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Clinical Presentation and Genetic Heterogeneity Including Two Novel Variants in Sri Lankan Patients With Infantile Sandhoff Disease

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Abstract

Infantile Sandhoff Disease (*i*SD) is a subtype of GM2 gangliosidosis, which is never been reported in Sri Lanka. Data of eight children, who were diagnosed with *i*SD during the period of 2017 to 2021, were analyzed retrospectively. The aim of this study was to analyze genotypic and phenotypic variations of native *i*SDs. Café-au-lait spots, mitral regurgitation and atrial septal defect were found in our patients but never reported in the literature. We found c.1417 + 5G>A and c.1303_1304insCT p.(Arg435Thrfs*10) novel variants of *HEXB* gene among the nine different gene mutations that were identified. The commonest *HEXB* gene variant identified in India was c.850 C4T (p.R284X) but was not noticed among Sri Lankan patients. In contrast to other studies, all our patients died within the age of two years. This is the first Sri Lankan study that expands the clinical and molecular basis of *i*SD with its novel findings.

Keywords

Infantile Sandhoff Disease, cardiac involvement, atrial septal defect, café-au-lait spots HEXB gene, novel variants

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Introduction

GM2 gangliosidosis is a subgroup of lysosomal storage disorders, which is further classified into Type O (Sandhoff disease), Type A (Tay-Sachs disease), and Type AB (GM2 activator deficiency).[1] Sandhoff disease (SD) is a rare autosomal recessive neurodegenerative disorder due to the lack of β-Hexosaminidase activity that leads to neural deposition of GM2 ganglioside.[2] It has three clinical subtypes depending on the age of onset (infantile, juvenile, and adult forms).

Infantile SD (*i*SD) presents early with progressive neurological impairment, truncal hypotonia, hyperacusis, developmental delay or regression, seizures, hepatosplenomegaly, cherry-red spots in the retina, and congenital dermal melanocytosis. However, cardiac involvement is extremely uncommon, and café-au-lait spots were not reported to date in SD.[3,4]

Though SD can be suspected from the clinical picture, molecular analysis and enzyme activity assessment are needed to confirm the diagnosis. The β-Hexosaminidase enzyme has two major hexaminidase isoenzymes named HexA and HexB. HexA is a heterodimer enzyme composed of a/b subunits, whereas HexB is a homodimer consisting of b subunits alone. These a and b subunits are encoded in the *HEXA* and *HEXB* genes. Variants in *HEXB* gene lead to SD. Therefore, the low or absent HexA and HexB activities are noted in SD,[5] but the former correlates well with the clinical severity.[6] Small residual hexaminidase activity also can be

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noted in patients with SD due to the presence of a subunit dimer called $HexS^6$

This report compares the typical and atypical clinical findings of eight diagnosed Sri Lankan children of *i*SD, their β-Hexosaminidase enzyme activity, and their *HEXB* gene variant heterogeneity.

Method

From 2017 to 2021, *i*SD diagnosed patients' referrals to the Chemical Pathology Department of Lady Ridgeway Hospital for Children in Sri Lanka were collected and analyzed retrospectively. We found eight unrelated children from different geographical backgrounds and ethnicity.

Case 1

An eleven-month-old girl with global developmental delay was found to have bilateral ankle joint contracture, marked hypotonia, intellectual disability, hyperacusis, and hepatosplenomegaly. She was able to fix an object but could not follow it and had cherry-red spots in both eyes.

She was a healthy child of sixth pregnancy to consanguineous parents with an uneventful antenatal period. The child's mother had first-trimester miscarriages in her first, third and fifth pregnancies. The rest of the two siblings were healthy.

Her two-dimensional echocardiogram (2D Echo) showed a myxomatous valve with grade 1 mitral regurgitation, and the blood picture revealed microcytic hypochromic anaemia with target and pencil cells. She passed away at the age of two years.

Case 2

A one-year and two-month-old girl under evaluation for seizure episodes with developmental regression and intellectual disability was noted to have a flat nasal bridge, hypertelorism, and congenital dermal melanocytosis. On admission, her head circumference (HC) was 48 cm (at + 2 SD), but at birth, she had a normal HC (34 cm; at the median) following an uneventful pregnancy. Her ophthalmic evaluation revealed visual impairment and a cherry-red spot on the macula with pallor optic disc. Mother had noticed that the child could not tolerate even an ordinary sound.

She was second born to consanguineous parents with a healthy elder sister. She had a family history of seizures and mental retardation. Her ultrasound scan (USS) of the brain was normal. We couldn't contact the child's family to get an update on her current status.

Case 3

Ten-month-old first-born boy was referred for generalized seizure with global developmental delay. His clinical examination revealed dystonic movements, hypotonia, microcephaly (HC = 41cm, < −3 SD), contracture in the bilateral metacarpophalangeal joint, hepatosplenomegaly, and a cherry-red spot on the macula. His brain imaging and 2D echo were normal. He had an uneventful antenatal period and expired in the following year of referral.

Case 4

After an uncomplicated full-term pregnancy of consanguineous parents, a first-born boy was hospitalized with fever and convulsions on his second day of life. He was treated for neonatal sepsis. Later, he was referred to us at the age of fifteen months with seizure episodes, developmental delay, hypertonia, exaggerated reflexes in bilateral lower limbs, failure to thrive, frontal bossing, low set ears, microcephaly (HC = 44 cm; < −3 SD), pectus excavatum and a cherry-red spot on the macula with disc pallor. He had a family history of seizure disorders.

His 2D echo showed a small atrial septal defect (ASD) with moderate to large left coronary to right ventricular fistula. Contrast-enhanced computed tomography (CECT) – brain showed bilateral lower attenuated area involving bilateral temporal region symmetrically. By the time we got his genetic report, he had lost the follow-up, and we couldn't contact his family to get an update about his current status.

Case 5

An otherwise healthy one-year-old girl consulted the paediatrician for an episode of generalized tonic-clonic movement, and she was referred to us six months later with developmental regression. Her physical examination showed café-au-laid spots, hypotonia with brisk reflex, hepatomegaly without splenic enlargement, and a cherry-red spot on the fundus examination.

She was the second child of non-consanguineous parents with a healthy sister. Her birth history, perinatal period, and family history were not contributory.

Her brain's T1 weighted magnetic resonance imaging (MRI) showed an extensive lack of myelination of both hemispheres and bilateral basal ganglia. She passed away at the age of two years.

Case 6

An eleven-month-old only child of non-consanguineous parents was presented with the first episode of generalized tonic-clonic convulsion. On admission, she was developmentally five months and found to have congenital dermal melanocytosis on the back of the trunks and cherry-red spots in the eyes. There was no organomegaly. The mother noticed hyperresponsiveness to startle sounds. Her antenatal period was uneventful. Despite normal routine biochemical investigations and imaging, the child is deteriorating now.

Case 7

A one-year and three-month-old girl presented with sudden onset of a focal seizure. On further evaluation, she had congenital dermal melanocytosis, developmental regression, hypertonia, macrocephaly (50 cm; > + 3 SD), failure to thrive, and cherry red spots on both eyes,

She was the third child of non-consanguineous parents following an unremarkable antenatal period. She had a sibling die due to congenital heart disease (CHD). Her 2D echo showed mild prominent left ventricle and no intra-cardiac anomaly. Her brain imaging was normal. She also died at the age of one year and nine months.

Case 8

A seven-month-old girl of consanguineous parents was documented to have a coarse face, flat nasal bridge, congenital dermal melanocytosis, hearing loss, bilateral cherry-red spots, umbilical hernia, capillary hemangioma over both eyelids, and developmental delay. Further details were not documented, and we could not trace the patient after receiving her genetic report.

Table 1. Clinical Features of all Eight Cases [F: Female, M: Male, +: Present; – :Absent and Uncommon Clinical Features Were Marked in Bold]
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	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Age at presentation	11 months	14 months	10 months	15 months	18 months	11 months	15 months	7 months
Sex	F	F	M	M	F	F	F	F
Consanguinity	+	+	–	+	–	–	–	+
Seizure	–	+	+	+	+	+	+	–
Developmental delay or regression	+	+	+	+	+	+	+	+
Hyper-/ Hypo- tonia	Hypo	Hypo	Hypo	Hyper	Hypo	Hypo	Hyper	–
Micro- / Macro-cephaly	–	Macro	Micro	Macro	–	–	Macro	–
Skin changes	–	+	–	–	+	+	+	+
Organomegaly	+	–	+	–	+	+	–	–
Hyperacusis	+	+	–	–	–	+	–	–
Cherry red spots on the macula	+	+	+	+	+	+	+	+
Cardiac involvement	+	–	–	+	–	–	–	–
Current status	Died at the age of 2 years	Could not contact	Died at the age of 18 months	Could not contact	Died at the age of 2 year	Alive. (1 year and 11 months)	Died at the age of 1 year and nine-month	Could not contact

Results

All eight unrelated cases were presented before age two, with female predominance. All of them were presented with developmental delay or regression associated with other typical and uncommon clinical features, as summarized in Table 1.

The total beta-hexosaminidase and beta-hexosaminidase A activity were measured by fluorimetry, where the normal levels of total beta-hexosaminidase and beta-hexosaminidase A were > 4.5 μmol/L/h and > 2.0 μmol/L/h, respectively. The enzyme activity and *HEXB* gene variant analysis were summarized in Table 2.

Discussion

SD affects 1 in 1,000,000 individuals and commonly affects the Creole population of Argentina, Metis citizens of Saskatchewan, Canada, and people with Lebanese ancestry.[7] Only very few cases of *i*SD have been reported in South Asia and those were mainly from Pakistan and India, not from Sri Lankan.[8] Usually, it affects males and females in equal numbers,[7] but six out of eight were females in our case series. The mean age of their presentation to the tertiary care center was 12.5 months, and most died at or around two years of age, which is far shorter than compared to other parts of the world.[8] Poor socio-economic status, less awareness about the illness, comparatively late presentation to a specialist center, inadequate management guidelines, poor compliance, and improper follow-up could be the reasons for this short life span of diagnosed patients. So, proper management guidelines and follow-up plans needed to be established, especially in developing countries like Sri Lanka, to improve the quality of life and life expectancies of those who affected by *i*SD.

Out of eight, four children were born to consanguineous parents. Only one patient's mother had previous miscarriages, one patient's sibling died due to CHD, and two patients had a family history of seizures. As it is an autosomal recessive disease with a very low prevalence, family screening for SD is still debatable in developing countries like Sri Lanka.

Cherry-red spots on fluoroscopy examination and hyperacusis are the essential clinical clues to diagnosing SD.[2,9] Here, cherry-red spots in all eight cases helped early suspicion of *i*SD, but hyperacusis was present only in three cases.

Congenital dermal melanocytosis was identified in three patients. It results from the entrapment of melanocytes in the dermis due to increased activity of nerve growth factors.[10] Apart from congenital dermal melanocytosis, café-au-lait spots were found in a patient (case 5), which is commonly associated with neurofibromatosis, McCune-Albright syndrome, Legius syndrome, Noonan syndrome, and constitutional mismatch repair deficiency syndrome.[11] To our knowledge, café-au-lait spots were not reported in patients with SD.

Our study is the first to report flat nasal bridge, hypertelorism, frontal bossing, low-set ears, capillary hemangioma and umbilical hernia in *i*SD, though they are commonly associated with genetic syndromes. The particular patient with these features had a novel variant in *HEXB* gene. Unfortunately, we could not contact the family to study extensively on this.

Hepatosplenomegaly was identified in three patients, and hepatomegaly alone was noted in one case. The absence of organomegaly does not preclude the presence of *i*SD.[12]

Lying on par with literature, almost all of them had developmental regression or delay; apart from two patients, others had seizure episodes. Though hypotonia is commonly associated with *i*SD, two patients had hypertonia.[2]

Table 2. Hexosaminidase Enzyme Activity and HEXB Gene Variant Analysis [Novel Mutations Were Marked in Bold].

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Total beta hexosaminidase (µmol/L/h)	0.4	0.4	0.9	0.7	0.7	0.4	0.6	< 0.35
Beta-hexosaminidase A (µmol/L/h)	0.4	< 0.31	0.7	1.1	1.9	1.9	3.3	< 0.31
HEXB variant NM_000521.3	**c.1417 + 5G>A**	c.771 + 5G>A	c.1058_1060 del p.(Gly353 del)	c.1058_1060 del p.(Gly353 del)	c.1303_1304 insCT p.(Arg435Thrfs*10)	c.611G>A p.(Gly204Glu) c.325dup p.(Tyr109Leufs*6)	c.1417 + 5G>A c.850C>T p.(Arg284*)	**c.1303_1304insCTp .(Arg435Thrfs*10)**
Zygosity	Homozygous	Homozygous	Homozygous	Homozygous	Homozygous	Compound Heterozygous	Compound Heterozygous	Homozygous
	Class I: Pathogenic	Class I: Pathogenic	Class I: Pathogenic	Class I: Pathogenic	Class I: Pathogenic	Class 2: likely Pathogenic	Class I: Pathogenic	Class I: Pathogenic

Three of eight children had macrocephaly; one had microcephaly, and others had normal HC. Only two patients had significant brain imaging findings among those with abnormal HC. The HC size does not play an essential role in diagnosing *i*SD.[12–14]

According to Lee *et al*, only six cases of cardiomyopathies, valvulopathies and cardiomegaly were reported.[3] But up to now, no cases of *i*SD associated with ASD have been reported. In this study, two patients had cardiac involvements, including a myxomatous valve with grade 1 mitral regurgitation and ASD with moderate to large left coronary to right ventricular fistula.

As mentioned in the literature, total hexosaminidase activity was low in all eight cases that differentiate SD from other types of GM2, including Tay Sach disease. Anyhow there is no correlation between the level of hexosaminidase activity and the phenotype.

Genetic study is essential for definite diagnosis and counselling.[5] Up to now, more than 50 variants have been identified in the *HEXB* gene that causes SD.[2] According to PM Tamhankar *et al*, c.850 C4T (p.R284X) mutation was the most shared mutation identified in Indian population who were diagnosed to have *i*SD, but in our study, non were found to have this specific type of mutation (9). In this case series, nine variants of the *HEXB* gene were identified, including two novel variants [c.1417 + 5G>A in case 1 and c.1303_1304insCT p.(Arg435Thrfs*10) in case 8]. Case 8 was found to have a *HEXB* variant c.1303_1304insCT p.(Arg435Thrfs*10), creating a shift in the reading frame starting at codon 435. The new reading frame ends in a stop codon nine positions downstream. When reporting this case, this variant was detected for the first time and not listed in the Genome Aggregation Database, Exome Sequencing Project, or the 100 Genome Browser. With the given clinical information and reduced enzyme activity, it was considered pathogenic according to the recommendations of CENTOGENE and ACMG. The same patient *HEXA* variant in exon 2, c.296A>C p.(His99Pro), was also detected for the first time and classified as a variant of uncertain significance according to the recommendation of Centogene and ACMG. Apart from c.611G>A p.(Gly204Glu) and c.325dup p.(Tyr109Leufs*6) variants that were identified in Case 6, all other variants were classified as pathogenic. Two *HEXB* gene variants were found in more than two patients [c.1417 + 5G>A in cases 1 and 7; c.1058_1060 del p.(Gly353 del) in cases 3 and 4], and two patients had (cases 6 and 7) more than two mutations in the *HEXB* gene.

Conclusion

This is the first Sri Lankan study that expands the clinical and molecular basis of *i*SD with its novel findings. Developmental delay or regression associated with seizure episodes as the presenting complaint and Cherry red spots on fundoscopy were consistent with the literature. And they play a crucial role in diagnosing *i*SD clinically. *i*SD should be considered a differential when a child presents with metabolic cardiomyopathy or café-au-laid spots with other striking features. Though there were nine *HEXB* variants, including two novel variants (c.1417 + 5G>A and c.1303_1304insCT p.(Arg435Thrfs*10)), the genetic variance did not influence the clinical pattern of *i*SD. In contrast to other

studies, all our patients died within the age of two years, thereby proper management guidelines and follow-up methods needed to be established.

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Author's Contribution

Siddiqa: Collected the data, interpreted the data, and drafted the manuscript
Subashinie: Collected the data
Surani: Coordinated the enzyme analysis and genetic study
Sabine: Critically revised the manuscript
Anura: Critically revised the manuscript
Eresha: Designed the study and critical revision of the manuscript

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. Sabine Schröder is an employee of Centogene GmbH, Rostock, Germany.

Ethical Approval

The work described is consistent with the Journal's guidelines for ethical publication.

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References

1. Rajkumar V, Dumpa V. 2022. Lysosomal Storage Disease. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing, https://www.ncbi.nlm.nih.gov/books/NBK563270/.
2. Tavasoli AR, Parvaneh N, Ashrafi MR, et al. Clinical presentation and outcome in infantile Sandhoff disease: a case series of 25 patients from Iranian neurometabolic bioregistry with five novel mutations. *Orphanet J Rare Dis*. 2018;13(1):130.
3. Lee HF, Chi CS, Tsai CR. Early cardiac involvement in an infantile Sandhoff disease case with novel mutations. *Brain Dev*. 2017;39(2):171-176.
4. Reza Ashrafi M, Tavasoli A, Shiva S, et al. Diffuse dermal melanocytosis in two patients with Sandhoff disease and mucopolysaccharidosis VI. *Int J Dermatol*. 2014;53(6):736-738.
5. Zampieri S, Cattarossi S, Oller Ramirez AM, et al. Sequence and copy number analyses of HEXB gene in patients affected by Sandhoff disease: functional characterization of 9 novel sequence variants. *PLoS One*. 2012;7(7):e41516.
6. Scriver C, Sly W, Childs B, et al. GM2 Gangliosidoses. In: *The metabolic and molecular bases of inherited disease*. McGraw-Hill, 2001: 3827-3876.
7. Sandhoff Disease. *National Organization for Rare Disorders*, https://rarediseases.org/rare-diseases/sandhoff-disease/ (2020).
8. Smith NJ, Winstone AM, Stellitano L, et al. GM2 Gangliosidosis in a UK study of children with progressive neurodegeneration: 73 cases reviewed. *Dev Med Child Neurol*. 2012;54(2):176-182.
9. Tamhankar PM, Mistri M, Kondurkar P, et al. Clinical, biochemical and mutation profile in Indian patients with Sandhoff disease. *J Hum Genet*. 2016;61(2):163-166.
10. Hackbart BA, Arita JH, Pinho RS, et al. Mongolian Spots are not always a benign sign. *J Pediatr*. 2013;162(5):1070.
11. Shah KN. The diagnostic and clinical significance of Café-au-lait Macules. *Pediatr Clin N Am*. 2010;57(5):1131-1153.
12. Gowda VK, Amoghimath R, Srinivasan VM, et al. Sandhoff disease without hepatosplenomegaly due to hexosaminidase B gene mutation. *J Pediatr Neurosci*. 2017;12(1):78-79.
13. Karimzadeh P, Jafari N, Nejad Biglari H, et al. GM2-Gangliosidosis (Sandhoff and Tay Sachs disease): diagnosis and neuroimaging findings (an Iranian pediatric case series). *Iran J Child Neurol*. 2014;8(3):55-60.
14. Sharawat IK, Saini L, Randhawa MS, et al. Extensive Mongolian spots and normocephaly: an uncommon presentation of infantile Sandhoff's disease. *BMJ Case Rep*. 2018;2018:bcr2018225013.