Beta-glucocerebrosidase gene mutation in a sample of Egyptian patients with Gaucher disease

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Background
Gaucher disease (GD) is an autosomal recessive lysosomal storage disorder resulting from inherited deficiency in the acid β-glucocerebrosidase enzyme (*GBA*). This defective activity causes accumulation of glucocerebroside in the lysosomes of cells derived from the monocyte/macrophage lineage. Glucocerebrosidase-engorged cells, termed Gaucher cells, infiltrate various organs, leading to multisystem abnormalities. Three major clinical types are delineated by the absence (type I) or presence (types II and III) of primary central nervous system involvement.

Objective
Identification of mutations of the whole coding region of β-glucocerebrosidase (*GBA*) gene among 24 Egyptian patients with GD.

Patients and methods
In this study, molecular assessment was carried out by sequencing of 11 exons of the *GBA* gene for the 24 studied patients from 24 families, their age ranged from 10 months to 10 years. Seventeen (70.8%) families out of 24 were with parental consanguinity.

Results
The disease-causing mutations were revealed in 16 patients. Eleven (45.8%) patients were homozygous for p.L483P mutation. Two (8.3%) patients were homozygous for p.N409S mutation. One (4.2%) patient showed a compound heterozygous for p.N409S/p.L483P mutation; one patient showed heterozygous p.L483P mutation while one patient showed homozygous p.R87W mutation (4.2%). The p.R87W mutation was not reported as one of the targeted mutations screening of all previous studies in Egyptian GD patients.

Conclusion
The study identified the most common mutation p.L483P followed by p.N409S located on exons 9 and 10, respectively. Interestingly, missense p.R87W mutation is the first time to be reported in Egyptian GD patients' genotype database using sequencing of the whole *GBA* gene.

Keywords:
allele, Gaucher disease, *GBA*, heterozygous, homozygous

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Introduction

Gaucher disease (GD) is the most common autosomal recessive lysosomal storage disorder. The acid β-glucocerebrosidase (*GBA*) gene encoding the lysosomal enzyme glucocerebrosidase is the gene known to be implicated in GD pathogenesis. The reduced enzyme activity results in the accumulation of glucocerebroside in the liver, spleen, bone marrow, brain, lungs, and the kidneys (James *et al*., 2006). The main clinical presentations involve hepatomegaly, splenomegaly, and anemia. Hypersplenism might lead to hemolysis and thrombocytopenia that are also bone barrow infiltrations and neurological complications. Less frequent manifestations involve pulmonary infiltrations, renal manifestations, and severe bone pain. Progressive infiltration of Gaucher cells in the bone marrow might also lead to Erlenmeyer flask deformity which can be detected by bone imaging (Huang *et al*., 2015).

GD is classified into three clinical subtypes on the basis of the absence or presence of neurological manifestations: type I (non-neuropathic), type II (acute neuropathic), and type III (chronic neuropathic) (Goker-Alpan *et al*., 2003; Grabowski, 2008). GD is a panethnic disorder affecting 1:40 000 to 1:60 000 individuals globally. GD type I is prevalent in the Ashkenazi Jewish population, with a disease prevalence of 1:855 and an estimated carrier frequency of 1: 18. Most of the type I affected individuals do not usually seek medical advice, which leads to underestimation of frequency of this type. Neuronopathic forms (types II and III) are rare variants of GD, with an

estimated incidence of less than 1 in 100 000 live births (Di Rocco *et al.*, 2013). In Egypt, most of the diagnosed patients are of type III as concluded from previous Egyptian studies (El-Beshlawy *et al.*, 2006; Tylki-Szymanska *et al.*, 2010; Khalifa *et al.*, 2011).

The *GBA* gene encoding the lysosomal enzyme glucocerebrosidase is 7.2 kb long, located on locus 1q21, and consists of 11 exons and 10 introns. The enzyme protein consists of 497 amino acids and has three isoforms (Horowitz and Zimran, 1994; Zampieri *et al.,* 2017). A highly homologous (96% identity) pseudogene (5 kb) sequence is located 16 kb downstream (Zimran *et al.*, 1991). More than 400 different *GBA* mutations have been detected in patients with GD (Siebert *et al.*, 2013). The identified mutant alleles include point mutations, deletions, splice-site mutations, and recombinant alleles. Recombination within the glucocerebrosidase locus appears to be enhanced by the high degree of sequence identity and the close physical proximity of the *GBA* pseudogene (Dandana *et al.*, 2016).

Different mutations can lead to nonfunctioning truncated proteins, unstable mRNA, or decreased enzyme activity as well as misfolded conformation of the resulting protein. Among the most commonly known reported mutations are p.L483P, p.N409S, IVS2+1G>A, and c. 84GG, which account for 90% of the mutant alleles in the Ashkenazi Jewish populations and about 50–60% of alleles in non-Jewish individuals (Pastores *et al.*, 2005). Although the genotype–phenotype correlation in GD is not always straightforward, some mutations have been linked to specific phenotypes. For example, the p.N409S mutation has been associated exclusively with type I GD (Goker-Alpan *et al.*, 2005).

The course of the disease was found to be milder in cases with the p.N409S mutant allele and excludes neurological involvement, with minor changes in protein structure, and hence catalytic activity (Lachmann *et al.*, 2004). The p.L483P mutation can occur as either a single base substitution or as a part of a complex allele (e.g., Rec NciI allele includes p.L483P +p.A495P and +p.V499V) and can be associated with all three types of GD (Pastores *et al.*, 2005). Homozygotes for the p.L483P mutation typically develop type III GD, whereas heterozygotes of the same mutation are more likely to develop type I or type II GD (Ron and Horowitz, 2005). Homozygosity for the c. 84GG or the IVS2+1 alleles leads to embryonic lethality, whereas heterozygous (84GG/IVS2+1) alleles showed progressive pulmonary involvement and usually patients do not survive past the first or second decade of life (Pastores *et al.,* 2005). Patients with homozygous p.D409H mutation exhibited a rare phenotype that involves cardiac calcifications and oculomotor abnormalities (Chabas *et al.*, 1998). The p.R496H mutation has been previously reported as a mild mutation (Beutler and Gelbart, 1998), whereas the p.V394L mutation is commonly seen in patients with myoclonic epilepsy, often together with a null or recombinant allele (Koprivica *et al.*, 2000; Park *et al.*, 2003; Kowarz *et al.*, 2005). Homozygous p.P266L leads to severe GD type II, with patients having neurological involvement, visceromegaly, and premature death (Alfonso *et al.*, 2001).

Early precise diagnosis of GD on biochemical and molecular basis are ultimately useful in prenatal as well as neonatal diagnosis allowing early therapeutic intervention. The enzyme-replacement therapy Cerezyme is known as the effective line of treatment for GD up till now (Weinreb *et al.*, 2002).

Patients and methods

Patients

The study included 24 unrelated Egyptian patients from 24 families. The patients were diagnosed biochemically with GD; their age ranged from 10 months to 10 years and were from different areas of Egypt. The patients were referred from the Biochemical Genetics Department at the National Research Centre. A written informed consent was signed by the patients' guardians according to the Medical Research Ethics Committee of the National Research Centre.

Methods

Clinical assessment started with recording family history and pedigree construction. Patients were clinically examined, and all needed laboratory investigations including complete blood count, reticulocyte count, and liver function tests were done.

Biochemical assay included assessment of β-glucocerebrosidase activity in peripheral leukocytes, which was performed using the artificial substrate 4-methylumbelliferyl-b-D-glucopyranoside (Peters *et al.*, 1977; Daniels *et al.*, 1981). In addition, assessment of plasma chitotriosidase using the artificial substrate 4-methylumbelliferyl-β-N-N, N'-triacetylchitotriose was performed (Hollak *et al.*, 1994; Guo *et al.*, 1995).

Mutational analysis included DNA extraction from peripheral blood leukocytes (Miller *et al.*, 1988), followed by PCR amplification of the 11 exons of the

GBA gene. Nested PCR was done for exons (1,2) (5,6) (8,9), and (10,11) in order to avoid amplification of pseudogene, while for exons 3, 4, and 7, conventional PCR was the method of choice (Alfonso *et al.*, 2001). PCR fragments were allowed to run on a 2% agarose gel to confirm successful amplification. The PCR products were purified using the QIAquick PCR purification kit (QIAGEN, Hilden, Germany). Cycle sequencing PCR was carried out using the BigDye Terminator kit (Applied Biosystems, Foster City, California, USA). Removal of dye terminators was done using CENTRI-SEP purification spin columns (Applied Biosystems). Sequencing was performed using a 310 ABI Prism DNA sequencer.

Bioinformatics were performed for the results obtained using different in-silico analysis tools. Data files obtained from the sequencer were shown by Finch TV, version 1.4.0. This was followed by aligning query sequences against those present in BLAST Basic Local alignment search tool (*http://blast.ncbi.nlm.nih.gov*) (Camacho *et al.*, 2009).

Results

The study group included 24 Egyptian GD patients, 14 (58.3%) men and 10 (41.7%) women. Ages of patients at the time of referral ranged from 10 months to 10 years. Seventeen (70.8%) patients were of consanguineous parents where the other seven (29.2%) patients were of nonconsanguineous parents. The patients were referred from the Biochemical Genetics Department at the National Research Centre and were referred from different areas of Upper and Lower Egypt.

Clinical features of the study group

All (100%) patients suffer from hepatomegaly, splenomegaly, anemia, and thrombocytopenia. No neurological manifestations appeared till the time of referral as shown in the supplementary table. Collective clinical data of the studied 24 GD patients are shown in Table 1.

Biochemical results

The results of the three biochemical tests performed for each patient revealed the following:

(1) Marked reduction of β-glucocerebrosidase activity (0.5364 ± 0.21) (normal laboratory reference values: 1–5 μmol/g/h)
(2) Marked elevation of chitotriosidase activity ranging from 133 to 25718 μmol/l/h (normal laboratory reference values: 4–80 μmol/l/h) was detected in all patients.

Table 1 Collective clinical data of the studied 24 Gaucher disease patients

Sex [*n* (%)]	Male	Female
	14/24 (58.3)	10/24 (41.7)
Consanguinity [*n* (%)]	Positive	Negative
	17/24 (70.8)	7/24 (29.2)
Clinical manifestations [*n*/*N* (%)]		
Hepatomegaly	24/24 (100)	
Splenomegaly	24/24 (100)	
Anemia	24/24 (100)	
Thrombocytopenia	24/24 (100)	

Biochemical assessments of GD patients are shown in Table 2.

Molecular results

PCR of *GBA* gene showed successful amplification of the 11 coding exons.

Sequencing analysis of the 11 coding exons of the *GBA* gene for the 24 studied patients revealed identification of pathogenic mutations in 16 patients. Sequencing of exon 3 revealed p.R87W/p.R87W genotype in one (4.2%) patient (Fig. 1), p.N409S/p.N409S genotype in exon 9 in two (8.3%) patients (Fig. 2), p.L483P/p.L483P genotype in exon 10 in 11 (45.8%) patients (Fig. 3), one (4.2%) patient carried p.L483P on one allele with an undetected mutation on the other allele, and p.N409S/p.L483P genotype in one (4.2%) patient (Fig. 4). Eight patients remain with unidentified mutations on both alleles (33.3%). Fig. 5 shows the detected mutations in this study and their positions on *GBA* gene. The most frequent mutation in the patients was p.L483P, which had a frequency of 50% followed by p.N409S with a frequency of 10.4%. The p.R87W allele had a frequency of 4.2%. Alleles with undetected mutations had a frequency of 35.4% (Table 3). Fourteen (87.5%) patients had homozygous mutations and two (12.5%) patients had heterozygous mutation (Table 4). Table 5 summarizes the detected mutations.

Discussion

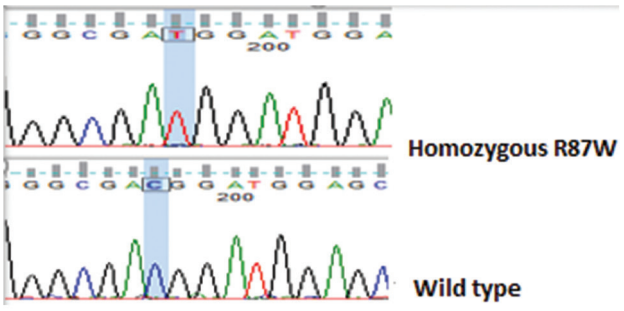
GD (MIM#230800, 230900, and 231000) is an autosomal recessive lysosomal storage disorder caused by deficiency in acid β-glucocerebrosidase (*GBA*). In patients, enzyme activity is usually less than 15% of normal that results in the lysosomal storage of a glycolipid named glucosylceramide (Zimran and Elstein, 2016). The most common clinical symptoms involve hepatomegaly, splenomegaly, anemia, and thrombocytopenia (Huang *et al.*, 2015). The *GBA* gene is the only gene known to be associated with GD (Zampieri *et al.*, 2017). According to HGMD, 2019 more than 400 mutations have been identified in

Table 2 Biochemical assessment of Gaucher disease patients

Patient number	Sex	Age at the time of referral	Enzyme levels	
			Beta-glucocerebrosidase assay level Reference level (1-5 µmol/g/h)	Chitotriosidase assay level reference level (4-80 µmol/l/h)
1	M	3 years	0.04	1472
2	F	2 years	0.7	3694
3	M	1 year 6 months	0.2	25718
4	M	1 year	0.5	2583
5	M	1 year 4 months	0.1	3711
6	F	10 years	0.55	543
7	F	1 year 3 months	0.9	4225
8	M	2 years	0.89	5720
9	M	1 year 2 months	0.1	3711
10	F	4 years	0.8	496
11	M	1 year 5 months	0.07	25560
12	F	3 years	0.7	5575
13	F	2 years	0.67	6850
14	M	10 months	0.3	2200
15	F	3 years	0.4	133
16	M	1 year 1 months	0.3	4709
17	M	1 year 2 months	0.1	5836
18	M	1 year 8 months	0.2	5395
19	F	2 years 8 months	0.19	5627
20	F	3 years	0.2	6214
21	F	2 years	0.3	5863
22	M	3 years	0.4	3720
23	M	1 year 4 months	0.6	4655
24	M	2 years 3 months	0.3	3650

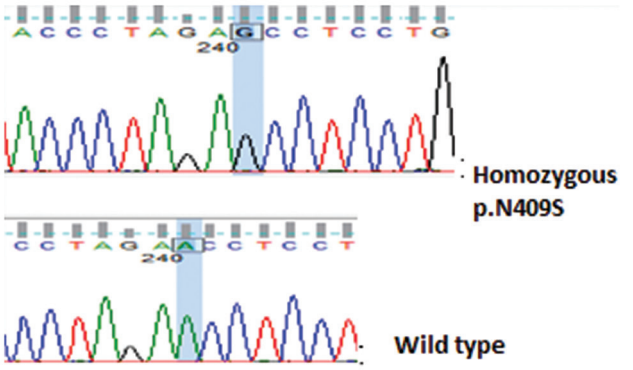
F, female; M, male.

Figure 1



Chromatogram of exon 3 of the *GBA* gene showing homozygous p.R87W (c. 259C>T) in patient 6.

Figure 2



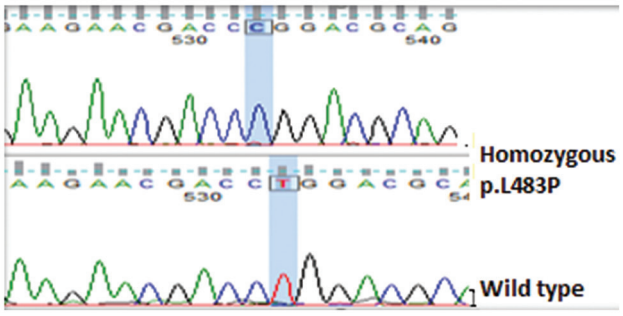
Chromatogram of exon 9 of the *GBA* gene showing homozygous p.N409S (c.1226A>G) in patients 2 and 13.

GD patients (■, ■) (*http://www.hgmd.cf.ac.uk/ac/gene.php?gene = GBA*).

The most common mutations reported worldwide were p.D448H, c. 84GG, IVS2+1G>A, p.P305L, p.L483P, p.N409S, p.V433L, and p.R535H (Sheth *et al.*, 2019). The c. 84GG, p.V433L, and p.R535H mutations were not detected in Egyptian patients. On the other hand, p.D448H, IVS2+1G>A, p.L483P, p.N409S, and p.P305L mutations were reported in Egyptian patients in previous studies (Fateen *et al.*, 2017). The p.L483P, p.N409S, c. 84GG, p.D448H, IVS2+1, and p.R535H mutations account for 90 and 75% of total mutant alleles observed among Jewish and non-Jewish patients with GD, respectively (Sheth *et al.*, 2019).

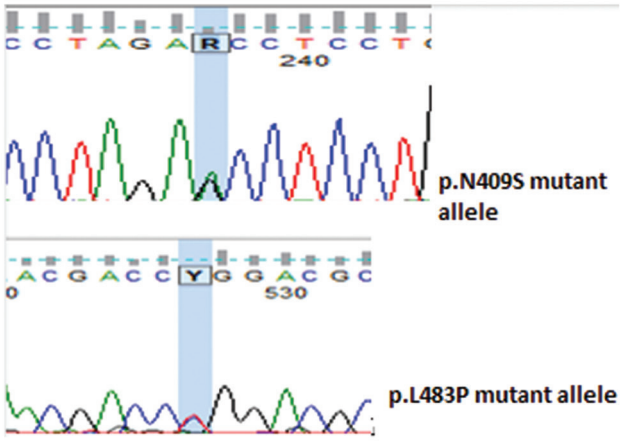
In this study, sequencing of the entire 11 coding exons was done for all GD patients. Interestingly, this is the first Egyptian study to do full sequencing for the *GBA* gene whereas all previous Egyptian studies performed either RFLP (Fateen *et al.*, 2017), sequencing for exons 9 and 10 (El-Morsy *et al.*, 2011) or strip hybridization-based assay (El-Gawhary *et al.*, 2007) in search for most common mutations. The study group consisted of 24 Egyptian GD patients with variable clinical features, 14 (58.3%) men and 10 (41.7%) women. Ages of patients at the time of presentation ranged from 10 months to 10 years. Enzyme levels of patients displayed a marked reduction in

Figure 3



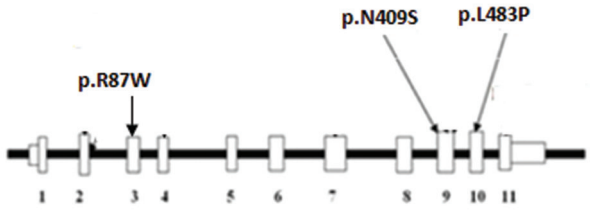
Chromatogram of exon 10 of the *GBA* gene showing homozygous p.L483P (c.1448T>C) in patients 11, 3, 5, 7, 9, 11, 12, 16, 17, 18, and 19.

Figure 4



Chromatogram of exons 9 and 10 of the *GBA* gene showing heterozygous p.N409S/p.L483P in patient 10.

Figure 5



Locations of mutations in the *GBA* gene identified in the Egyptian GD patients. NB: Exons are represented by boxes. GD, Gaucher disease.

β-glucocerebrosidase activity (0.5364 ± 0.21) and marked elevation of chitotriosidase activity ranging from 133 to 25718 μmol/l/h.

Although GD is an autosomal recessive disorder, male predominance was found in this study (1.4: 1) and in three previous Egyptian studies (Ragab *et al.*, 2000; El-Morsy *et al.*, 2011; Khalifa *et al.*, 2011; Fateen *et al.*, 2017; Fateen and Abdallah, 2019). This reflects the culture of the Egyptian society that values the male

Table 3 Allele frequency among 24 patients (48 alleles)

Mutation type	Number of alleles	Allele frequency (%)
p.L483P	24	50
p.N409S	5	10.4
p.R87W	2	4.2
Undetected alleles	17	35.4

Table 4 Frequency of homozygous and heterozygous mutations with respect to patients with positive molecular results

Mutation status	Number of patients [*n* (%)]
Homozygous mutation (p.L483P/p.L483P, p.N409S/p.N409S, p.R87W/p.R87W)	14 (87.5)
Heterozygous mutation (p.N409S/p.L483P, p.L483P/?)	2 (12.5)

Table 5 Different mutations detected in the study group patients (24 patients)

Genotype	Number of patients	Frequency (%)
p.L483P/p.L483P	11	45.8
p.N409S/p.N409S	2	8.3
p.N409S/p.L483P	1	4.2
p.R87W/p.R87W	1	4.2
p.L483P/undetected allele	1	4.2
Undetected mutation	8	33.3

siblings to the extent of giving them more medical attention and care (Fateen *et al.*, 2014).

The age range of patients in this study at the time of diagnosis was between 10 months and 10 years covering the infantile and juvenile spectrum of GD. This agrees with most of the previous Egyptian studies whose age range also covered the infantile and juvenile spectrum of GD.

In this study, 11 patients were less than 4 years and had the homozygous p.L483P/p.L483P mutation and two patients carried p.L483P in a heterozygous form. This mutation is a missense mutation, p.L483P (c.1448T>C). Leucine at position 483 was replaced by proline and it was found in patients 1, 3,5, 7, 9, 11, 12, 16, 17, 18, and 19 in a homozygous form and patients 10 and 20 in a heterozygous form. Clinically, they were classified as type I due to the absence of neurological manifestations at the time of referral. Long-term follow-up is important because neurological symptoms might appear among type I GD and this will reclassify them into type III even under enzyme-replacement therapy which delayed the appearance of the neurologic signs but did not prevent them (Khalifa *et al.*, 2011). Two patients carried homozygous p.N409S/p.N409S, one patient carried compound heterozygous p.N409S/p.L483P, and one patient was homozygous for p.R87W/p.R87W mutation, which was not detected in any previous Egyptian GD patient. All the patients were diagnosed by type I GD which agrees with previous studies

stating that these mutations were known to have mild phenotypic presentations (Ortiz-Cabrera *et al.*, 2016; El-Beshlawy *et al.*, 2017).

All (100%) patients irrespective to their genotypes suffered from hepatomegaly, splenomegaly, anemia, and thrombocytopenia. Anemia in GD resulted from hemolysis secondary to hypersplenism and presented with severe form may be in advanced cases (postsplenectomy) and thrombocytopenia occurred as a result of hypersplenism and/or bone marrow infiltrations. These symptoms were consistent with those described by Weinreb *et al*. (2013).

The most common mutation in this study was the homozygous p.L483P which in 11 patients was in a homozygous form and in two patients in a heterozygous form with an allele frequency of 50%. This finding is close to other previous Egyptian studies as shown in Table 6. Close differences in frequencies may be due to the different sample size. Collectively, the p.L483P in a homozygous form was found as 52.3% among 195 patients (204 alleles out of 390) in the previously mentioned Egyptian studies including ours.

The high frequency of p.L483P mutation agrees with being the most common mutation worldwide according to the previous ICGG Gaucher Registry reports (Charrow *et al*., 2000; Tylki-Szymanska *et al*., 2010; Grabowski *et al*., 2015). Of the 34 countries represented in El-Beshlawy *et al*. (2017), the majority of patients were from European countries (32.4%), the United States (26.9%), and Egypt (20.9%). Patients from the JAPAC region (China, India, Japan, Korea, Malaysia, Philippines) and Latin America accounted for 10.3 and 5.9% of patients, respectively. Among the 202 patients with *GBA* genotype data reported, 163 (80.7%) had at least one p.L483P allele, with 122 (60.4%) being homozygous for p.L483P.

The second detected mutation in this study was the missense p.N409S mutation. Asparagine at position 409 was replaced by serine and it was found in two patients (patients 2 and 13) in a homozygous form and patient 10 in a heterozygous form. The frequency of the mutation was 8.3%, which is in agreement with the previous Egyptian studies as shown in Table 6. The p.N409S allele was found as three (0.06%) alleles in El-Beshlawy *et al.* (2006) and in three (0.49%) alleles in El-Beshlawy *et al*. (2017). Collectively, the p.N409S allele was found as 8.7% among 195 patients (34 alleles out of 390) in all Egyptian studied patients.

The mutation history follow-up of p.N409S suggested that this mutation occurred first in non-Jewish and then passed into the Jewish population. Consequently, it is not surprising that the p.N409S mutation is also relatively frequent in the Southern Mediterranean region due to geographical and historical reasons, and was likely introduced into North Africa during the Roman Empire (Dandana *et al*., 2016). It is suggested that the p.N409S mutant allele is found mainly in individuals of European or West Asian ancestry (Riboldi and Di Fonzo, 2019). This is evidenced by the findings that the p.N409S mutant allele was found in higher percentages among other populations like in 44% of Tunisian patients which is also similar to European populations, 36.5% of Italian patients, and 55% of Spanish patients (Dandana *et al*., 2016). Higher frequencies are encountered among Portuguese patients (62.5%) (Geraldo *et al*., 2012) and Ashkenazi Jewish patients (77%) (Jaffe *et al.,* 2019).

The compound heterozygous mutation p.N409S/p.L483P was found in this study in one patient with a frequency of 4.2%. These two alleles were rarely found together in the previous Egyptian studies except for one patient reported by Khalifa *et al*. (2011). On the other hand, this genotype appears to be more frequent among Spanish and Portuguese patients (Geraldo *et al*., 2012).

Homozygous p.R87W (c.259C>T) was found in one (4.2%) patient (patient 6) in this study. This is the first time for this mutation to be reported among the Egyptian GD patients. This mutation is considered to be a rare mutation where it has been described in 1 (0.55%) out of 181 Spanish patients in a heterozygous form p.R87W/p.N409S (Ortiz-Cabrera *et al*., 2016), in two Bedouin Arabs (Rockah *et al.*, 1997; Choy *et al.*, 2007), two Lebanese patients (El-Zahabi *et al*., 2007), and an Indian patient (Sheth *et al.*, 2019).

Table 6 Comparison between frequency of mutations detected in this study and previous Egyptian studies

References	p.L483P/p.L483P (%)	p.N409S/p.N409S (%)	p.R87W/p.R87W (%)	p.N409S/p.L483P (%)	Number of patients
El-Beshlawy *et al.* (2006)	40.9	0	0	0	22
El-Gawhary *et al.* (2007)	40	5	0	0	20
El-Morsy *et al.* (2011)	17.6	0	0	0	17
Khalifa *et al.* (2011)	56.4	13.03	0	4.3	23
Fateen *et al.* (2017)	38.2	5.8	0	0	34
Saleem *et al.* (2017)	30.8	15.4	0	0	26
Elmonem *et al.* (2016)	66.6	3.3	0	0	30
This study	45.8	8.3	4.2	4.2	24

IVS2+1G>A was previously detected in more than one Egyptian study in GD patients (El-Gawhary *et al.*, 2007; Fateen *et al.*, 2017), but it was not detected in our study. It is worth saying that the technique used previously was either strip assay or restriction fragment length polymorphism which are less accurate techniques than the Sanger sequencing technique used in this study.

In our study, eight patients remained with undetected mutations (33.3%) in addition to one patient who had p.L483P on one allele and undetected mutation on the other allele. More than one step should be taken toward further mutation analysis for those patients. First, the multiplex ligation-dependent probe amplification technique should be used for detecting large deletions or duplications in the *GBA* gene which could not be detected by the Sanger sequencing technique. Those deletions and duplications together with gene–pseudogene complex rearrangements represent the main cause of pitfalls in GD mutational analysis (Cozar, 2011). This is affirmed by the study by Amico *et al.*(2016) where two partial gene deletions (3–9 exon del and 7–10 exon del) and a recombinant allele involving *GBA-GBAp* genomic sequences between exons 6 and 9 were detected after being uncharacterized by the conventional sequencing method.

A second way to go is the screening of PSAP gene (MIM#176801) responsible for the production of saposin C protein, which is an essential activator for glucocerebrosidase enzyme. Saposin C deficiency can be a cause for GD although cases stated in the literature are few, it should be taken into consideration for patients with wild-type *GBA* gene (Tamargo *et al.*, 2012).

GD encompasses a spectrum of clinical findings from a perinatal lethal form to an asymptomatic form. Phenotype–genotype correlation has been a debatable issue among studies since some molecular studies could find correlation between the severity of the disease and mutation type while other studies could not find this correlation (Grabowski, 2008; Migdalska-Richards and Schapira, 2016). Some studies have concluded that patients with the same genotypes may exhibit different phenotypes of GD (El-Gawhary *et al.*, 2007; Hassan *et al.*, 2018). Even if there are few common features, some exceptions exist, making it difficult to use genotyping to determine the disease prognosis (El-Morsy *et al.*, 2011). The presence of modifier genes may be a good explanation for the wide range of phenotypic variability among patients sharing the same genotype (Davidson *et al.*, 2018).

The homozygous p.L483P mutation may be associated with type I (Tammachote *et al.*, 2013; El-Beshlawy *et al.*, 2017; Sheth *et al.*, 2019), type II (Wan *et al.*, 2006; Fateen *et al.*, 2017), and type III (Khalifa *et al.*, 2011; Geraldo *et al.*, 2012; Elmonem *et al.*, 2016; Saleem *et al.*, 2017; Schwartz *et al.*, 2017). It is also likely that many of type I GD patients with p.L483P genotype may develop neurological symptoms and are reclassified as type III later in life (Sheth *et al.*, 2019). Patients of homozygous P.L483P/p.L483P in our study were diagnosed as having type I GD. This might be due to their young ages (<4 years) and absence of neurological manifestations. So, long-term follow-up is recommended for these patients as neurological manifestations are expected to appear later on changing the classification of GD patients to type III.

The p.N409S allele was found in two patients in a homozygous form and in one patient in a heterozygous form, all were found with type I GD (10.4%) with no neurological symptoms. This finding was similar to that published previously concerning the presence of the p.N409S on one allele appears to be protective of the development of a neuropathic form (El-Beshlawy *et al.*, 2017; Fateen *et al.*, 2017; Riboldi and Di Fonzo, 2019) and the genotype p.N409S/p.N409S is considered the most common among type I patients and especially among Ashkenazi Jewish patients in whom disease manifestations are milder (Balwani *et al.*, 2010).

The p.R87W has been described as a mild mutation (Ortiz-Cabrera *et al.*, 2016), based on the absence of neurologic manifestations when reported to occur with severe mutations like p.L483P, and confirmed by the mild clinical phenotype seen in a patient who is homozygous for p.R87W in our study. Our patient showed improvement in hepatosplenomegaly while on treatment, in addition to thrombocytopenia and anemia but with no bone involvement unlike the patient mentioned in Baris *et al.* (2015) who experienced long bone crises in childhood.

In this study, the consanguinity rate was high (70.8%) as is evident by homozygous mutations found in a frequency of 87.5%. One of the highest rates of consanguineous marriage worldwide occurs in the Middle East. Consanguineous marriage is fairly common in Egypt (21–33%). But even couples who consider themselves unrelated, especially in rural areas, may show high levels of homozygosity because marriage within the narrow community is a nationally long-established tradition (Elmonem *et al.*, 2016). Hence, genetic counseling is essential for providing families with information on the nature, inheritance, and implications of GD to help them make medical and personal decisions.

Conclusion

In conclusion, the most common mutation among Egyptian patients was p.L483P (50%), followed by p.N409S (10.4%) and p.R87W (4.2%). Consequently, p.L483P and p.N409S should be the first mutations to be tested in any Egyptian Gaucher patient. Gross deletions, insertions, and recombinant mutations might be the cause of undetected mutations in seven patients. It is worth including multiplex ligation-dependent probe amplification assay in GD mutational analysis when the conventional diagnostic PCR-based approaches show no mutations in the *GBA* gene. Genetic counseling (including discussion of potential risks to offspring and reproductive options) for the purpose of early diagnosis and treatment is highly recommended.

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Conflicts of interest

There are no conflicts of interest.

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