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ORIGINAL ARTICLE

Prevalence of lysosomal storage disease (LSD) in Malaysia

Affandi OMAR[1], Salina ABDUL RAHMAN[1], Rosnani MOHAMED[1], Fatimah Diana AMIN NORDIN[1], Norashareena MOHAMED SHAKRIN[1], Sofwatul MUKHTAROH NASOHAH[2], Nor Shuhaili SALLIH[3], Nor Azimah ABDUL AZIZE[4], Siti Aishah ABDUL WAHAB[4], Seok Hian LUA[4], Yusnita YAKOB[4], Wan Ahmad Syazani MOHAMED[5], Mohd Shihabuddin AHMAD NOORDEN[6]* and Julaina ABDUL JALIL[1]

[1]Inborn Errors of Metabolism & Genetics Unit, NMCRC, Institute for Medical Research, National Institutes of Health (NIH), Ministry of Health Malaysia, Selangor, Malaysia; [2]Biochemistry Unit, SDC, Institute for Medical Research, National Institutes of Health (NIH), Ministry of Health Malaysia, Kuala Lumpur, Malaysia; [3]Rehabilitation Department, Hospital Sultan Idris Shah Serdang, Selangor, Malaysia; [4]Molecular Diagnostic Unit, SDC, Institute for Medical Research, National Institutes of Health (NIH), Ministry of Health Malaysia, Kuala Lumpur, Malaysia; [5]Nutrition Unit, NMCRC, Institute for Medical Research, National Institutes of Health (NIH), Ministry of Health Malaysia, Selangor, Malaysia; [6]Faculty of Pharmacy, Universiti Teknologi MARA, Selangor, Malaysia

Abstract

Lysosomal storage disorders (LSD) are storage disorders involving the malfunction of degradation enzymes in the lysosome. This study aimed to calculate the birth prevalence and carrier frequency of LSDs in the Malaysian population, to compare our results with previously reported epidemiologic data from other populations, and to describe the mutation spectrum in Malaysia. Between 2008 and 2017, 2.1% (92/4338) of suspected patients were diagnosed with LSD. The prevalence of LSD and carrier frequency in Malaysia were 0.43 per 100,000 live births and 1 in 241, respectively. The combined prevalence of mucopolysaccharidoses (MPS) and its carrier frequency were 0.34 per 100,000 live births and 1 in 271, respectively. Among this MPS group, MPS II presented the highest calculated birth prevalence of 0.45 per 100,000 male live births with a carrier frequency of 1 in 236. Within the group of sphingolipidoses, the combined prevalence was 0.13 per 100,000 live births with a carrier frequency of 1 in 439. Fabry disease was the most common disorder with a calculated prevalence of 0.52 per 100,000 male live births and a carrier frequency of 1 in 220 followed by metachromatic leukodystrophy (MLD) (0.2 per 100,000 live birth and carrier frequency 1 in 352). MLD is more common among people of Iban ethnicity with a prevalence of 14.33 per 100,00 live births and a carrier frequency of 1 in 42. Pompe and mucolipidosis type II are the less common subtypes of LSD with a prevalence of 0.06 per 100,000 live births and a carrier frequency of 1 in 651 and 0.04 per 100,000 live births with carrier frequency of 1 in 747, respectively. Overall, although the prevalence of LSD in Malaysia may be underestimated, the prevalence of MPS is consistent with reports done in other Asian countries.

Keywords: Lysosomal storage disease, prevalence, Malaysia

INTRODUCTION

Lysosomal storage disease (LSD) is a constellation of genetic storage disorders characterised by the deficiencies of lysosomal enzymes. Subsequently, it will result in the malfunction of the macromolecules' degradation mechanisms. Genetically, LSD is mostly an autosomal recessive disorder, except Fabry and mucopolysaccharidoses (MPS) II which are X-linked and noted to be more common in consanguineous marriages.[1] LSD is also notably known as one of the rarest diseases worldwide, with a prevalence ranging from 12 to 25 per 100,000 live births.[2,3]

Currently, there are more than 50 types and sub-types of LSD have been discovered which can be divided into the following groups: (i) MPS, (ii) sphingolipidoses, (iii) oligosaccharidoses,

**Address for correspondence:* Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia. Tel: +603-32584843; Email: *shiha@uitm.edu.my*

(iv) mucolipidoses, (v) lipoprotein storage disorders, (vi) lysosomal transport defects and (vii) neuronal ceroid lipofuscinoses and others. Within its vast classification, several subtypes were discovered within each group. For example, in MPS group, there are six subtypes: MPS type I, MPS type II, MPS type III (with sub subtype of MPS IIIA, MPS IIIB, MPS IIIC, MPS IIID), MPS type IV (with sub subtype of MPS IVA and MPS IVB), MPS type VI and MPS type VII. As for Fabry, Gaucher, ganglioside monosialic 1 (G_{M1}) gangliosidoses, ganglioside monosialic 2 (G_{M2}) gangliosidoses, metachromatic leukodystrophy (MLD) and Krabbe disease, all were categorised under sphingolipidoses group.

Patients who have suffered with LSD may appear normal at birth but deteriorate in the first years of life as they can present with several clinical manifestations such as coarse facies, mental retardation, hepatosplenomegaly, dysostosis multiplex, valvular heart disease, respiratory pathology, and renal impairment. The mortality of the affected patients may increase during their adolescence if the treatment remains unaddressed.[4] Thus, treatments such as enzyme replacement therapy (ERT) have been extensively used in some LSD such as Fabry, Gaucher and Pompe diseases as it can improve the quality of life and prognosis of the patients.[5-7] Hence, early diagnosis of LSD is crucial for successful treatment and to prevent further complications.

Due to the fact that the public understanding of LSD is still lacking as these diseases are rare, it may prompt an urgency to evaluate the prevalence and mutation spectrum of LSD.[8] In Malaysia, a preliminary study on selective screening of MPS was carried out between 2014-2016.[9,10] Despite that, the current prevalence of LSD in Malaysia is still unidentified and incomprehensively described. Therefore, the current study aims to evaluate the birth prevalence of LSD with its carrier frequencies, along with its unique mutation spectrum in Malaysian population.

MATERIALS AND METHODS

Study Design

The study is conducted in a cross-sectional design, in which retrospective laboratory records-based analyses of patients diagnosed with LSD were retrieved and reviewed between 2008 to 2017. The blood and urine samples of all LSD patients within Malaysia, which included biochemical assessments and genotyping were referred to Institute for Medical Research (IMR) as part of our diagnostic routine. Patients were included in the study only if the diagnosis of LSD was confirmed with both screening and confirmatory testing.

Geographical origin of the case studied

As IMR is the only reference centre in Malaysia that provides comprehensive tests and diagnosis for LSD, we received the samples from all of Malaysia's healthcare facilities, including government and private hospitals. The received samples cover six main regions: i) the central region includes the Federal Territory of Kuala Lumpur, the Federal Territory of Putrajaya, the State of Selangor and Negeri Sembilan; ii) The northern region consists of the states of Perak, Penang, Kedah and Perlis; iii) The states of Kelantan, Terengganu and Pahang form the eastern region; iv) The southern region includes the states of Melaka and Johor and v) The region of Sabah and the region of Sarawak (Malaysian Borneo).

Variables and measurement

Variable measures in this study include gender, ethnicity, regionality, age at diagnosis, frequency of LSD, LSD group, and LSD type.

Laboratory processes and tests

The diagnostic workflow outlined in Table 1 reflects a tiered approach, beginning with broad biochemical screening followed by targeted confirmatory testing. Initial screening tests such as glycosaminoglycans (GAGs) quantitation, qualitative oligosaccharidosis analysis and Pompe screening using dried blood spots (DBS) were chosen for their utility in detecting early biochemical analysis associated with LSDs. Multiplex enzyme assays using tandem mass spectrometry allow simultaneous evaluation of multiple conditions from a single DBS sample, optimising efficiency and cost. Confirmatory testing includes individual enzyme assays performed on plasma or leukocytes to establish definitive biochemical diagnoses with higher specificity. Where enzyme activity was insufficient to distinguish subtypes or confirm inheritance patterns, genetic testing via PCR and bidirectional sequencing of relevant genes was conducted. This multilevel strategy ensures diagnostic accuracy, facilitates early intervention and supports genetic counselling and family studies.

Table 1: Overview of the Diagnostic Workflow and Testing Methods for LSD Diagnosis

Level	Test	Sample type	Methodology	Disease(s) covered	Equipment(s)
Screening	Quantitation of glycosaminoglycans	Urine	Spectrophotometry and characterisation of GAGs using one-dimensional high-resolution electrophoresis	Mucopolysaccharidosis (MPS) subtype	Spectrophotometer and one-dimensional high resolution electrophoresis
	Qualitative oligosaccharidoses	Urine	Thin layer chromatography (TLC)	- α-mannosidosis - α-fucosidosis, - GM_1 gangliosidosis, - GM_2 gangliosidosis, - β-mannosidosis, - Pompe/Glycogen Storage Disease Type II - Schindler disease	Thin layer chromatography (TLC)
	Pompe screening	Dried blood spots (DBS)	Fluorometry method	Quantitation of α-glucosidase enzyme in DBS	Fluorometer
	Multiplex enzyme assay	Dried blood spots (DBS)	Tandem mass spectrometry	Screening: - Niemann-Pick - Fabry - Pompe - Krabbe - Gaucher	Tandem mass spectrometry
Confirmatory	Individual enzyme assay	• Plasma • Leucocyte	Colourimetry or fluorescence method	- MPS Type I - MPS Type II - MPS Type IIIA - MPS Type IIIB - MPS Type IIIC - MPS Type IIID - MPS Type IVA - MPS Type IVB - MPS Type VI - MPS Type VII - GM1 gangliosidosis - GM2 gangliosidosis (Tay-Sachs) - GM2 gangliosidosis (Sandhoff)	Spectrofluorometer

Table 1: Overview of the Diagnostic Workflow and Testing Methods for LSD Diagnosis

Level	Test	Sample type	Methodology	Disease(s) covered	Equipment(s)
Confirmatory	Individual enzyme assay	• Plasma • Leucocyte	Colourimetry or fluorescence method	- Fabry - Gaucher - Krabbe - Metachromatic Leukodystrophy - Niemann-Pick - α-Mannosidosis - β-Mannosidosis - Aspartylgluco-saminuria - Fucosidosis - Schindler - Mucolipidoses - Galactosialidosis - Multiple sulphatase - Neuronal Ceroid Lipofuscinosis	Spectrofluorometer
	Mutational analysis of respective genes	DNA extraction from whole blood	Polymerase Chain Reaction (PCR) and bidirectional sequencing	- Gaucher - Pompe - MPS Type VI - MPS Type IVA - Metachromatic Leukodystrophy (MLD) - Fucosidosis	- Mastercycler Pro (Eppendorf, Germany) - Veriti (Applied Biosystems, USA) - 3500 Genetic Analyzer (Applied Biosystems, USA)

Data Analysis

The number of live births was obtained from the Department of Statistics Malaysia (https://www.dosm.gov.my) and its prevalence was expressed as the number of patients per 100,000 live births[11] and calculated using the method reported by Poorthuis *et al.*[3] Meanwhile, the birth period (as defined as the time interval between the year of birth of the oldest patient and the year of birth of the youngest patient) of each LSD type was calculated using the birth period method reported by Pinto *et al.*[2] Subsequently, the data was compared with other reference countries with a high frequency of LSD, which include the United Arab Emirates (UAE) (an Asian country); while the Czech Republic, the Netherlands, and Northern Portugal representing European countries.[2,3,12,13]

As for mutation analysis, the sequencing results obtained were evaluated by aligning with the human reference sequence retrieved from the NCBI database; and the pathogenicity of variants detected was classified by using in-silico prediction software, i.e., MutationTaster and available information from publications as well as clinical and population frequency databases such as gnomAD. Carrier frequency was calculated using the Hardy-Weinberg equation online calculator at http://perinatology.com/calculators/Hardy-Weinberg.htm.

Descriptive statistics were presented as numbers and percentages for categorical variables. Patients with missing gender and age data were excluded from the descriptive statistics. A median with an interquartile range (IQR) was used for data that was not normally distributed. Data collected was tabulated using IBM SPSS Statistics version 22 software packages (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp).

RESULTS

Patient demographics

The demographics of Malaysian patients are presented in Table 2, in which 44.6% of total patients originated from the central region. Out of 4338 patients who were clinically suspected of having LSD, 92 patients (2.1%) were diagnosed with 15 different subtypes. Demographically, the median age at diagnosis was corresponding to 2.54 years, ranging from 0.01 (4 days) to 40 years, with most patients (93.6%) being below the age of 18 years at diagnosis. Meanwhile, the gender distribution was almost balanced between males and females with 55.4% and 44.6%, respectively. In terms of ethnicities, the result showed that Chinese were 17 times more likely to have Pompe disease compared to others (OR 16.98, 95% CI 3.52-81.88, $p<0.05$).

Distribution of LSD subtypes

In this study, we have diagnosed fifteen subtypes of LSD. The MPS (51.1%) was the most common subtype followed by sphingolipidoses (33.7%), Pompe (9.8%) and mucolipidoses type II (5.4%) (Table 2). In relation to the age of diagnosis, patients with Pompe disease were diagnosed as early as 0.58 years while it took approximately 9.33 years to diagnose patients with MPS IVA. Only one case was found in patients with Krabbe and Fabry disease, respectively. No patient was diagnosed with α-mannosidosis, β-mannosidosis, Schindler disease, MPS Type IIIC/IIID and MPS Type VII.

In the MPS group, MPS Type II (31.9%) represented more than a quarter of all MPS cases followed by MPS Type IVA (21.3%), MPS Type VI (19.1%), MPS Type I (14.9%), MPS Type IIIA (8.5%) and MPS Type IIIB (4.3%). Nearly 50% of cases were diagnosed with the adult form of MPS IV and mostly originated from the central region (44.6%). As for the sphingolipidoses group, out of 31 patients, nearly half of the cases were diagnosed as MLD (48.4%), followed by fucosidosis (12.9%), G_{M1} gangliosidosis (12.9%), Gaucher (12.9%), G_{M2} gangliosidosis (Sandhoff) (6.5%), Krabbe (3.2%) and Fabry (3.2%). Nine patients were diagnosed with Pompe disease, and the majority of them had infantile onset.

The comparison of LSD prevalence between Malaysia with one Asian country (UAE) and three European regions*:* Czech Republic, Northern Portugal and the Netherlands is shown in Table 3. The prevalence of LSD among Malaysian was 0.43 per 100,000 live births or 1 in 231,904 live births, in which nearly 60-fold lower as compared to UAE and Northern Portugal. This prevalence also reflects the low number of diseases diagnosed under LSD in Malaysia, such as Mucolipidosis II, Pompe Disease, Sphingolipidoses and MPS. There were also between-country differences in the prevalence of individual types of diagnosed LSD, not only between Malaysia, UAE and European countries, but also between UAE and the European countries. For example, Fabry and MPS II were the most prevalent in Malaysia, while G_{M2} and Pompe were common in Northern Portugal and the Netherlands respectively.

Table 2: Demographic characteristic of patients with lysosomal storage disease in Malaysia

Disorders	Gender (Frequency)			Ethnicity (Frequency)						Regional (Frequency)					Median age of diagnosis (range), years
	Male	*Female*	*Total*	*Malay*	*Chinese*	*Indian*	*Sabah Native*	*Sarawak Native*	*Others*	*Central*	*Northern*	*East Coast*	*Southern*	*Sabah*	*Sarawak*	
Total MPS	29	18	47	32	0	2	3	1	0	25	9	4	3	3	3	2.50 (0.01-24.00)
MPS I	2	5	7	4	1	0	2	1	0	1	0	2	0	2	2	1.33 (0.67-4.00)
MPS II	15	0	15	14	0	0	0	0	0	8	4	2	0	1	0	3.33 (0.17-9.00)
MPS IIIA	3	1	4	4	2	0	0	0	0	1	2	0	1	0	0	5.50 (0.25-11.00)
MPS IIIB	0	2	2	0	4	0	0	0	0	1	0	0	1	0	0	2.08 (1.17-3.00)
MPS IVA	5	5	10	5	2	1	0	0	0	8	2	0	0	0	1	9.33 (0.01-24.00)
MPS VI	4	5	9	5	9	1	1	0	0	6	1	0	1	0	0	2.00 (0.21-16.83)
Mucolipidoses Type II	0	5	5	5	0	0	0	0	0	1	1	1	2	0	0	2.50 (1.00-22.00)
Pompe	4	5	9	0	7	2	0	0	0	6	2	0	0	0	1	0.58 (0.17-23.92)
Total Sphingolipidoses & Oligosaccharidoses	18	13	31	16	3	0	1	10	1	9	5	3	1	2	11	2.58 (0.08-40.00)
Fucosidosis	2	2	4	0	2	0	0	2	0	1	0	0	0	0	3	3.17 (1.42-6.58)
Gaucher	1	3	4	4	0	0	0	0	0	2	0	0	1	1	0	4.50 (0.14-7.00)
G_{M1}	2	2	4	4	0	0	0	0	0	4	0	0	0	0	0	1.58 (0.77-10.00)
G_{M2} (Sandhoff)	2	0	2	1	0	0	1	0	0	0	1	0	0	1	0	1.13 (1.00-1.25)
MLD	10	5	15	4	1	0	0	9	1	1	4	3	0	0	7	2.67 (0.08-14.00)
Fabry	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	40.00
Krabbe	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0.69
Total LSD	**51**	**41**	**92**	**53**	**19**	**4**	**4**	**11**	**1**	**41**	**17**	**8**	**6**	**5**	**15**	**2.54 (0.01-40.00)**

Abbreviation: MPS (Mucopolysaccharidoses), MLD (Metachromatic leukodystrophy), GM_1 (GM_1 gangliosidosis), GM_2 (GM_2 gangliosidosis)
Others referring other minorities e.g., Sikh, Portuguese etc. Central: State of Selangor, Negeri Sembilan, Federal Territory of Kuala Lumpur and Putrajaya; Northern: State of Perak, Pulau Pinang, Kedah and Perlis; East Coast: State of Pahang, Terengganu, and Kelantan; Southern: State of Melaka and Johor

Table 3: Birth prevalence of LSDs in Malaysia and comparison of data in different populations

Disease	No. of patients 2008-2017	Years of birth	No. of live births	Carrier frequency^	Birth prevalence (per 100,000)				
					This study	United Arab Emirates (UAE)	Czech Republic	Northern Portugal	The Netherlands
Mucolipidosis II	5	1994-2015	11,147,581	747	0.04	1.35	0.22	0.81	0.16
Pompe	9	1988-2017	15,251,705	651	0.06	2.66	NA	0.17	2.00
Oligosaccharidoses									
Fucosidosis	4	2006-2011	2,917,609	427	0.14	2.02	0.00	0.00	0.05
Sphingolipidoses (all types)	27	1974-2016	20,810,991	439	0.13	NA	5.00	12.6	6.2
Gaucher	4	2009-2017	4,550,140	534	0.09	0.25	1.13	1.35	1.16
GM1	4	2002-2016	7,403,711	681	0.05	4.66	0.26	0.62	0.41
GM2 (Sandhoff)	2	2010-2013	1,983,105	498	0.10	1.21	0.19	3.13	0.41
MLD	15	2000-2014	7,417,704	352	0.20	1.50	0.69	1.85	1.42
MLD (Iban origin only)	9	2009-2013	62,825	42	14.33	-	-	-	-
Fabry	1	1974	193,203*	220	0.52*	0.25	1.00*	0.12	0.42*
Krabbe	1	2014	511,865	358	0.20	0.00	0.40	1.21	1.35
MPS (all types)	47	1990-2016	13,742,837	271	0.34	NA	3.72	4.80	4.50
MPS I	7	2010-2016	3,524,309	355	0.20	0.25	0.72	1.33	1.19
MPS II	15	2004-2016	3,321,377*	236	0.45*	0.00	0.83*	1.09	1.30*
MPS IIIA	4	2003-2015	6,400,970	633	0.06	0.00	0.47	0.00	1.16
MPS IIIB	2	2010-2012	1,479,191	431	0.14	1.05	0.02	0.72	0.42
MPS IVA	10	1990-2014	12,713,498	564	0.08	1.41	0.71	0.60	0.22
MPS VI	9	1998-2014	8,464,679	485	0.11	2.51	0.05	0.42	0.15
Total LSD	92	1974-2017	21,335,178	241	0.43	26.87	12.25	25	14

*Male live birth; ^expressed as 1 carrier per number of shown live births

Among Asian population, G_{M1} was five times higher in UAE compared with Malaysia while MPS II was relatively high in Malaysia compared to UAE.

Mutation analysis

Based on Table 4, there were 23 mutations associated with LSD identified in Malaysia. The mutations detected include missense, nonsense, small deletions, duplications and splicing. In patients with MLD, two missense mutations, c.746 T>C p.(Phe249Ser) and c.922T>C p.(Tyr308His) were observed in the *ARSA* gene which were also reported in patients with metachromatic leukodystrophy.[14,15]

We noted that out of nine Pompe patients, seven were detected in Chinese and the c.1935C>A p.(Asp645Glu) was the commonest pathogenic mutation. Among these patients, five missense mutations (c.1A>G p.(Met1Val), c.1561G>A p.(GLu521Lys), c.1843G>A p.(Gly615Arg), c.1935C>A p.(Asp645Glu) and c.2238G>C p.(Trp746Cys), two small nucleotide deletions (c.2815_2816delGT p.(Val939Leufs*78) and c.2024_2026delACA p.(Asn675del) in *GAA* gene. One patient demonstrated a splice site mutation at c.1551+1G>A in *GAA* gene. This mutation is at the canonical splice site sequence, predicting to lead exon skipping during pre-mRNA splicing and may eventually resulting in aberrant protein synthesis.

In patients with MPS IVA, one splicing mutation (c.1364+1 G>A) p.(?), two missense mutations c.953T>G p.(Met318Arg) and c.503G>T p.(Gly168Val), one small nucleotides deletion c.106-111delCTGCTC p.(?) and 3 nonsense mutations c.473_477delAGTGG p.(Glu158Valfs*12), c.551G>A p.(Trp184*) and c.502G>T p.(Gly168*l) were found in *GALNS* gene. Most of the mutations were found to be clustered in exon 5 of the *GALNS* gene. While in Gaucher, c.1389-3 C>G p.(?), c.1448T>G p.(Leu483Arg) and c.1448 T>C p.(Leu483Pro) mutations were detected in the *GBA* gene.

In regards to fucosidosis, two homozygous nonsense mutations were presented in four

Table 4: Several genotypes detected in selected LSDs in Malaysia

Disease	Ethnicity*	Phenotype	Gene (OMIM number#)	Genotype				References
				Nucleotide change	Amino acid changes	Exon/Intron	Clinical Impact/ Mutation Type	
Pompe	Indian (1)	Cardiomyopathy	*GAA* (606800)	c.1551+1G>A; c.1561G>A	p.(Glu521Lys)	IVS 10; 11	Missense	[26-27]
	Indian (1)			c.1A>G	p.(Met1Val)	2	Missense	[32]
	Chinese (1)			c.1843G>A; c.2238G>C	p.(Gly615Arg); p.(Trp746Cys)	13; 16	Missense	[28-29]
	Chinese (1)			c.1843G>A; c.2815_2816delGT	p.(Gly615Arg); p.(Trp746Cys)	13; 20	Missense	[29]
	Chinese (1)			c.1935C>A	p.(Asp645Glu)	14	Missense	[30]
	Chinese (1)			c.1935C>A; c.2024_2026delACA	p.(Asp645Glu); p.(Asn675del)	14	Missense	[30-31]
	Chinese (1)			c.1935C>A; c.2238G>C	p.(Asp645Glu); p.(Trp746Cys)	14; 16	Missense	[28; 30]
Fucosidosis	Chinese (1)	Hepatosplenomegaly, coarse facies	*FUCA1* (612280)	c.393T>A	p.(Tyr131*)	2	Nonsense	[16]
	Chinese (1) Iban (2)			c.1295G>A	p.(Trp432*)	8	Nonsense	[20]
Gaucher	Malay (1)	Hepatosplenomegaly	*GBA* (606463)	c. 1389-3 C>G	p.(?)	IVS 10	Splicing	[33]
	Malay (1)			c.1448 T>C	p.(Leu483Pro)	11	Missense	[34]
				c.1448 T>G	p.(Leu483Arg)	11	Missense	[35]
MLD	Malay (1)	Neuro regression, leukodystrophy	*ARSA* (607574)	c.746 T>C	p.(Phe249Ser)	4	Missense	[14]
	Malay (1)			c.922T >C	p.(Tyr308His)	5	Missense	[15]
MPS IVA	Malay (1)	Coarse facies, Clawed hand, scoliosis, kyphoscoliosis	*GALNS* (611222)	c.1364+1 G>A	p.(?)	IVS 12	Splicing	[36]
	Malay (1)			c.473_477delAGTGG	p.(Glu158Valfs*12)	5	Frameshift	[39]
	Malay (1)			c.502G>T	p.(Gly168*)	5	Nonsense	[39]
	Malay (1)			c.503G>T	p.(Gly168Val)	5	Missense	[39]
	Chinese (1)			c.106_111delCTGCTC	p.(?)	1	Frameshift	[37]
	Chinese (1)			c.953T>G	p.(Met318Arg)	9	Missense	[38]
	Indian (1)			c.551G>A	p.(Trp184*)	5	Nonsense	[39]
	Indian (1)			c.218 A>G	p.(Tyr73Cys)	2	Missense	[40]

**Number in parenthesis represent number of patient(s) harbouring mutation in this study*

#OMIM number is a unique six-digit identifier assigned to each entry in the Online Mendelian Inheritance in Man database, which catalogues human genes and genetic disorders

unrelated patients, c.393T>A p.(Tyr131*) and c.1295G>A p.(Trp432*) in the *FUCA1* gene. Changes from T to A at nucleotide 393 were predicted to create a truncated α-L-fucosidase protein. This mutation was previously reported in two separate studies involving patients of Chinese origin.[16,17] The c.1295G>A mutation is located in a highly conserved region and causing substitution from amino acid Trp to a stop codon that may lead to a truncated protein as well.

Table 4 also shows distribution of the selected LSD mutations in Malaysian ethnicities. Pompe disorder was mainly presented in Chinese ethnicity. The three distinct mutations in the *GBA* gene were found only in Malay ethnicity. As for MPS IVA, the mutations c.473_477delAGTGG and c.502G>T were found only in Malay ethnicity whereas mutations c.218A>G and c.551G>A were revealed in Indian ethnicity. Meanwhile, a few patients from Chinese descendant showed mutations c.106-111delCTGCTC and c.953T>G. Interestingly, fucosidosis is mainly presented in three unrelated Iban origins and inherited from their parents involving a homozygous p.(Trp432*) mutation in the *FUCA1* gene.

DISCUSSION

Our analysis suggested that the prevalence of LSD in Malaysia is quite low, 0.43 per 100,000 live births with carrier frequencies of 1:241 as compared to United Arab Emirates, 26.87 per 100,000 live births, Czech Republic, 12.25 per 100,000 live births; Northern Portugal, 25 per 100,000 live births and the Netherlands, 14 per 100,000 live births.[2,3,12,13] We also observed that the most prevalent LSD subtypes for MPS and sphingolipidoses in Malaysia were MPS type II and Fabry, and the most prominent nucleotide change was fucosidosis with c.1295G>A.

The low prevalence of LSD may be due to the short period of data retrieval (2008-2017) as compared to the reference countries. One factor contributing to the low prevalence of LSD in Malaysia may be the lack of clinical suspicion of LSD in patients among Malaysia's medical health practitioners, particularly general physicians and paediatricians. Studies have highlighted limited awareness and diagnostic capacity for rare diseases, including LSDs, among Malaysian general practitioners and paediatricians, often leading to delayed or missed diagnoses.[8] A national policy brief further emphasises that most frontline clinicians lack adequate training and exposure to rare diseases, underscoring the need for structured educational initiatives.[43] Increased awareness of LSD and sufficient laboratory facilities can help in detecting underdiagnosed patients.

Geographical factors also play some role, particularly for patients in remote areas of Malaysia, such as in Sabah and Sarawak. Limited access to genetic clinics, specialised diagnostic laboratories and treatment centres in these regions can delay or even prevent the diagnosis of LSD. As a result, the true prevalence of LSDs in these areas may be underestimated due to underdiagnosis. Contributing barriers include long travel distances, limited public transport and inadequate local awareness among healthcare providers. To address these challenges, some initiatives such as flying doctors, telemedicine consultations and limited mobile health services - have been introduced.[41,42] However, these efforts remain isolated and under-resourced, and a more structured nationwide strategy is still lacking.

The diagnosis of several LSDs was found to be more delayed in Malaysia compared to Australia. For instance, the median age at diagnosis of MPS type IVA and mucolipidosis type II in Malaysia was 9.3 years and 2.5 years, respectively; substantially higher than in Australia (1.6 years and 0.2 years).[23] Similarly, MPS type IIIB and Gaucher disease were diagnosed later in Malaysia (2.1 years vs 4.5 years, and 4.5 years vs 16.4 years, respectively). Interestingly, the range of age at diagnosis in Malaysia was narrower than in Australia (e.g., MPS type IIIB: 1.17–3 years vs 1.4–39.6 years; Gaucher: 0.14–7 years vs 0.01–75.3 years), which may reflect a smaller diagnosed patient pool or clustering of cases within certain referral centres.

Several factors likely contribute to this diagnostic delay. First, limited access to specialised diagnostic laboratories - especially for enzyme assays and molecular testing - often leads to prolonged referral chains and testing turnaround times.[8] In past years, Malaysia lacked the capability to conduct in-house testing. Most lysosomal disorder tests were outsourced to laboratories in Australia and Taiwan. However, starting in 2014, these tests have been performed locally at the Institute for Medical Research, significantly shortening the turnaround time for results.

Second, there are gaps in clinical awareness and training among general paediatricians and primary care doctors, which may lead to misdiagnosis or delayed referrals.[43,44] Third, the absence of streamlined national diagnostic

protocols and algorithms for LSDs means that early signs are not systematically investigated. Currently, the Malaysian Paediatric Association has published general national diagnostic protocol guidelines. This includes a diagnostic algorithm for inborn errors of metabolism and not specifically for LSD.[45] Additionally, some diagnostic resources are concentrated in urban tertiary centres, further disadvantaging patients in rural or remote regions.[44]

Notably, for MPS type I, MPS type II, fucosidosis, G_{M1} gangliosidosis, and G_{M2} gangliosidosis, the median age at diagnosis was comparable between Malaysia and Australia, possibly reflecting increased clinical recognition of these particular subtypes or the presence of specific screening efforts.

In Malaysia, mucopolysaccharidoses were the most common type of LSD (47 of 92 patients, or ~51%) with the prevalence of 0.34 per 100,000 live births. MPS type II accounted for the most among other MPS which showed similarity with China and Taiwan.[18,19] Although sphingolipidoses were not common (0.13 per 100,000), the prevalence of Fabry disease was almost similar to that in the Netherlands and twice as high as that in the UAE.[3,12]

We found that MLD was more common in the Iban ethnic population of Sarawak. The Iban population was 44 times more likely to have MLD compared to the non-Iban population (OR 43.53, 95% CI 15.29-123.93, $p<0.05$). Therefore, the prevalence of MLD among the Iban population was estimated to be 14:100,000 live births. Few other studies have been conducted among ethnic populations around the world. Holve *et al.*[24] reported that the incidence of MLD among Navajo tribes in the United States was 1 in 2,520 live births, while the Habbanite Jews constituted a high-risk population for MLD with a reported incidence of 1 in 75 live births.[24,25] In addition, it was noted that Pompe disease was more frequent in Chinese. However, we were unable to calculate the prevalence of Pompe among the Chinese population due to inadequate data on live births among that population between 1988 to 2000.

Over a 10-year period from 2008 to 2017, fucosidosis was the only disease detected (n=4) in the oligosaccharidosis group. It was noted that three out of four patients with fucosidosis originated from the state of Sarawak. The p.(Trp432*) mutation, to cause the amino acid substitution from Trp to stop codon in exon 8 of the *FUCA1* gene was predicted for a formation of a truncated protein.[20]

A similar study conducted by UAE also supported this hypothesis, whereby the higher number of fucosidosis cases was most likely due to consanguineous marriage. It was known that consanguineous marriage among Emiratis is common, which leads to a higher birth prevalence in autosomal recessive diseases with the presence of a 'founder mutation'.[12] We believe that this finding may support our hypothesis of a founder gene p.(Trp432*) mutation among the Sarawak population.[21] While the overall national rate of consanguineous marriages in Malaysia is relatively low at around 6%, this figure does not capture regional or ethnic variation.[46] Certain communities in Malaysia, including some indigenous groups, still practice forms of consanguineous unions. For instance, within the Iban community in the remote area of Sarawak, there is a traditional ceremony called *Jadi Mali*, which is performed to bless the universe during unforeseen circumstances, such as when a marriage occurs between close relatives, has been documented as part of customary practices.[47] Holguin Province in Cuba has also been observed to have the highest incidence of fucosidosis with a single mutation of p.(Gln422*) among its population.[22]

While our study focuses on LSD prevalence from 2008 to 2017 (0.43 per 100,000; n=92), more recent data from 2024 shows a figure of 0.46 per 100,000 (n=6) (unpublished data). Since 2017, there have been incremental advances in diagnostic capabilities in Malaysia, including greater availability of molecular testing and enzyme assays in IMR. These findings emphasise that with the advancement of diagnostic facilities, a higher number of cases are likely to be detected, enabling earlier and more accurate diagnosis. To further enhance diagnostic yield and address unresolved or complex cases, the incorporation of next-generation sequencing (NGS) methods such as whole exome sequencing (WES) may be considered, as these approaches can identify disease-causing variants when traditional biochemical or targeted genetic tests are inconclusive.[48-50]

CONCLUSION

In conclusion, our findings highlight the need for more comprehensive research on the prevalence and distribution of LSDs in Malaysia. Future efforts should include the establishment of a national LSD registry to

systematically collect and analyse patient data, which would support better resource allocation and disease management strategies. Additionally, a nationwide screening program—particularly targeting high-risk or underserved populations—could help identify undiagnosed cases. Exploring the feasibility of incorporating LSDs into the existing newborn screening framework is also recommended, as early diagnosis has the potential to significantly improve clinical outcomes through timely interventions. These steps are essential to advance the understanding and management of LSDs in Malaysia.

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