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

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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).

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 mucopolipidosis 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_1278 insTATC, 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]	<i>Type 1</i> – chronic non-neuropathic: severe hepatosplenomegaly, chronic anemia, and thrombocytopenia; Gaucher cells present in bone marrow <i>Type 3</i> – subacute neuropathic: degenerative of central nervous system, peripheral symptoms similar to type 1	6 M–13 Y	425	60 (15.5 %)	0.0–4.6 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	425	02 (0.51 %)	0.0–4.0 8.0–32.0	Not yet carried out
		10 M–3 Y	465	39 (10 %)	0.0–23.4 80.4–410.0	<i>Earlier reported mutations^a</i> c.340G>A (E114K) [exon 2]/c.340G>A (E114K) [exon 2] (<i>n</i> = 1) c.964G>A (D322N) [exon 8]/c.964G>A (D322N) [exon 8] (<i>n</i> = 1) c.964G>A (D322N) [exon 8]/c.1277_1278insTATC [exon 11] (<i>n</i> = 1) c.964 G>T (D322Y) [exon 8]/c.964G>T (D322Y) [exon 8] (<i>n</i> = 3) c.1178C>G (R393P) [exon 11]/c.1178 C>G (R393P) [exon 11] (<i>n</i> = 1) c.1385A>T (E462V) [exon 12]/c.1385 A>T (E462V) [exon 12] (<i>n</i> = 6) c.1432G>A (G478R) [exon 13]/c.672+30T>G [intron 6] (<i>n</i> = 1) c.1277_1278insTATC [exon 11]/c.1277_1278insTATC [exon 11] (<i>n</i> = 3) c.508C>T (R170W) [exon 5]/c.508C>T (p.R170W) [exon 5] (<i>n</i> = 1) c.805+1 G>C [intron 7]/c.805+1G>C [intron 7] (<i>n</i> = 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
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		<i>Novel mutations</i> c.788C>T (T263I)/c.788C>T (T263I) [exon 7] (<i>n</i> = 1) c.1121A>C (Q374P)/c.1121 A>C (Q374P) [exon10] (<i>n</i> = 1) c.1421G>A (W474X)/c.1420 G>A (W474X) [exon 12] (<i>n</i> = 1) c.1454G>A (W485X)/c.1454G>A (W485X) [exon 13] (<i>n</i> = 2) c.898-905delTTTCATGAG/c.899-906delTTTCATGAG [exon 8] (<i>n</i> = 1) 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 NPD B – non-neuropathicLate onset as compared to type A, hepatosplenomegaly, cherry red spot in macula, 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
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)	<i>MPS IH – Hurler syndrome</i> 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
[4-MU- α -L-iduronide]						
Hunter syndrome (MPS II) (α -iduronidase-sulfatase)	<i>MPS IHS-Hurler-Scheie syndrome</i> 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
[4-MU- α -L-iduronide-2-sulfate]						
Sanfilippo syndrome A (MPS IIIA) (Heparan sulfamidase)	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
[4-MU- α -D-sulfoglucosaminide]						
Sanfilippo syndrome B (MPS IIIB) (N-acetyl- α -glucosaminidase)	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
[4-MU-N-Ac- α -glucosaminide]						
Morquio syndrome A (MPS IVA) (β -galactosidase-6-sulfate-sulfatase)	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
[4-MU- β -galactose-6-sulfate]						
Morquio syndrome B (MPS IVB) (β -galactosidase)	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
[4-MU- β -D-galactopyranoside]						
Maroteaux-Lamy syndrome (MPS VI) (Arylsulfatase B)	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
[P-nitro-catechol-sulfate]						
Sly syndrome (MPS VII) (β -glucuronidase)	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
[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-galactocerebrosidase) [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
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Defects in lysosomal transporters

Sialic acid storage disorder [N-acetyl-l-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							
Mucopolipidosis 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	<i>HEXA</i>	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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