SUPPLEMENTARY MATERIALS

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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′	MspI	931
	5′-GGGCTTACGTCGCTGTAAGCTCACACCGGC-3′		
R463C (c.1504C>T)	5′-CTGAACCCCGAAGGAGGACC-3′	MspI	931
	5′-GGGCTTACGTCGCTGTAAGCTCACACCGGC-3′		
N370S (c.1226A>G)	5′-TGTCTCTTT GCCTTTGTCCTTACCCTCGA-3′	AvaI	115
	5′-GACAAAGTTACGCACCCAATT-3′		
IVS2+1G-A (c.115+G>A)	5′-GCATCATGGCTGGCAGCCTCACAGGACTGC-3′	HpHI	255
	5′-GCCCAGGCA ACAGAGTAAGACTCTGTTTCA-3′		
Exon 2	F.P- 5′-GGAGAGGGGCTTGCTTTTCA-3′	—	371
	R.P- 5′-GGAGGCAGAGGTTGGAATGA-3′		
Exons 3–4	F.P- 5′-CAAGGGGTGAGGAATTTTGA-3′	—	696
	R.P- 5′-CACCACTGCACTCCTGTCTC-3′		
Exons 5–6	F.P- 5′-TGGCCCTGACTCAGACACTA-3′	—	788
	R.P-5′-CTGATGGAGTGGGCAAGATT-3′		
Exon 7	F.P- 5′-GGCTGTTCTCGAACTCCTGA-3′	—	473
	R.P-5′-ATAGTTGGGTAGAGAAATCG-3′		
Exon 8	F.P- 5′-AGTTGCATTCTTCCCGTCAC-3′	—	466
	R.P- 5′-ATCATGGTTCCCCAGAGTTG-3′		
Exon 9	F.P- 5′-CAGCTGCCTCTCCCACAT-3′	—	381
	R.P- 5′-GTGTGCCTCTTCCGAGGTT-3′		
Exons 10–11	F.P- 5′-GAGAGCCAGGGCAGAGCCTC-3′	—	569
	R.P- 5′-CTCTTTAGTCACAGACAGCG-3′		

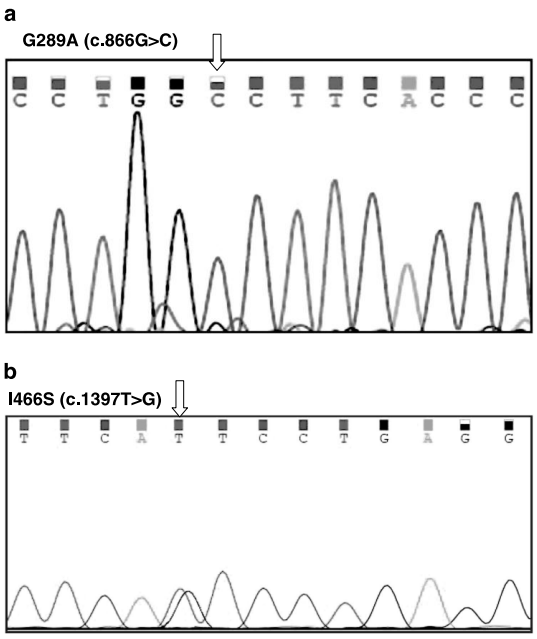

Figure 1 DNA sequencing chromatogram showing novel mutations: (**a**) G289A (c.866G>C) in homozygous state in exon 7; (**b**) I466S (c.1397T>G) in heterozygous state in exon 10. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

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

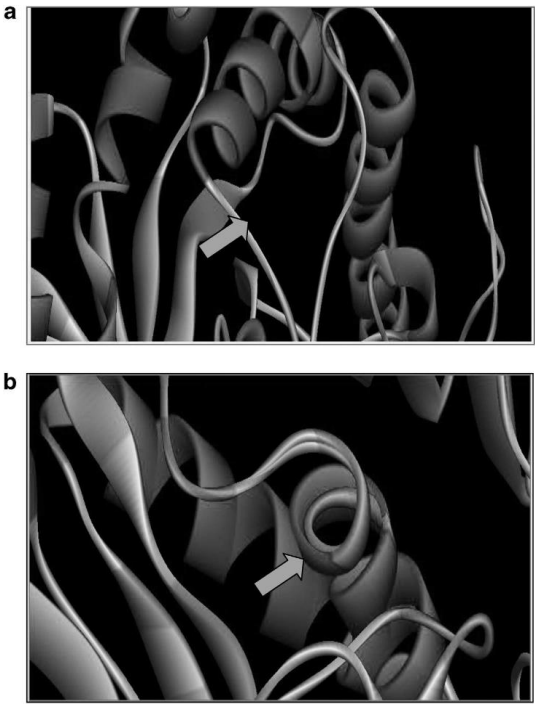

Figure 2 Superimposed native structures (green) and mutant structure (red) of the acid β-glucosidase (GlcCerase) produced using Accelrys Discovery Studio software: (**a**) mutation p. G289A located in β_4 strand and destabilize the protein structure; (**b**) mutation p.I466S has extra turn in α_8 helices 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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RESEARCH REPORT

Burden of Lysosomal Storage Disorders in India: Experience of 387 Affected Children from a Single Diagnostic Facility

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Abstract Lysosomal storage disorders (LSDs) are considered to be a rare metabolic disease for the national health forum, clinicians, and scientists. This study aimed to know the prevalence of different LSDs, their geographical variation, and burden on the society. It included 1,110 children from January 2002 to December 2012, having coarse facial features, hepatomegaly or hepatosplenomegaly, skeletal dysplasia, neuroregression, leukodystrophy, developmental delay, cerebral-cerebellar atrophy, and abnormal ophthalmic findings. All subjects were screened for I-cell disease, glycolipid storage disorders (Niemann-Pick disease A/B, Gaucher), and mucopolysaccharide disorders followed by confirmatory lysosomal enzymes study from leucocytes and/or fibroblasts. Niemann-Pick disease-C (NPC) was confirmed by fibroblasts study using filipin stain. Various storage disorders were detected in 387 children (34.8 %) with highest prevalence of glycolipid storage disorders in 48 %, followed by mucopolysaccharide disorders in 22 % and defective sulfatide degradation in 14 % of the children. Less common defects were glycogen degradation defect and protein degradation defect in 5 % each, lysosomal trafficking protein defect in 4 %, and transport defect in 3 % of the patients. This study demonstrates higher incidence of Gaucher disease (16 %) followed by GM2 gangliosidosis that includes Tay-Sachs disease (10 %) and Sandhoff disease (7.8 %) and mucopolysaccharide disorders among all LSDs. Nearly 30 % of the affected children were born to consanguineous parents and this was higher (72 %) in children with Batten disease. Our study also demonstrates two common mutations c.1277_1278insTATC in 14.28 % (4/28) and c.964G>T (p.D322Y) in 10.7 % (3/28) for Tay-Sachs disease in addition to the earlier reported c.1385A>T (p.E462V) mutation in 21.42 % (6/28).

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Introduction

Lysosomal storage disorders (LSDs) are the consequence of an abnormal storage of undigested cellular debris, proteins, fats, carbohydrates, and nucleic acids within the cell (Parkinson-Lawrence et al. 2010). They occur due to mutations in the genes that encode for lysosomal hydrolases, resulting in an attenuated enzyme activity and/or their transport to the lysosomes (Saftig and Klumperman 2009; Vellodi 2005). To date, nearly 50 such enzyme deficiencies are responsible for around 40 known storage diseases that have been identified (Vellodi 2005; Futerman and van Meer 2004).

Individually, these disorders are rare but collectively they are as common as other metabolic disorders with a reported incidence of 1:7,700 in Australia (Meikle et al. 1999). A newborn screening program in Taiwan identified a surprisingly high frequency of Taiwanese males with Fabry disease (~ 1 in 1,250). Hwu et al. (2009) identified IVS4+919G>A cryptic mutation in 86 % of the Fabry patients having a late-onset cardiac phenotype. Similarly, Gaucher disease is also one of the most common genetic disorders in Ashkenazi Jews, with a frequency of 1 in 855 live births (Staretz-Chacham et al. 2009). In the Ashkenazi Jews, 94–98 % patients with Tay-Sachs disease have three common mutations: c.1277_1278 insTATC, c.1421+1G>C, and c.805 G>A (p.G269S) (Myerowitz and Costigan 1988; Kaback et al. 1993), while a 7.6 kb deletion is the major mutation causing Tay-Sachs disease in the French Canadian population (Myerowitz and Hogikyan 1986).

A few Indian studies (Sheth et al. 2004; Verma 2000; Nalini and Christopher 2004; Verma et al. 2012) have partly addressed the incidence of LSDs in India along with mutation detection for Tay-Sachs disease (Mistri et al. 2012) and metachromatic leukodystrophy (MLD) (Shukla et al. 2011). Considering the large population and a high frequency of consanguineous marriages, the incidence of LSDs is likely to be higher in India. Hence, this study has been planned to know the burden of LSDs in high-risk group of children and identify common mutation for Tay-Sachs disease in the country, which is more commonly seen in schedule caste (SC) community of Gujarat (Mistri et al. 2012).

Material and Methods

This work is a prospective randomized study of 1,110 children referred from various Indian states and a couple of neighboring countries (Sri Lanka and Afghanistan) from January 2002 to December 2012. It comprises of 938 (84.50 %) children from western, 121 (10.9 %) from southern, 30 (2.7 %) from northern, 1 (0.09 %) each from eastern and central parts of India, 18 (1.62 %) from Sri Lanka, and 1 (0.09 %) from Afghanistan in the age range of 1 day to 16 years. This study includes 738 male and 372 female children. The most common phenotypes for the referral were coarse facial features, hepatomegaly or hepatosplenomegaly, skeletal dysplasia, neurological involvement including developmental delay or neuroregression, spasticity, seizures, leukodystrophy, cerebral-cerebellar atrophy, and abnormal ophthalmic findings such as cherry red spot, vision loss, and corneal clouding. An institutional ethics committee approval and patient informed consent was obtained to carry out this study.

Random urine and peripheral whole blood samples were collected in all the cases and were initially processed for the screening test followed by confirmatory enzyme study. The screening study was carried out from urine samples for glycosaminoglycans (GAGs) [both quantitative and qualitative] (Dembure and Roesel 1991; Hopwood and Harrison 1982) and plasma was analyzed for chitotriosidase activity (Aerts et al. 2008; Sheth et al. 2010) and mucolipidosis II/III [ML II/III] screening (Sheth et al. 2012). Leukocytes were isolated from whole blood and were processed by standard protocol for enzyme assay using 4-MU fluorometric assay or PNCS spectrophotometric synthetic substrate as outlined by Shapria et al. (1989); Filocamo and Morrone (2011); and Sheth et al. (2004). Niemann-Pick disease-C (NPC) study was carried out on cultured skin fibroblasts in lipid-deficient medium using filipin stain (Kruth and Vaughan 1980; Sheth et al. 2008).

The mutation analysis was carried out in 28 children with enzymatically confirmed diagnosis of Tay-Sachs disease. Genomic DNA was extracted by the standard salting out method (Miller et al. 1988) and investigated for common mutations c.1277_1278insTATC, c.1421+1 G>C, 7.6 kb deletion, the two pseudo-deficiency alleles c.739C>T (p.R247W) and c.745C>T (p.R249W) of *HEXA* gene followed by exon and intron flanking sequencing for known, rare, and private mutations in all the cases. Prediction of functional effects of non-synonymous single nucleotide substitutions (nsSNPs) was done using softwares SIFT (Sorting Intolerant From Tolerant) and Polyphen2 (Polymorphism Phenotyping v2).

Results

Out of 1,110 children, 387 (34.8 %) were found to be affected with different storage disorders (Table 1 and Fig. 1). Of these, 115 children (29.6 %) were born to consanguineous parents. Glycolipid storage disorders were the most commonly diagnosed LSDs (48 %) in this study population followed by mucopolysaccharide (MPS)

Table 1 Clinical and biochemical study of 387 children affected with different lysosomal storage disorders in India

Disease name/(enzyme name)/[substrate name]	Phenotype observed	Age at diagnosis	Number of cases investigated	Number of affected cases confirmed by enzymatic analysis	Enzyme activity detected in affected individuals (nmol/h/mg protein) Enzyme activity from leukocytes observed in normal individuals (nmol/h/mg protein)	Genotype analysis
Defects in degradation of glycolipids						
Gaucher disease (β-glucosidase) [4-MU-β-D-glucopyranoside]	*Type 1* – chronic non-neuropathic: severe hepatosplenomegaly, chronic anemia, and thrombocytopenia; Gaucher cells present in bone marrow	6 M–13 Y	425	60 (15.5 %)	0.0–4.6 8.0–32.0	Not yet carried out
	Type 3 – subacute neuropathic: degenerative of central nervous system, peripheral symptoms similar to type 1	10 M–3 Y	425	02 (0.51 %)	0.0–4.0 8.0–32.0	Not yet carried out
Tay-Sachs disease (β-hexosaminidase A) [4-methylumbelliferyl-N-acetyl-β-D-glucosamine-6-sulfate (MUGS)]	Neuroregression after 6 months of age, cherry red spot, vision loss, seizures, hypotonia, exaggerated startle response, progressive deafness, delayed mile stone, microcephaly, cerebral atrophy, partial optic atrophy	10 M–3 Y	465	39 (10 %)	0.0–23.4 80.4–410.0	*Earlier reported mutations*[a] c.340G>A (E114K) [exon 2]/c.340G>A (E114K) [exon 2] ($n = 1$) c.964G>A (D322N) [exon 8]/c.964G>A (D322N) [exon 8] ($n = 1$) c.964G>A (D322N) [exon 8]/ c.1277_1278insTATC [exon 11] ($n = 1$) c.964 G>T (D322Y) [exon 8]/c.964G>T (D322Y) [exon 8] ($n = 3$) c.1178C>G (R393P) [exon 11]/c.1178 C>G (R393P) [exon 11] ($n = 1$) c.1385A>T (E462V) [exon 12]/c.1385 A>T (E462V) [exon 12] ($n = 6$) c.1432G>A (G478R) [exon 13]/ c.672+30T>G [inton 6] ($n = 1$) c.1277_1278insTATC [exon 11]/ c.1277_1278insTATC [exon 11] ($n = 3$) c.508C>T (R170W) [exon 5]/c.508C>T (p.R170W) [exon 5] ($n = 1$) c.805+1 G>C [intron 7]/c.805+1G>C [intron 7] ($n = 2$)

(continued)

Table 1 (continued)

Disease name/(enzyme name)/[substrate name]	Phenotype observed	Age at diagnosis	Number of cases investigated	Number of affected cases confirmed by enzymatic analysis	Enzyme activity detected in affected individuals (nmol/h/mg protein) Enzyme activity from leukocytes observed in normal individuals (nmol/h/mg protein)	Genotype analysis
						Novel mutations c.788C>T (T263I)/c.788C>T (T263I) [exon 7] ($n = 1$) c.1121A>C (Q374P)/c.1121 A>C (Q374P) [exon10] ($n = 1$) c.1421G>A (W474X)/c.1420 G>A (W474X) [exon 12] ($n = 1$) c.1454G>A (W485X)/c.1454G>A (W485X) [exon 13] ($n = 2$) c.898-905delTTCATGAG/c.899-906delTTCATGAG [exon 8] ($n = 1$)
Sandhoff disease (β-hexosaminidase T) [4-MU-N-Ac-β-D-glucosaminide]	Neuroregression after 6 months of age, cherry red spot, vision loss, seizures, hypotonia, exaggerated startle response, progressive deafness, delayed mile stone, peripheral neuropathy, and organomegaly	7 M–3.5 Y	489	30 (7.75 %)	33.0–382.0 723.4–2700.0	Not yet carried out
Niemann-Pick disease A/B (Sphingomyelinase) [Hexadecarbonylamino-p-nitro-phenyl phosphoryl choline]	NPD A – neuropathicEarly life progressive loss of motor and intellectual capacity, hepatosplenomegaly, cherry red spot in macula, pneumonia, foamy histiocytes in bone marrow	5 M–2.3 Y	374	07 (1.8 %)	0.1–0.35 0.77–2.33	Not yet carried out
	NPD B – non-neuropathicLate onset as compared to type A, hepatosplenomegaly, cherry red spot in macula, foamy histiocytes in bone marrow	3–13 Y	374	17 (4.4 %)	0.25–0.46 0.77–2.33	Not yet carried out
GM1 gangliosidosis (β-galactosidase) [4-MU-β-D-galactopyranoside]	Cherry red spot, neuroregression, mild to moderate dysostosis multiplex, coarse facies, hypotonia, delayed mile stone, hepatosplenomegaly, mongolian spot on back, MRI shows diffuse dysmyelination of white matter with bilaterally symmetric thalamic signal change	7 M–4 Y	1,045	30 (7.75 %)	0.0–15.3 79.6–480.0	Not yet carried out

Defects in degradation of mucopolysaccharides						
Hurler syndrome (MPS I) (α-iduronidase) [4-MU-α-L-iduronide]	*MPS IH – Hurler syndrome* Progressive mental and physical disabilities, corneal clouding, coarse facies, dysostosis multiplex, stiff joints, organomegaly, hernia	7 D–2.1 Y	355	12 (3.1 %)	0.0–12 32.0–105.5	Not yet carried out
	MPS IHS-Hurler-Scheie/Scheie syndrome Progressive physical disabilities, corneal clouding, coarse facies, dysostosis multiplex, stiff joints, glaucoma, hernia	3–9 Y	355	09 (2.32 %)	2.1–12 32.0–105.5	Not yet carried out
Hunter syndrome (MPS II) (α-iduronidase-sulfatase) [4-MU-α-L-iduronide-2-sulfate]	Severe mental retardation, dysostosis multiplex, coarse facies, organomegaly	6 M–7 Y	30	09 (2.32 %)	0.0–9.3[b] 600–1616[b]	Not yet carried out
Sanfilippo syndrome A (MPS IIIA) (Heparan sulfamidase) [4-MU-α-D-sulfoglucosaminide]	Aggressive behavior, mental retardation, joint stiffness, hirsutism	2.5–9 Y	32	08 (2.0 %)	0.01–0.24[c] 1.3–6.8[c]	Not yet carried out
Sanfilippo syndrome B (MPS IIIB) (N-acetyl-α glucosaminidase) [4-MU-N-Ac-α-glucosaminide]	Similar to MPS type IIIA	5–6.5 Y	128	02 (0.51 %)	0.6–4.7[b] 300–600[b]	Not yet carried out
Morquio syndrome A (MPS IVA) (β-galactosidase-6-sulfate-sulfatase) [4-MU-β-galactose-6-sulfate]	Skeletal abnormality, short stature, short neck, prominent lower ribs, odontoid anomalies, coarse facies	1.6–9 Y	54	18 (4.65 %)	4.0–5.2 14.0–32.0[c]	Not yet carried out
Morquio syndrome B (MPS IVB) (β-galactosidase) [4-MU-β-D-galactopyranoside]	Mild skeletal dysplasia, short stature, corneal clouding, coarse facies	6 M–10 Y	1,045	04 (1.03 %)	2.2–24.2 79.6–480.0	Not yet carried out
Maroteaux-Lamy syndrome (MPS VI) (Arylsulfatase B) [P-nitro-catechol-sulfate]	Severe dysostosis multiplex, corneal clouding, coarse facies, cardiomyopathy	1 M–10 Y	389	18 (4.65 %)	0.0–0.25 0.65–8.5	Not yet carried out
Sly syndrome (MPS VII) (β-glucuronidase) [4 MU-β-D-glucuronide]	Mental retardation, coarse facies, organomegaly, corneal clouding, mild skeletal abnormality	5 M–12 Y	372	05 (1.29 %)	0.0–27.8 80–400.0	Not yet carried out

(continued)

Table 1 (continued)

Disease name/(enzyme name)/[substrate name]	Phenotype observed	Age at diagnosis	Number of cases investigated	Number of affected cases confirmed by enzymatic analysis	Enzyme activity detected in affected individuals (nmol/h/mg protein) Enzyme activity from leukocytes observed in normal individuals (nmol/h/mg protein)	Genotype analysis
Defects in degradation of sulfatides						
Metachromatic leukodystrophy (MLD) (Arylsulfatase A) [P-nitro-catechol-sulfate]	Peripheral neuropathy, absence of deep tendon reflexes, slowly progressive dementia, gait disturbance, convulsion, behavior changes. MRI shows diffuse symmetric abnormalities of periventricular myelin with hyperintensities on T_2-weighted images	1 M–16 Y	483	38 (9.82 %)	0.0–0.3 0.6–4.5	Not yet carried out
Krabbe disease (β-galactocerebrosidse) [6-hexadeconylamino-4-methylumbelliferyl-β-D-Galactopyranoside]	Progressive psychomotor retardation, convulsion, spasticity, absence of deep tendon reflexes, irritability, hyperthermia; progressive, diffuse, and symmetric cerebral atrophy is observed in neuroimaging	1 M–15 Y	85	17 (4.39 %)	0.0–5.50[c] 15.2–114.9[c]	Not yet carried out
Defects in degradation of glycoproteins						
Neuronal ceroid lipofuscinosis I (NCL I) (Palmitoyl protein thioesterase)[4-MU-6-sulfo-palmitoyl-β-D-glucoside]	Early onset psychomotor impairment, vision loss, GTC convulsion, dementia, spasticity; MRI shows variable cerebral atrophy and thalamic hypointensity in the white matter and basal ganglia	6 M –2 Y	39	05 (1.28 %)	3.0–9.3 25.5–215	Not yet carried out
Neuronal ceroid lipofuscinosis II (NCL II) (Tripeptidyl peptidase 1) [Alanyl-alanyl-phenylalanyl-7-amido-4-methylcoumarin (AAF-AMC)]	Late onset psychomotor impairment, vision loss, GTC convulsion, dementia, spasticity; MRI shows progressive cerebral and cerebellar atrophy with normal basal ganglia and thalami	2–16 Y	84	13 (3.08 %)	4.3–22.5 32.8–233	Not yet carried out

Defects in degradation of glycogen						
Pompe disease (α-1-4-glucosidase) [4-MU-α-D-glucopyranoside]	Recurrent hypoglycemic seizures, cardiomegaly and hypertrophic cardiomyopathy, delayed milestone, muscle weakness, noisy breathing, respiratory distress, mild hepatosplenomegaly, gait disturbance	1 D –13 Y	384	19 (4.9 %)	0.0–0.19[d] > 0.25–0.63[d]	Not yet carried out
Defects is lysosomal transporters						
Sialic acid storage disorder [N-acetyl-neuraminic acid (NANA)]	Regression motor and physical milestone, cherry red spot, coarse facial features, hepatosplenomegaly, gum hypertrophy, inguinal hernia	1 M –9 Y	45	07 (1.80 %)	Free NANA[e] 7.83 (0–6 M) 4.4–17.22 (7–24 M) 4.75 (61–120 M) Total NANA[e] 8.48 (0–6 M) 3.9–20.0 (7–24 M) 5.75 (0–6 M) Free NANA[e] 0.42–1.70 (0–6 M) 0.26–1.39 (−24 M) 0.1–0.38 (61–120 M) Total NANA[e] 0.92–3.0 (0–6 M) 0.55–2.31 (7–24 M) 0.2–0.64 (61–120 M)	Not yet carried out
Galactosialidosis (β-galactosidase) [4-MU-β-D-galactopyranoside] and [N-acetyl-neuraminic acid total (Total NANA)]	Regression of motor milestone, seizures, coarse facies, organomegaly, mongolian spot, mild skeletal abnormality	3 M –2 Y	09	03 (0.77 %)	β-galactosidase 79.6–480.0 Free NANA[e] 0.6–0.7 (0–6 M) 0.51 (13–24 M) Total NANA[e] 3.5–8.25 (0–6 M) 2.8 (13–24 M) β-galactosidase 79.6–480.0 Free NANA[e] 0.42–1.70 (0–6 M) 0.31–0.79 (13–24 M) Total NANA[e] 0.92–3.0 (0–6 M) 0.55–1.47 (13–24 M)	Not yet carried out

(continued)

Table 1 (continued)

Disease name/(enzyme name)/[substrate name]	Phenotype observed	Age at diagnosis	Number of cases investigated	Number of affected cases confirmed by enzymatic analysis	Enzyme activity detected in affected individuals (nmol/h/mg protein) Enzyme activity from leukocytes observed in normal individuals (nmol/h/mg protein)	Genotype analysis
Defects in lysosomal of trafficking proteins						
Mucolipidosis II/III	Delayed milestone, coarse features, kyphosis, mild scoliosis, hypertonia, skin pigmentations, low-set ears, mild hepatosplenomegaly, mongolian spot, thick skin	7 M–6.5 Y	179	11 (1.78 %)	Aryl sulfatase-A (416.2–2828.0)[b] β-hexosaminidse T (66560–333300)[b] β-glucuronidase (18700–40546)[b] α-fucosidase (26500–32000)[b] Aryl sulfatase-A (28–85)[b] β-hexosaminidse T (12000–30149)[b] β-glucuronidase (420–2054)[b] α-fucosidase (334–1275)[b]	Not yet carried out
Niemann-Pick disease C[f]	Psychomotor symptoms occurring in early age, neonatal jaundice, organomegaly, hypertonic limbs, facial dyskinesia	1–6 Y	11	4 (1.03 %)	Depressed cholesterol esterification and abnormal filipin staining Normal cholesterol esterification and normal filipin staining	Not yet carried out

Y Years; *M* Months

[a] (Mistri et al. 2012)

[b] Enzyme activity was carried out in plasma and activity was expressed in nmol/h/ml plasma

[c] Enzyme activity was expressed in nmol/17 h/mg protein

[d] Enzyme activity is expressed by calculating ratio of with acarbose and without acarbose

[e] Enzyme activity was carried out in urine mmol/g creatinine

[f] NPD-C was carried out in skin fibroblasts using filipin staining

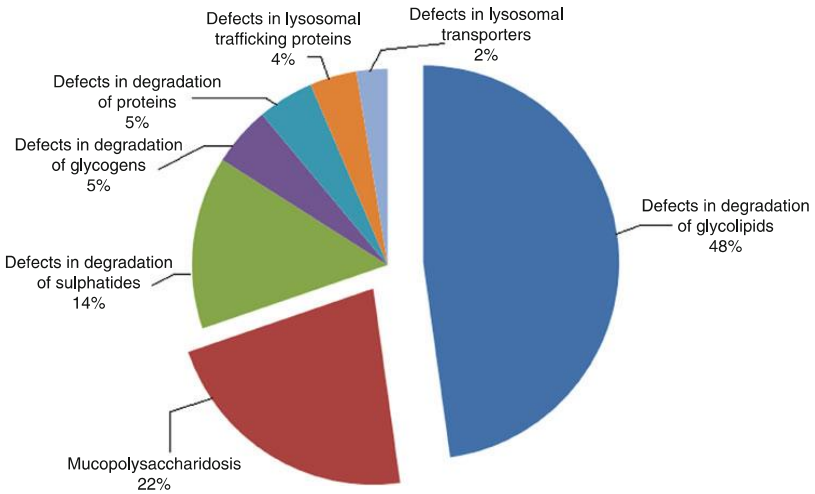

Fig. 1 Distribution of storage disorders in India

disorders (22 %) and sulfatide degradation defect (MLD and Krabbe disease) in 14 % of the patients. Glycogen storage disorder type II (Pompe disease), protein degradation defect (Batten disease), lysosomal trafficking protein defect (ML II/III and NPC), and lysosomal transporter defect (sialic acid storage disorder and galactosialidosis) were found to be comparatively less common (Fig. 1). Interestingly, all confirmed cases of Batten disease were from South India where consanguineous marriages were observed in 72 % of the cases.

Gaucher disease was one of the most common glycolipid storage disorders observed with a high frequency in Maharashtra 64.5 % (40/62) followed by GM2 gangliosidosis (Tay-Sachs disease and Sandhoff disease), which was more prevalent in Gujarat 49.7 % (34/69). Among MPS disorders, MPS I (Hurler, Hurler-Scheie, and Scheie syndrome) was the most common, followed by MPS IV (Morquio A and Morquio B), MPS VI (Maroteaux-Lamy syndrome), MPS II (Hunter syndrome), MPS III A and B (Sanfilippo syndrome A and B), and MPS VII (Sly syndrome). The remaining were sulfatide degradation defects with MLD and Krabbe disease, protein degradation defect with Batten disease (NCL I and II), lysosomal transporter defect with sialic acid storage disorder, and lysosomal trafficking protein deficiency with ML II/III and NPC (Table 1).

Mutation analysis was carried out for 28 children with confirmed diagnosis of Tay-Sachs disease. Common mutation c.1277_1278insTATC was detected in four cases and was confirmed by bidirectional sequencing. In the remaining 24 cases, exon sequence study revealed 14 different mutations, except one. This includes nine earlier reported mutations c.340G>A (p.E114K), c.1178C>G (p.R393P), c.1432G>A (p.G478R), c.508C>T (p.R170W) in one each, c.964G>A (p.D322N) in two, c.964G>T (p.D322Y) in three, and c.1385A>T (p.E462V) in six cases. Intron 7 showed variant c.805+1 G>C in two cases and intron 6 demonstrated c.672+30 T>G in one case. Additionally, two novel deleterious missense mutations c.788C>T (p.T263I) and c.1121A>C (p.Q374P) along with one small novel deletion c.898_905delTTCATGAG was detected in one case each. Two novel nonsense mutations c.1421G>A (p.W474X) and c.1454G>A (p.W485X) were observed in one and two cases, respectively. Pathological significance of novel missense mutations was confirmed using softwares SIFT and Polyphen2. Mutation spectrum of Tay-Sachs disease detected in various states of India is shown in Table 2, which also includes one case from Iran.

Discussion

This study demonstrates glycolipid storage disorders as one of the most common LSDs in India, similar to that observed in Portugal, Australia, and Czech Republic (Pinto et al. 2004; Meikle et al. 1999; Poupetova et al. 2010) with higher frequency of Gaucher disease (Fig. 2). The high prevalence of Gaucher disease (16 %) is in accordance with previously reported study (14.4 %) from northern India (Verma et al. 2012) and Mappila Muslims from southern India encompassing Kerala (Feroze et al. 1994).

GM2 gangliosidosis accounted for 10 % of Tay-Sachs and 7.8 % of Sandhoff patients, respectively. This was in accordance to the Portugal cohort especially for Tay-Sachs disease (13.8 %), while lower prevalence was

Table. 2 Regional distribution of mutation spectrum in Tay-Sachs disease

Disease condition	Gene involved	Mutation detection	Number of cases	Region – Country
Tay-Sachs disease	*HEXA*	c.1385 A>T (p.E462V)	6	Gujarat – India
		c.1277_1278insTATC (p.Y47IfsX5)	2	Gujarat – India
			2	Maharashtra – India
		c.964 G>T (p.D322Y)	2	Gujarat – India
			1	Uttar Pradesh – India
		c.964 G>A (p.D322N)	1	Gujarat – India
			1	Uttar Pradesh – India
		c.805+1G>C	1	Maharashtra – India
			1	Andhra Pradesh – India
		c.340G>A (p.E114K)	1	Maharashtra – India
		c.1432G>A (p.G478R)	1	Maharashtra – India
		c.672+30T>G	1	Maharashtra – India
		c.1178 C>G (p.R393P)	1	Tamil Nadu – India
		c.508C>T (p.R170W)	1	Iran
		c.788C>T (p.T263I)	1	Maharashtra – India
		c.1454G>A (p.W485X)	2	Maharashtra – India
		c.1121A>C (p.Q374P)	1	Maharashtra – India
		c.1421G>A (p.W474X)	1	Maharashtra – India
		c.898_905delTTCATGAG	1	Maharashtra – India

reported from Australia and Czech Republic (4.1 % and 2.5 %, respectively). High prevalence of GM2 gangliosidosis (Tay-Sachs disease and Sandhoff disease) has also been reported in children with neurological disorders from the southern region of India, where consanguinity is more common (Nalini and Christopher 2004). Higher incidence of Tay-Sachs in our study could be due to the presence of founder mutation in *HEXA* gene in the SC community of Gujarat (Mistri et al. 2012).

Mucopolysaccharidosis was the second most common LSD, which is in accordance with several other groups (Pinto et al. 2004; Meikle et al. 1999; Poupetova et al. 2010; Verma et al. 2012). Among these groups, MPS I was the most prevalent. This finding is similar to that observed by Verma et al. (2012). Although identification of MPS IV B in four cases is unique in our study, this could be either due to ethnic diversity or referral bias. A study from Czech Republic (Poupetova et al. 2010) reported only one case of MPS IV B out of 394 affected patients. Nonetheless, the prevalence of MPS IV A was found to be higher as compared to IV B which is in concordance with other reported studies (Pinto et al. 2004; Meikle et al. 1999; Poupetova et al. 2010). Occurrence of MPS III-A and III-B in this study is lower than that reported from Australia and Portugal (Pinto et al. 2004; Meikle et al. 1999) (Fig. 2). Milder clinical presentation, lack of clinical awareness, and diagnostic facility could be one of the reasons for underdiagnosis of MPS III disorders in this work.

In this study, the frequency of Pompe disease (GSD II) was higher (5 %) as compared to Czech Republic, Australia, and Portugal (Fig. 2). While Batten disease [Neuronal ceroid lipofuscinosis I and II (NCL I and II)] was observed in lower frequency (4 %) as compared to 7.5 % in Czech Republic, it was higher than that reported in Portugal (1.5 %). Interestingly, all cases of Batten disease in our study belonged to a single region of South India where higher incidence of consanguineous marriages is seen (72 %).

High carrier frequency of Fabry disease has been observed in Czech Republic and Taiwan, however, not a single case was detected in this study (Fig. 2) (Poupetova et al. 2010; Staretz-Chacham et al. 2009). This could be due to lack of awareness among clinicians involved in the cardiac and/or renal management of adults with Fabry disease. Unlike in Czech Republic, Australia, and Portugal (Meikle et al. 1999; Poupetova et al. 2010; Pinto et al. 2004), NPC was also the least observed lysosomal trafficking disorder. This could be a result of overlapping clinical phenotypes such as hepatosplenomegaly, neonatal jaundice, and psychiatric disturbances (Wenger et al. 2003) and limited investigational facilities for NPC.

In general, low prevalence of storage disorders like Fabry, NPC, and MPS III in our study is likely to be due to the lack of awareness among the clinicians and a dearth of enzyme diagnostic facilities in most parts of the country. In countries with higher incidences of aforementioned

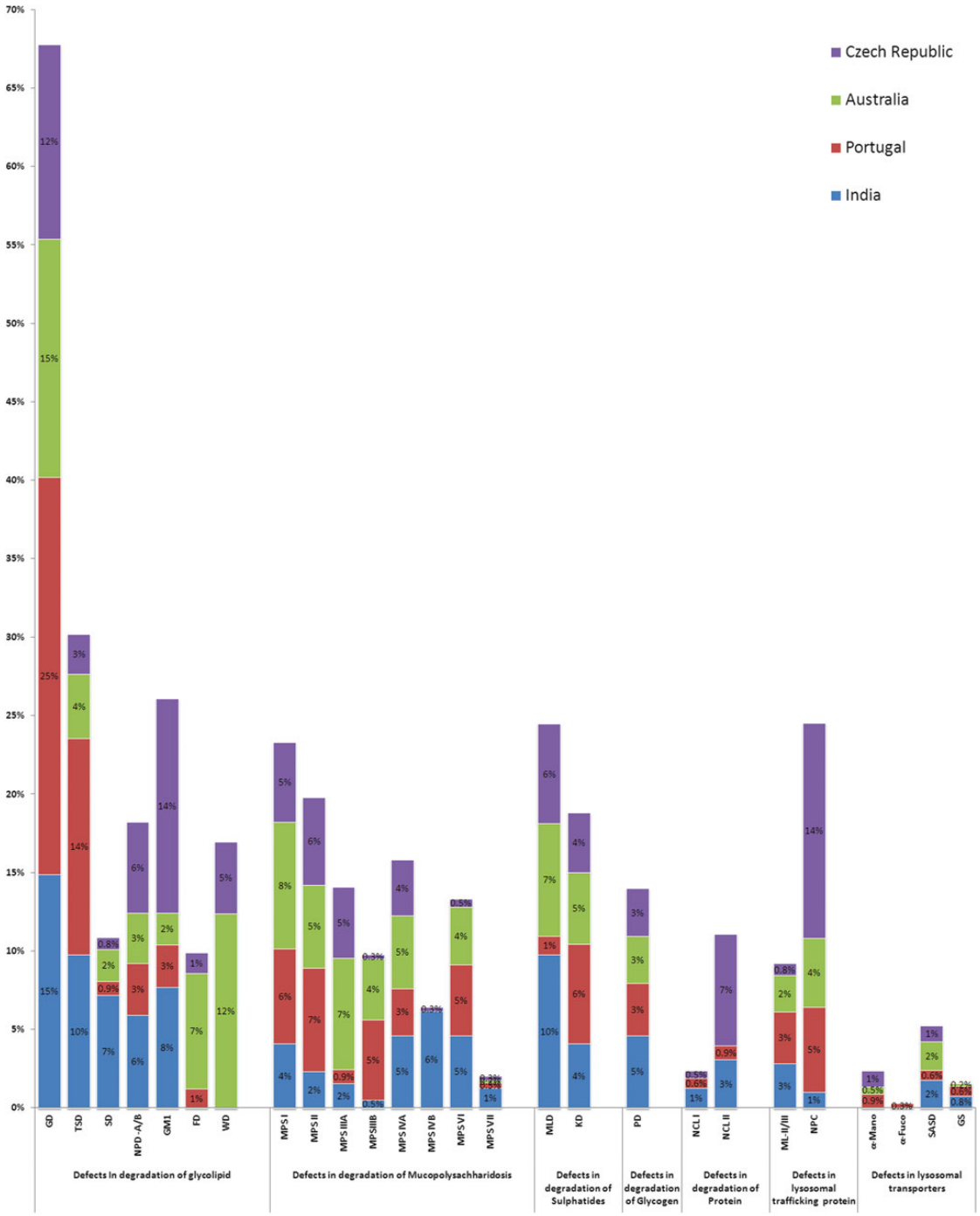

Fig. 2 Incidence of various LSDs in different countries

disorders, easy accessibility of specialized enzyme laboratory for confirming clinical diagnosis exists. Additionally, in these countries (Australia, Portugal, and Czech Republic) special working groups on LSDs increase awareness among the medical fraternity. Also patient support groups advocate the disease magnitude and support the affected families (Meikle et al. 1999; Pinto et al. 2004; Poupetova et al. 2010). In India, very recently Indian Council of Medical Research (ICMR) has set up a special task force on LSDs which will focus on the magnitude of these disorders in different parts of the country, increase awareness among the clinicians by organizing regional training program, and establish common mutation spectrum for different LSDs.

The ethnicity predilection is known to be associated with a specific lysosomal disease like Gaucher, Tay-Sachs, Niemann-Pick type A, and ML IV disease in Ashkenazi Jewish descendants (Marsden and Levy 2010); Salla disease and aspartylglucosaminuria in Finnish population; and Gaucher type III disease in people of Swedish descent (Marsden and Levy 2010). In this study, the prevalence of Gaucher disease was found to be higher in Maharashtra region, whereas GM2 gangliosidosis (Tay-Sachs) was more in Gujarat province.

Among all the LSDs, molecular analysis was carried out in 28 cases of Tay-Sachs disease by mutation identification in *HEXA* gene. Molecular study in 15 of these cases has been reported earlier with high prevalence of p.E462V mutation in a particular community (SC) of Gujarat (Mistri et al. 2012). In the remaining 13 cases, 6 aforementioned novel mutations have been observed in patients from Maharashtra region (Table 2) of the country that include two missense mutations in exon 7 and exon 10, two nonsense mutations in exon 12 and exon 13, and one small deletion (8 bp deletion) in exon 8. Deleterious effect of these mutations has been confirmed by SIFT and Polyphen2 software programs. The remaining seven patients have shown earlier reported mutations with c.1277_1278insTATC in three, p.D322Y in two, p.D322N and c.805+1G>C in one each. Thus, this study shows that p.E462V, p.D322Y, and c.1277_1278insTATC identify nearly 45 % (13/28) of the disease causing mutations in patients affected with Tay-Sachs disease from India.

Overall, the study demonstrates that glycolipid storage and MPS are the most common LSDs in India. By providing appropriate genetic counseling and offering prenatal diagnosis during subsequent pregnancies, the burden of these diseases could be reduced. Mutations p.E462V, p.D322Y, and c.1277_1278insTATC are possibly the commonest ones detected for Tay-Sachs disease in the Indian population and can be used as a part of common mutation screening program.

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Synopsis

This cross-sectional study provides an up-to-date description of the current scenario of different lysosomal storage disorders (LSDs) and its burden on the society and highlights the common mutation spectrum for Tay-Sachs disease in India.

Conflict of Interest

The authors declare that they have no conflict of interest.

Informed Consent

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.

Details of the Contributions of Individual Authors

JS designed the experiment and standardized the protocols. MM, NO, and CA were involved in processing of the samples. MJ provided the technical guidelines. RS, AB, KG, NN, CD, MK, and SM were involved in collection of the clinical details. MM, JS, and FS prepared the manuscript. All the authors read and approved the manuscript.

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Case Report

Splenomegaly, Cardiomegaly, and Osteoporosis in a Child with Gaucher Disease

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A 15-month-old girl, born to the consanguineous parents, was referred with the sign of massive splenomegaly associated with thrombocytopenia and anemia. Plasma Chitotriosidase estimation was carried out as a screening test and was found to be normal with reduced activity of β-glucosidase in leucocytes suggestive of Gaucher disease. At the age of 4 years, severe osteoporosis and cardiomegaly with pulmonary congestion were observed in the child. Molecular analysis for GBA gene has revealed homozygous status for L444P (c.1448C) in the proband, whereas parents and two elder sisters were found to be heterozygote. Prenatal study during the fourth pregnancy was carried out from cultured chorionic villi for β-glucosidase, which was in the carrier range. Further confirmation of the carrier status was carried out from amniotic fluid DNA and was found to be heterozygous for L444P (c.1448C) in the *GBA* gene. This case demonstrates that children with the sign of splenomegaly with anemia and thrombocytopenia need to be screened for Gaucher disease, and molecular study can further help to confirm the heterozygous status, where prenatal study by enzyme investigation demonstrate heterozygous condition.

1. Introduction

Gaucher disease (GD) is an autosomal recessive sphingolipid disorder resulting from the accumulation of glucocerebroside in the cells of macrophage-monocyte system as a result of a deficiency in lysosomal enzyme β-glucosidase [glucocerebrosidase, E.C. 3.2.1.45], which is encoded by the GBA gene on chromosome-1 [1]. The three main clinical types have been delineated according to the absence (type 1, nonneuronopathic) or the presence (type 2, acute neuronopathic, and type 3, sub acute neuronopathic) of neurological involvement [2]. Glucosylceramide, the accumulated glycolipid, is primarily derived from the phagocytosis and degradation of senescent leukocytes and, to a lesser extent, from erythrocyte membranes. The glycolipid storage gives rise to the characteristic Gaucher cells, which are typically present in the bone marrow, liver, spleen, lungs, and other organs. This contributes to pancytopenia, massive hepatosplenomegaly, and at times diffuse infiltrative pulmonary disease. However, signs like anemia, severe splenomegaly, and hepatomegaly were observed more frequently in younger patients [3].

2. Case Report

A 15-month-old female child was referred to our centre with the clinical sign of massive splenomegaly associated with thrombocytopenia and anemia. She was delivered after full-term normal pregnancy. She had seizure immediately after 2 hours of birth and was kept under observation for a day. Development of the child was normal till 1 year of age. Later on, her parents noticed sporadic crying and regression of milestones especially when she stopped walking. Her two elder sisters were healthy, and there was no family history of blood transfusion, splenectomy, and recurrent jaundice. She was referred for plasma chitotriosidase estimation at the age of 15 months, which was 64.12 nmol/hr/mL plasma (normal range: 28.66–62.94 nmol/hr/mL plasma). Ultrasound scanning at 15 months showed the spleen of about 11-12 cm. No active bleeding, hepatomegaly, or lymphadenopathy was observed. X-ray of the pelvis with lower limbs was found to be normal. Subsequently, colour Doppler was carried out, which had shown huge splenomegaly with normal splenic echotexture and splenic vein measuring 4 mm at splenic

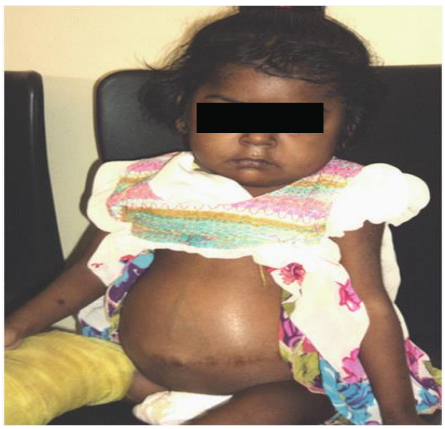


Figure 1: Proband with hepatomegaly at 4 years of age.

hilum. There was no evidence of periportal varices observed, and fundoscopy examination was found normal. On systemic examination, central nervous system (CNS) report showed consciousness with normal eye movements. She had no focal deficit, power was normal, mild hypotonia with deep tendon reflex (DTR)-brisk was noted. Proband was referred once again at the age of 2.5 years due to difficulty in walking with persistence of a large abdomen and her histopathology examination from liver biopsy also showed characteristic Gaucher cells occupying the sinusoids, and the spleen biopsy section revealed large cells with fibrillary cytoplasm and small eccentric nuclei populating red pulp. Plasma chitotriosidase study was reconfirmed and was found normal 32.00 nmol/hr/mL plasma, and β-glucosidase activity from leucocytes was low 3.8 nmol/hr/mg protein (normal range: 8.0–32.0 nmol/hr/mg protein) suggesting GD in the proband. At the age of 4 years, due to septicemia and bleeding from the mouth, proband was operated for splenectomy. At this time, X-ray of the chest posterior-anterior view showed cardiomegaly with pulmonary congestion and electrocardiogram (ECG) report revealed mild left ventricular systolic dysfunction with 40–50% of left ventricular ejaculation function. Ultrasound scanning at this time showed an enlarged liver measuring 15.5 cm in size (Figure 1). X-ray of the right tibia/fibula anteroposterior and lateral view revealed osteoporosis and fracture (Figure 2(a)) and X-ray of the right humerus revealed severe osteoporosis with cortical irregularity and fracture seen along midshaft of the humerus (Figure 2(b)). Hematogram investigation at this time showed Hb-6.7 (N.R.: 13.5–18.0 g/dL), total WBC count-29484 (N.R.: 4,000–11,000/cmm), and platelet count-45,000 (N.R.: 1, 50,000–4, 50,000/cmm).

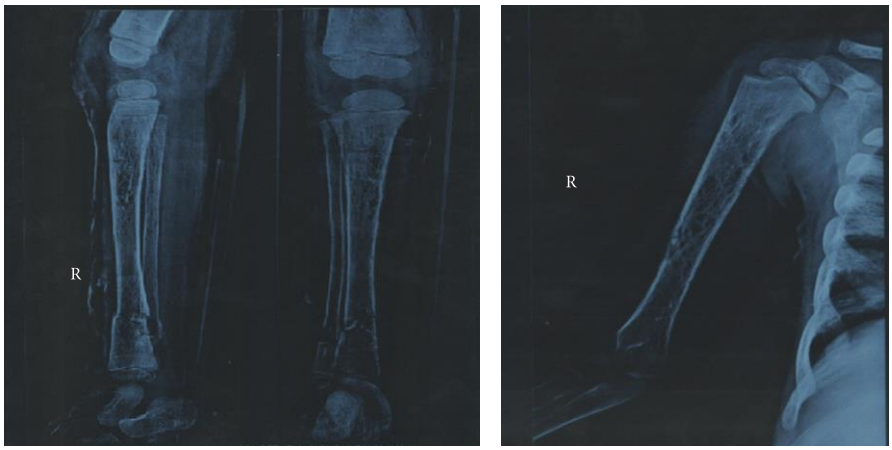
Further confirmation of the disease was carried out by molecular analysis of *GBA* gene. This has shown homozygous status for L444P (c.1448C), confirming GD. Two unaffected elder sisters and parents revealed heterozygous status for L444P (c.1448C) mutation. During the fourth pregnancy cultured chorionic villus study was carried out for β-glucosidase, which was in the carrier range 139.58 nmol/hr/mg protein with control value of 328.3 nmol/hr/mg protein. Further confirmation was carried out from amniotic fluid DNA, which has confirmed heterozygous status of the fetus for the said mutation.

3. Discussion

The most common signs and symptoms noted in GD are splenomegaly (95%), hepatomegaly (87%), radiological bone disease (81%), thrombocytopenia (50%), anemia (40%), growth retardation (34%), bone pain (27%), and bone crisis (9%). A skeletal manifestation is found more often in older children [3]. The femoral head and the femoral shaft are by far the most frequently involved sites in bone crisis. However, these episodes occur with some frequency in humeral heads, vertebral bodies, and ischium of pelvis [4]. In the present case, all these symptoms were observed and in addition osteoporosis and fracture in lower shaft tibia/fibula, severe osteoporosis with cortical irregularity and fracture were seen along the midshaft of the humerus. Cardiomegaly with pulmonary congestion was also observed at this time, and it is likely to occur due to frank infiltration of lungs by Gaucher cells [4].

Plasma chitotriosidase, which acts as a screening marker and shows marked elevated level in Gaucher disease, was found to be normal in present case [5]. This can be explained by the fact that 5-6% of the population who lack this enzyme as a result of genetic deficiency due to an expressional mutation in the human chitotriosidase gene that occurs with high polymorphic frequency and approximately one-third of patients with GD are heterozygous for this null allele, and thus the extent to which GD may increase the activity of chitotriosidase in the plasma is reduced in these individuals [6, 7]. Although β-Glucosidase activity in leucocytes or fibroblasts is the confirmative test for GD followed by mutation identification in GBA gene [8]. Our study is in accordance with this observation with significantly low activity of the enzyme in leucocytes followed by identification of L444P mutation in the proband. Identification of the genotype may help in predicting phenotypic expression, therapeutic response, and carrier screening for genetic counseling. Presence of L444P (c.1448C) homozygous status in the proband was useful in detecting carrier status in both parents, two unaffected sisters, and during prenatal diagnosis. This is one of the most common mutation observed in non-Jewish populations with a frequency of 31.43% and resulted in substitution of proline for leucine at position 444(L444P) [9]. This mutation was first found in neuronopathic form (Type II and type III) but later on it was also found to be associated with the nonneuronopathic form (Type I) [10].

It has been reported that nonneuronopathic GD is the most prevalent form (94%) and is differentiated from the acute neuronopathic (1%) and chronic neuronopathic (5%) forms by the absence of central nervous system involvement [3]. In the present report, hepatosplenomegaly, anemia, thrombocytopenia, osteoporosis, cardiomegaly, mild hypotonia, and homozygous for L444P (c.1448C) mutation and



(a) (b)

Figure 2: (a) X-ray of RT T/F AP/LAT shows osteoporosis and fractures. (b) X-ray of RT humerus shows severe osteoporosis.

normal central nervous system examination suggest Type-I Gaucher disease.

In such cases the long-term clinical picture is critical in light of data demonstrating that the efficacy of enzyme replacement therapy (ERT) with placental-derived preparation, alglucerase or the recombinant form, imiglucerase and substrate reduction therapy (e.g., miglustat) can prevent progressive manifestations of GD and completely or partly ameliorates disease-associated anemia, thrombocytopenia, organomegaly, bone pain, and bone crises [11].

As per our knowledge, this is the only case study from India where screening to confirmative molecular analysis has been carried out in the family. It clearly demonstrates that primary screening by plasma chitotriosidase followed by confirmative enzyme study of β-Glucosidase together with mutation identification can help the family to corroborate the prenatal and postnatal diagnosis for an early therapeutic approach, before irreversible clinical manifestation occurs.

Authors' Contribution

J. J. Sheth was involved in the designing of the study and in preparation of paper. J. J. Sheth will act as a guarantor. C. M. Ankleshwaria and M. A. Mistri were involved in processing of sample and writing of paper; N. Nanavaty and S. J. Mehta were involved in clinical data collection.

Conflict of Interests

The authors declare that there is no conflict of interests.

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