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ORIGINAL ARTICLE

Novel mutations in the glucocerebrosidase gene of Indian patients with Gaucher disease

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Gaucher disease (GD) is the most common glycolipid storage disorder resulting from glucocerebrosidase deficiency due to mutations in the *GBA* gene. Study was performed in 33 unrelated patients with low β-glucosidase activity in leukocytes and/or fibroblasts. The exons and exon–intron boundaries of the *GBA* gene were bidirectionally sequenced using an automated sequencer. Mutations were confirmed in parents and were looked up in public databases, and *in* silico analysis was carried for novel mutations. We identified two novel missense mutations G289A (c.866G>C) and I466S (c.1397T>G) in exons 7 and 10, respectively, in two (6.06%) patients that destabilize the protein structure. L444P (c.1448T>C) was the most common mutation identified in 20/33 (60.60%) non-neuronopathic and 1/33 (3.03%) sub-acute neuronopathic form based on clinical presentation at the time of investigation. Other nine rare mutations were: R463C (c.1504C>T), R395C (c.1300C>T), R359Q (c.1193G>A), G355D (c.1181G>A), V352M (c.1171G>A) and S356F (c.1184C>T) found in each patient (18.18%). Compound heterozygous mutation L444P (c.1448T>C)/R496C (c.1603C>T) in exon 10/11 and L444P (c.1448T>C)/R329C (c.1102C>T) were observed in exon 10/8 in one each patient (6.06%). Two patients (6.06%) from Sri Lanka showed E326K (c.1093G>A) mutation in exon 8. We conclude that L444P is the most common mutant allele with exons 8 and 10 as the hot spot region of *GBA* gene observed in Indian GD patients.
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Keywords: Gaucher disease; *GBA* gene; India; L444P; LSD mutation

INTRODUCTION

Gaucher disease (GD) is the most common glycolipid storage disorder because of inherited deficiency of lysosomal enzyme acid β-glucosidase (glucocerebrosidase (GC), E.C.3.2.1.45),[1,2] that cleaves the glycolipid GC into glucose and ceramide. The enzymatic defect leads to the systemic accumulation of glucosylceramide, mainly within the cells of monocyte/macrophage lineage, leading to hepatosplenomegaly, anemia, thrombocytopenia and various skeletal complications.[3,4] The presence and severity of neurological symptoms define three types of GD as non-neuronopathic type 1, neuronopathic type 2 and sub-acute neuronopathic type 3.[2] Although 95% of GD patients presented as type 1 (MIM no. 230800) with onset at childhood or adulthood[5] may progress as type 3 GD with advancing age.[6] Neuronopathic variants are type 2 GD (MIM no. 230900), the more severe form, characterized by early onset and survival up to 2 years of age, and type 3 GD (MIM no. 231000) presenting as infantile or juvenile onset and a less ominous course with survival into adulthood.[5]

The gene encoding for human GC (*GBA*) has 11 exons (GenBank-J03059) with a highly homologous pseudogene sequence located 16 kb downstream (GenBank-J03060). Both genes are in the same orientation and share 96% exonic homology[7] responsible for recombinant allele formation. To date, more than 350 mutations have been reported for GD. These include missense and nonsense mutations, small insertions or deletions resulting in frameshifts or in-frame alterations, splice junction mutations and complex alleles carrying two or more mutations.[8] The frequency of specific mutant allele varies in different populations. In Ashkenazi Jewish ancestry, four mutations, N370S (c.1226 A>G), L444P (c.1448 T>C), 84insG (c.84dupG) and IVS2 +1 G>A (c.115+ 1 G>A), account for nearly 90% of the disease alleles. Whereas in non-Jewish patients, these mutations account for about 50–60%, and there is a broad spectrum of other mutations in different ethnic groups.[9,10]

Biochemical diagnosis of GD can be ascertained by plasma chitotriosidase followed by confirmative enzyme β-glucosidase study

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from leukocytes or fibroblasts.[11–14] Nonetheless, carrier frequency of the disease cannot be ascertained by enzyme study; this makes it imperative to identify the mutant allele in the *GBA* gene. This can further provide genotype/phenotype correlation and may help to know the severity of disease prediction. Hence, present study was planned to establish the most frequent mutations in our group of patients and identification of novel or/and known mutations. This information can also be used for population screening and provide comprehensive approach of enzymatic and molecular study during prenatal diagnosis.

MATERIALS AND METHODS

Patient's selection

The study has included cohort of 33 unrelated patients with confirmed diagnosis of GD comprising of 20 males and 13 females including 4 adults in the age range of 10 months to 40 years. Twenty two percent of these were born to the consanguineous parents. The inclusion criteria were low β-glucosidase activity in leukocytes and/or fibroblasts, or patients already diagnosed and receiving enzyme replacement therapy (ERT) or those found to have Gaucher cells in bone marrow. The most common clinical phenotypes observed were hepatomegaly, splenomegaly, anemia and thrombocytopenia. At the time of diagnosis, based on clinical presentation, 32 patients (10 months to 40 years) were of type 1, and 1 patient (3 years) was of type 3 GD with hepatosplenomegaly and affected cranial nerves. In 10/33 (30.30%) GD patients, Gaucher cells were seen in bone marrow, 6/33 (18.18%) patients were receiving ERT while referred to us and in 1/33(3.03%) patient bone marrow transplantation was done. In remaining 16/33 (48.48%) patients, bone marrow biopsy was not done but chitotriosidase was 1000-fold elevated with reduced enzyme activity.

An informed written consent was obtained either from patients or by their legal guardians as per the protocol approved by the Institutional Ethics Committee. Peripheral blood (6 ml) was collected in two different containers—heparin for plasma/leukocyte enzyme assay and EDTA for DNA extraction. Screening for GD was carried by plasma chitotriosidase assay using 4-methyl umbelliferyl β-D-N, N, N′, N″-triacetylchitotriosidase substrate and enzyme activity was expressed in terms of nmol h^{-1} ml^{-1} plasma[10] and confirmative study by β-GC enzyme from leukocytes using plasma 4-methylumbelliferyl-β-D-glucopyranoside fluorescence substrate and enzyme activity was expressed in terms of nmol h^{-1} mg^{-1} protein.[15]

Screening procedure for common mutations and DNA sequencing

Genomic DNA was extracted from whole blood by salting-out method (Miller *et al.*).[16] Mutation screening was carried out for the common mutant allele N370S, L444P, R463C and IVS2 +1 G>A by PCR-RFLP (restriction fragment length polymorphism). The exons and exon–intron boundaries of *GBA* gene were bidirectionally sequenced using automated sequencer. Primers for common mutations and bidirectional DNA sequencing are described in Table 1.

PCR was performed in a total volume of 20 μl, containing 250 ng of genomic DNA, 20 pmol of each primer, 10× Cetus buffer, dNTPs (2 mM) and 1 U Taq polymerase. The amplification protocol for N370S, L444P, R463C and IVS2 (+1) was denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 45 s, annealing at 61 °C (N370S, L444P and R463C) and 68 °C (IVS2 (+1)) for 45 s, and extension at 72 °C for 45 s, and 10 min final elongation at 72 °C. PCR products were run on the 2% agarose and visualized under ultraviolet transilluminator.

PCR products were sequenced by bidirectional Sanger sequence method using exon-specific primers. This has allowed us to confirm the previously identified common mutation by PCR–restriction fragment length polymorphism method together with the recombinant allele and identification of other known and novel mutations. PCR was performed in a total volume of 20 μl, containing 250 ng of genomic DNA, 10 pmol of each primer, 10× Cetus buffer, dNTPs (2 mM) and 1 U Taq polymerase. The amplification protocol for exons 3–11 was denaturation at 96 °C for 2 min; 33 cycles of denaturation at 96 °C for 30 s, annealing at 58 °C for 30 s, and extension at 74 °C for 60 s; and 5 min final elongation at 74 °C. The amplification protocol for exon 2 was denaturation at 94 °C for 4 min; 33 cycles of denaturation at 94 °C for 30 s, annealing at 65 °C for 30 s, and extension at 72 °C for 30 s; and 10 min final elongation at 72 °C. PCR products were purified by column purification using Qiagen PCR purification kit and sequenced on automated sequencer ABI 3730XL (Applied Biosystems, Carlsbad, CA, USA). Identified mutations were confirmed in parents and looked up in public domain of Human Gene Database (http://www.hgmd.cf.ac.uk) for pathogenicity. The novel mutations in the *GBA*-coding region were analyzed using PolyPhen (http://genetics.bwh.harvard.edu/pph)[17] and SIFT/PROVEAN (http://sift.jcvi.org)[18] to assess their potential pathogenicity. The mutants (G289A, I466S) were built using build mutant protocol of Accelrys Discovery studio using the 1OGS native and 1OGS mutant PDB structure of acid β-glucosidase.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

RESULTS

Of 33 subjects, plasma chitotriosidase was markedly elevated (6500–86 000 nmol h^{-1} ml^{-1} plasma) in 30(90.90%), undetectable in 1 (3.03%) and normal (21–107 nmol h^{-1} ml^{-1} plasma) in 2 patients (6.06%) and not done in 1 patient. Confirmative diagnosis was carried out in leukocytes by β-glucosidase activity in all subjects and had shown significantly reduced activity of the enzyme (10–30% of the normal activity). In 21 patients, enzyme activity was >30% that include three patients on ERT, seven patients with Gaucher cells in bone marrow and one with bone marrow transplantation. In remaining 10 patients although enzyme activity was nearly 30%, plasma chitotriosidase activity was 1000-fold elevated and mutation was identified in all of them.

Our study has identified novel mutations G289A (c.866G>C) in homozygous state and I466S (c.1397T>G) in heterozygous state in each patient (6.06%). The first novel sequence alteration is caused by G to C change at position 866 of the cDNA (exon 7 of *GBA*), leading to a glutamine to alanine substitution at residue 289 of the protein (G289A). This mutation was found in a homozygous state in female of type 1 GD patient born to consanguineous parents. Hepatosplenomegaly (liver 8 cm and spleen 8 cm size) is among the clinical feature of this patient. The other novel sequence alteration is caused by T to G change at position 1397 of the cDNA (exon 10 of *GBA*), leading to isoleucine to serine substitution at residue 466 of the protein (I466S). This mutation was found in a heterozygous state in female of type 1 GD patient and other mutation could not be identified. Moderate hepatomegaly with severe splenomegaly and pallor were among the clinical features of this patient. DNA sequencing chromatogram of novel mutations are shown in Figure 1. Novel variants were also ruled out as the polymorphism by sequencing the corresponding exons in 60 normal unrelated individuals.

We also observed 10 previously reported mutations in remaining 31 patients (93.93%). Among them, L444P in exon 10 was identified as the most common one in homozygous state in 20/33 (60.60%) non-neuronopathic and 1/33 (3.03%) in sub-acute neuronopathic patients, followed by rare mutations R463C (c.1504C>T) in exon 10, R395C (c.1300C>T) in exon 9, R359Q (c.1193G>A), G355D (c.1181G>A), V352M (c.1171G>A) and S356F (c.1184C>T) in exon 8 in homozygous state in each (18.18%) patient. Combined heterozygous mutation L444P (c.1448T>C)/R496C (c.1603C>T) in exon10/11 and L444P (c.1448T>C)/R329C (c.1102C>T) in exon 10/8 was observed in one each patient (6.06%). Two patients (6.06%) from Sri Lanka had shown E326K (c.1093G>A) mutation in homozygous state in exon 8.

Table 1 Primers for identification of common mutation and PCR amplification of the *GBA* gene and product sizes

Common mutations/exon	*Primer sequences (5′–3′)*	*Restriction site*	*Size (bp)*
L444P (c.1448T>C)	5′-CTGAACCCCGAAGGAGGACC-3′ 5′-GGGCTTACGTCGCTGTAAGCTCACAC**C**GGC-3′	MspI	931
R463C (c.1504C>T)	5′-CTGAACCCCGAAGGAGGACC-3′ 5′-GGGCTTACGTCGCTGTAAGCTCACAC**C**GGC-3′	MspI	931
N370S (c.1226A>G)	5′-TGTCTCTTT GCCTTTGTCCTTACCC**T**CGA-3′ 5′-GACAAAGTTACGCACCCAATT-3′	AvaI	115
IVS2+1G-A (c.115+G>A)	5′-GCATCATGGCTGGCAGCCTCACAGGA**C**TGC-3′ 5′-GCCCAGGCA ACAGAGTAAGACTCTGTTTCA-3′	HpHI	255
Exon 2	F.P- 5′-GGAGAGGGGCTTGCTTTTCA-3′ R.P- 5′-GGAGGCAGAGGTTGGAATGA-3′	—	371
Exons 3–4	F.P- 5′-CAAGGGGTGAGGAATTTTGA-3′ R.P- 5′-CACCACTGCACTCCTGTCTC-3′	—	696
Exons 5–6	F.P- 5′-TGGCCCTGACTCAGACACTA-3′ R.P-5′-CTGATGGAGTGGGCAAGATT-3′	—	788
Exon 7	F.P- 5′-GGCTGTTCTCGAACTCCTGA-3′ R.P-5′-ATAGTTGGGTAGAGAAATCG-3′	—	473
Exon 8	F.P- 5′-AGTTGCATTCTTCCCGTCAC-3′ R.P- 5′-ATCATGGTTCCCCAGAGTTG-3′	—	466
Exon 9	F.P- 5′-CAGCTGCCTCTCCCACAT-3′ R.P- 5′-GTGTGCCTCTTCCGAGGTT-3′	—	381
Exons 10–11	F.P- 5′-GAGAGCCAGGGCAGAGCCTC-3′ R.P- 5′-CTCTTTAGTCACAGACAGCG-3′	—	569

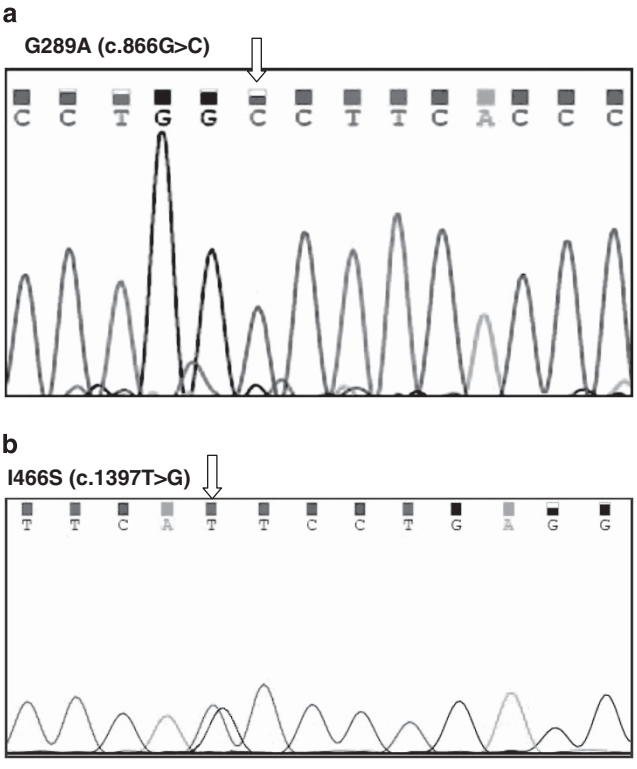

Figure 1 DNA sequencing chromatogram showing novel mutations: (**a**) G289A (c.866G>C) in homozygous state in exon 7; (**b**) I466S (c.1397T>G) in heterozygous state in exon 10. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Phenotype correlation with genotype demonstrated that L444P mutant allele was observed in 20 (95.23%) type 1 and one with type 3 (4.76%) GD.Type 1 GD patients in the present study were presented with severe hepatosplenomegaly (95%), anemia (53%), thrombocytopenia (32%), saccadic eye movement (5%), delayed milestones (10%), severe bone disease (15%) and cardiomegaly with pulmonary congestion (5%), and three patients were receiving ERT while referred to us for the molecular study. One patient with homozygous L444P genotype had type 3 GD with cranial nerves being affected in addition to the hepatosplenomegaly. Type 1 GD patients with L444P genotype may later on develop symptoms of type 3 GD that need follow-up study. R463C homozygous mutation was seen in one patient (3.03%) with type 1 GD with severe bone osteomylitis of femur, hepatosplenomegaly and chronic anemia, and splenectomy was carried out because of severely enlarged spleen. R496C and R395C mutation in homozygous state was seen in two patients (6.06%) with phenotype of hepatosplenomegaly, anemia and thrombocytopenia in type 1 GD with later mutation in an adult patient of 40 years. R359Q homozygous mutation was found in one type 1 GD patient with coarse features, hepatosplenomegaly (spleen 33 cm), scaly skin, and bone marrow with severe erythroid hyperplasia, large cells with fibrilly cytoplasm and thrombocytopenia. This patient was on ERT and responded well to the therapy with reduction in spleen size to 12 cm. G355D and S356F mutant allele were observed in one each patient with type 1 GD with the phenotype of hepatosplenomegaly. Both these patients were receiving ERT during study inclusion. One adult patient with L444P/R329C mutation was presented with the phenotype of avascular necrosis as the primary sign with mild hepatosplenomegaly. E326K mutation was seen in two patients with phenotype of mild to moderate hepatomegaly, severe splenomegaly and poor vision in one of them. The genotype–phenotype correlations of 33 Indian GD patients examined in this study are shown in Table 2.

DISCUSSION

Present study describes molecular analysis of 33 patients with GD from India with most common type 1 in 32 (96.96%) patients and

Table 2 Genotypes, clinical findings and origin of Indian GD patients

Patient no.	*Age at diagnosis*	*Geographic origin*	*Clinical features at the time of diagnosis*	*Plasma chitotriosidase (nmol h^{-1} ml^{-1} plasma)*	*β-glucosidase (nmol h^{-1} mg^{-1} protein) in leukocytes*	*Type of GD*	*Genotype*
1[a]	9 years	Gujarat	H, S, A, ERT	42748	8.3	I	Father: L444P (c.1448T>C/−) Mother: L444P (c.1448T>C/−)
2	2.5 years	Maharashtra	H, S, SME	29923	2.1	I	L444P (c.1448T>C)/L444P (c.1448T>C)
3	19 years	Maharashtra	H, S, A, SX, SB	6412	3.44	I	R463C (c.1504C>T)/R463C (c.1504C>T)
4	2.5 years	Maharashtra	H, S, DM, ERT	27786	4.4	I	L444P (c.1448T>C)/L444P (c.1448T>C)
5	3 years	Gujarat	H, S, T, ERT	38473	4.8	I	R359Q (c.1193G>A) /R359Q (c.1193G>A)
6	15 months	Gujarat	H, S, A, T, BD	32	3.8	I	L444P (c.1448T>C)/L444P (c.1448T>C)
7	21 years	Maharashtra	ERT	8763	NA	I	G355D (c.1181G>A)/G355D (c.1181G>A)
8	3 years	Maharashtra	H, S, GC, CN	85496	3.0	III	L444P (c.1448T>C)/L444P (c.1448T>C)
9	10 years	Maharashtra	NA	37404	4.4	I	L444P (c.1448T>C)/L444P (c.1448T>C)
10[a]	15 months	Gujarat	H, S, A, T, ERT	79000	1.96	I	Father: L444P (c.1448T>C/−) Mother: L444P (c.1448T>C/−)
11	1 year	Gujarat	H, S, A, GC	73205	3.23	I	L444P (c.1448T>C)/L444P (c.1448T>C)
12	3 year	Maharashtra	H, S, GC, DM	10687	0.0	I	L444P (c.1448T>C)/L444P (c.1448T>C)
13	4.5 year	Kerala	H, S, GC	19236	2.6	I	L444P (c.1448T>C)/L444P (c.1448T>C)
14	8 year	Maharashtra	H, S, A, T	19770	3.3	I	L444P (c.1448T>C)/L444P (c.1448T>C)
15	6 years	Maharashtra	S, GC, A, T	37618	2.85	I	L444P (c.1448T>C)/L444P (c.1448T>C)
16	10 months	Kerala	H, S, A, T	0.0	3.39	I	L444P (c.1448T>C)/L444P (c.1448T>C)
17	2 years	Maharashtra	H, S	9725.1	2.7	I	L444P (c.1448T>C)/L444P (c.1448T>C)
18	2.5 years	Karnataka	H, S	9083.9	2.9	I	L444P (c.1448T>C)/L444P (c.1448T>C)
19	40 years	Maharashtra	H, S, A, T	54503.7	1.5	I	R395C (c.1300C>T)/R395C (c.1300C>T)
20	6 years	Maharashtra	H, S	ND	2.2	I	V352M (c.1171G>A)/V352M (c.1171G>A)
21	2 years	Maharashtra	H, S	24580.1	2.9	I	L444P (c.1448T>C)/L444P (c.1448T>C)
22	2.5 years	Gujarat	H, S, GC, A, T	13465.6	2.6	I	L444P (c.1448T>C)/L444P (c.1448T>C)
23	2 years	Maharashtra	H, S, GC	1.4	2.1	I	L444P (c.1448T>C)/L444P (c.1448T>C)
24[a]	2 years	Maharashtra	H, S	14961.8	1.8	I	Father: L444P (c.1448T>C/−) Mother: L444P (c.1448T>C/−)
25	10 months	Sri Lanka	H, S, A, BD	19770	3.5	I	L444P (c.1448T>C)/L444P (c.1448T>C)
26	7 years	Maharashtra	H, S, GC, ERT	10473.2	Reduced activity	I	S356F (c.1184C>T)/S356F (c.1184C>T)
27	4.5 years	Sri Lanka	H, S, GC, A, GR	12824.4	3.2	I	E326K (c.1093 G>A)/E326K (c.1093 G>A)
28	11 years	Sri Lanka	H, S, GC, BD, GR, SX	18167.9	1.98	I	E326K (c.1093 G>A)/E326K (c.1093 G>A)
29	1 years	Delhi	H, S, DM	16030.5	2.8	I	G289A (c. 866 G>C)/G289A (c. 866 G>C)
30	1 years	Maharashtra	H, S, bone marrow transplantation	10794	4.0	I	Father: L444P (c.1448T>C/-) Mother: L444P (c.1448T>C/−)
31	20 years	Gujarat	H, S, GC, AVN, A, T	54503.7	2.5	I	L444P (c.1448T>C)/L444P (c.1448T>C)
32	22 months	Maharashtra	H, S, T	19236.0	1.98	I	L444P (c.1448T>C)/L444P (c.1448T>C)
33	9 years	Maharashtra	H, S	8015.25	1.00	I	I466S (c.1397 T>G)/?

Abbreviations: A, anemia; AVN, avascular necrosis; BD, bone disease; CN, cranial nerves affected; DM, delayed milestones, ERT, enzyme replacement therapy; GC, Gaucher cells in bone marrow; GR, growth retardation; H, hepatomegaly; MR, mental retardation; NA, not applicable; ND, not done; S, splenomegaly; SCE, saccade movement of eye; SX, splenectomy; T, thrombocytopenia.
Reference range: Plasma chitotriosidase: 28.66–62.94 nmol h^{-1} ml^{-1} plasma; β-glucosidase: 8.0–32.0 nmol h^{-1} mg^{-1} protein.
[a]DNA of index case is not available.

1 patient (3.03%) with type 3, which is in accordance with the earlier reported cases in International Collaborative Gaucher Group Gaucher Registry.[19]

The protocol described here allowed us to identify two novel sequence variants of the *GBA* gene in patients with GD. These include two missense mutations (G289A and I466S). *In silico* analysis was carried using Polyphen2, and showed G289A and I466S as probably damaging with a score of 0.963 (sensitivity: 0.78 and specificity: 0.95) and 1.0 (sensitivity: 0.00 and specificity: 1.00), respectively. SIFT/PROVEAN Human program further confirmed G289A and I466S mutation as deleterious with the score of −3.233 and −5.045 (http://genetics.bwh.harvard.edu/pph2 and http://sift.jcvi.org), respectively. Accelrys Discovery Studio software generated superimposed 10GS PDB native structures and 10GS PDB mutant structure of the acid-β -glucosidase (GlcCerase) as shown in Figure 2. It shows that mutation G289A located in β_4 strand and RMSD (root-mean-square deviation) value for superimposition is very small (0.009 Angstrom), which suggests that this mutation has little effect on the structure and mutation I466S has extra turn in α_8 helices. Both these mutations destabilize the protein structure.

We also observed that L444P mutation was common among the patients in this study, whereas the other common mutations N370S, IVS2 + 1G>A and 84GG were not detected in any of our patient. This is partly in disagreement with the previous study from India where L444P, N370S, IVS2 + 1G>A,D409H and 55Del mutant allele were observed in ∼50% of the GD patients with L444P as the most frequently identified, followed by D409H.[20] This is likely to be because of different ethnic group of northern origin with

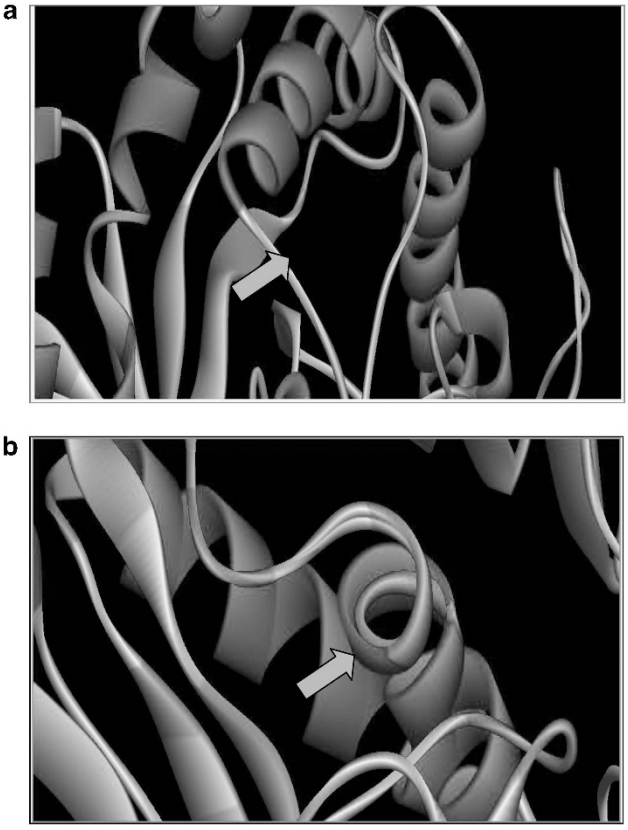

Figure 2 Superimposed native structures (green) and mutant structure (red) of the acid β-glucosidase (GlcCerase) produced using Accelrys Discovery Studio software: (**a**) mutation p. G289A located in β_4 strand and destabilize the protein structure; (**b**) mutation p.I466S has extra turn in α_8 helices and destabilize the protein structure. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

comparatively less number of patients, whereas ours is mainly consisting of western origin with fairly large number of patients.

Our study also demonstrate that L444P was the most common genotype in type 1 GD that is in accordance with the Taiwanese population with homozygous L444P mutation prevalent in 52.6%,[21] whereas German, Spanish and Portuguese patients were shown to have low prevalence of this mutation and higher prevalence of N370S mutation. L444P mutation was identified in 60% of mutant allele in Thai patients.[22] Study by Ida *et al.*[23] had shown L444P mutation as the second most common in GD among non-Jewish patients, accounting for 37% of the total mutations surveyed. L444P mutation was also found to be associated with all three groups of GD[24] in Spanish population. Aforementioned mutation was also demonstrated in neuronopathic form in Swedish, Pole, Ashkenazi Jewish and other Caucasian population, whereas in non-neuronopathic form in Taiwanese–Chinese[6] that is in agreement with present study. The expression of mutant allele on phenotype in different population could be because of effect of modifier gene on mutant allele as demonstrated by Alfonso *et al.*[10] in Spanish population.[9] It is also likely that many of our type 1 GD patients with L444P genotype may develop neurological symptoms at the later stage as has been observed in Turkish population.[6]

R463C mutation was observed with less frequency in type 1 GD that is similar to that observed in Turkish population.[6] This mutation was previously reported in 3.57% of non-Jewish population and not observed in Jewish population. Although the severity of this mutation is unknown but is reported to be associated with type 1 and type 3 GD.[25] R463C/Rec Nci I mutation was also reported in one type 3 GD patient from India.[26] R395C and R359Q are considered to be the mild mutation associated with non-neuronopathic type 1 GD patients with lesser frequency, which has been previously observed in Brazilian and Spanish type 1 GD patient.[27] G355D mutant allele was observed in type 1 GD in one patient, whereas the same was observed in type 2 Taiwanese children. This could be owing to ethnic diversity and some effect of modifier gene on the mutant allele.[28] V352M mutation was observed in one type 1 GD patient that was previously reported from Russia.[29]

Presence of S356F mutant allele was seen with severe phenotype type 1 except neuronal involvement in Turkish[24] patient, and in our case also this mutation was found with severe phenotype in one patient (3.03%). This has been reported earlier in one patient from Northern India[30] also.

The most variable of all the symptoms attributed to GD is that of skeletal involvement in these patients ranges from asymptomatic disease, with or without radiological signs, to symptomatic disease, which can be severe and engender considerable pain and disability. In our study, L444P, R463C and E326K mutations in type 1 GD patients are found to be associated with bone disease in one each. In one patient, L444P genotype was found to be associated with Erlenmeyer flasking of distal femora, pulmonary congestion and severe hepatosplenomegaly, and another patient with L444P/R329C genotype had shown phenotype of avascular necrosis and hepatosplenomegaly. Although none of our patients with severe bone disease had shown N370S as has been observed earlier.[31] L444P genotype in one of our type 3 GD had phenotype in which cranial nerves were affected. Phenotypic variations were descibed among patients with identical genotypes that is likely to be due to genetic, environmental or developmental factors that may contribute to the clinical expression of GD.[32]

Thus, present study provides an insight into the molecular bases of GD in Indian patients. The distribution of mutant alleles and the frequency with which particular genotypes were encountered displayed some specific particularities that may be related to the ethnic characteristics of our population. The identification of mutant alleles is crucial for advancing knowledge of the worldwide *GBA* mutation spectrum, and should contribute to a better understanding of the molecular basis of the disease. Such information will help in establishing genotype–phenotype correlations as well as in genetic counseling and/or in customized molecular analyses for families at risk.

CONCLUSION

To summarize, present study demonstrate L444P as the most common mutation in 63.63% (21/33) of Indian GD with severe type 1 phenotype except one that can be used as a primary molecular screening. Further it also demonstrates that exons 8 and 10 are the hot spot region of the *GBA* gene with 93.93% of mutant allele, and remaining mutation in exons 7 and 9 accounts for 6.06% of patients. Overall study provides the insights into the molecular basis of the disease that can be utilized for the molecular screening and understand the disease severity associated with all types of GD.

CONFLICT OF INTEREST

Jayesh Sheth is a scientific adviser to the Genzyme Sanofi India. While other authors have no conflict of interest.

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Author contributions: JS was involved in the designing of the study, standardization of technical procedure, preparation of manuscript and will also act as a guarantor. CA was involved in processing the sample, standardization of molecular techniques and preparation of the manuscript. MAM was involved in processing of sample for enzymes study. PT helped in the standardization of molecular technique by providing positive controls and independently confirmed L444P mutation in four samples. PT and FS have critically evaluated the manuscript. VK helped in protein modeling of novel mutations. AB, MM, UD, SN, SEK were involved in clinical data collection. SG helped in technical support. All the authors read and approved the manuscript.

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