characterized 246/250 mutant alleles, identified 28 different mutations, including 7 novel mutations, and 37 different genotypes.

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Individual contribution to the article.

Evangelia Dimitriou was involved in the assaying of the activity of chitotriosidase and β-glucocerebrosidase as well as the writing of the paper.

Marina Moraitou was involved in DNA preparation, the investigation of mutations using PCR and restriction enzyme analysis, as well as the writing of the paper.

Mónica Cozar, Jenny Serra-Vinardell, Lluïsa Vilageliu and Daniel Grinberg were involved in automated sequencing of *GBA1* and site-directed mutagenesis.

Lluïsa Vilageliu and Daniel Grinberg were also involved in the writing of the paper.

Irene Mavridou was involved in fibroblast cultures as well as the writing of the paper.

Helen Michelakakis was involved in designing, coordinating the study, evaluation of the results as well as the writing of the paper.

Declaration of Competing Interest

None.

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