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Childhood-Onset Myopathy With Preserved Ambulation Caused by a Recurrent *ADSSL1* Missense Variant

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Abstract

Background and Objectives

Distal myopathies are a heterogeneous group of primary muscle disorders with recessive or dominant inheritance. *ADSSL1* is a muscle-specific adenylosuccinate synthase isoform involved in adenine nucleotide synthesis. Recessive pathogenic variants in the *ADSSL1* gene located in chromosome 14q32.33 cause a distal myopathy phenotype. In this study, we present the clinical and genetic attributes of 6 Indian patients with this myopathy.

Methods

This was a retrospective study describing on Indian patients with genetically confirmed *ADSSL1* myopathy. Details were obtained from the medical records.

Results

All patients presented in their first or early second decade. All had onset in the first decade with a mean age at presentation being 17.7 ± 8.4 years (range: 3–27 years) and M:F ratio being 1:2. The mean disease duration was 9.3 ± 5.2 years ranging from 2 to 15 years. All patients were ambulant with wheelchair bound state in 1 patient due to respiratory involvement. The median serum creatine kinase (CK) level was 185.5 IU/L (range: 123–1564 IU/L). In addition to salient features of ptosis, cardiac involvement, bulbar weakness, and proximo-distal limb weakness with fatigue, there were significant seasonal fluctuations and decremental response to repetitive nerve stimulation, which have not been previously reported. Muscle histopathology was heterogenous with the presence of rimmed vacuoles, nemaline rods, intracellular lipid droplets along with chronic myopathic changes. Subtle response to pyridostigmine treatment was reported. While 5 of 6 patients had homozygous c.781G>A (p.Asp261Asn) variation, 1 had homozygous c.794G>A (p.Gly265Glu) in *ADSSL1* gene.

Discussion

This study expands the phenotypic spectrum and variability of *ADSSL1* myopathy with unusual manifestations in this rare disorder. Because the variant c.781G>A (p.Asp261Asn) is the most common mutation among Indian patients similar to other Asian cohorts, this finding could be useful for genetic screening of suspected patients.

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Glossary

CES = clinical exome sequencing; **CK** = creatine kinase; **LL** = lower limb; **UL** = upper limb; **WES** = whole-exome sequencing.

Introduction

Distal myopathies are a heterogenous group of inherited primary muscle diseases, which are variously classified based on the onset, clinical features, and muscle histopathology. With the advent of next-generation sequencing, there has been an expansion of data on causative/pathogenic variants causing distal myopathies. The common autosomal recessive distal myopathies include *GNE*, dysferlinopathies (*DYSF*), nebulin (*NEB*), anoctamin 5 (*ANO5*), and *ADSSL1* myopathies. Pathogenic variants in *ADSSL1* gene leading to deficiency of adenylosuccinate synthetase–like 1 muscle-specific isozyme causes a type of distal myopathy.[1] It catalyzes the conversion of inosine monophosphate to adenylosuccinate, which is the initial step in adenine nucleotide synthesis.[2] The functions of *ADSSL1* are the processes involved in energy transfer, synthesis of nucleic acids, and regulation of metabolism. The previously reported clinical phenotype of *ADSSL1* myopathy includes adolescent-onset distal leg weakness with mild facial weakness, mild creatine kinase (CK) elevation,[3] and proximo-distal nemaline myopathy.[4] This report describes Indian patients with *ADSSL1* myopathy and their characteristic clinicopathologic features.

Methods

This retrospective descriptive study was conducted at a quaternary hospital for neurologic disorders in India on patients with genetically diagnosed *ADSSL1* myopathy. The patients were evaluated between 2016 and 2022. The baseline demographic details, laboratory findings including muscle MRI, histopathology details were collected from the medical records and analyzed. Genetic testing was performed in patients and available family members as follows: PI-1, PI-2, patients and parents underwent clinical exome sequencing (CES) at Children's Hospital, Los Angeles; P-II and parents—whole-exome sequencing (WES) was performed by the genomics platform at the Broad Institute of MIT and Harvard, Cambridge. The exome data were then processed at the Centro Nacional de Análisis Genómico, Barcelona, Spain, and variant prioritization was conducted on the RD-Connect GPAP (platform.rd-connect.eu)[5]; P-III underwent WES at Stanford Health care, California; P-IV CES underwent WES at Sandor diagnostics, India; and P-V and parents underwent WES at Institute of Human genetics, Hamburg, Germany. *ADSSL1* variants reported were classified as per ACMG guidelines and annotated based on muscle-specific Ref seq transcript NM_152328.[4] Informed consent was obtained from all patients, and ethical approval was obtained from the Institution ethics committee. Permission was obtained for unmasking of faces in the photographs.

Data Availability

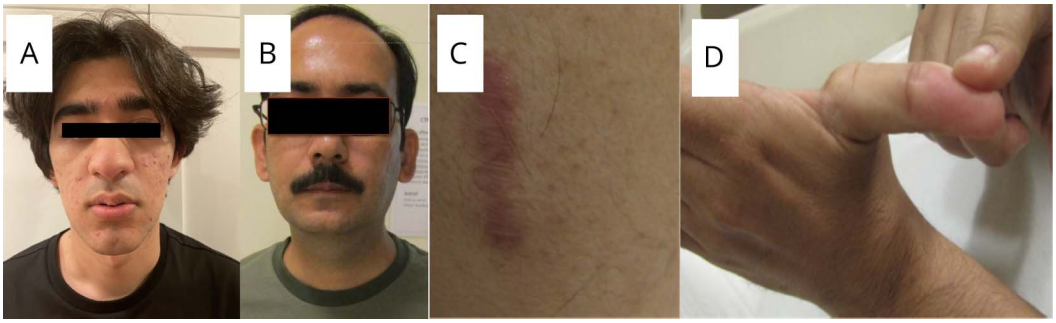
Anonymized data can be made available for qualified investigators on request to the corresponding author. Except for P-II, other patients came with genetic results obtained from other centers.

Results

Clinical Features

We included 6 patients from 5 families. All patients had onset of symptoms in the first decade with a mean age at presentation of 17.7 ± 8.4 years (range: 3–27 years). The M:F ratio was 1:2. All had onset of symptoms in early childhood with slow walking and difficulty in running. The major clinical features are given in eFigure 1 (links.lww.com/NXG/A669). Weakness of distal lower limbs (LL) with frequent tripping

Figure 1 Clinical Images of Patients



(A) Clinical image of patient I-2 showing elongated myopathic facies. (B) Clinical image of patient III showing absent nasolabial folds. (C) Atrophic scar of skin of patient III. (D) Distal laxity of joints of patient III.

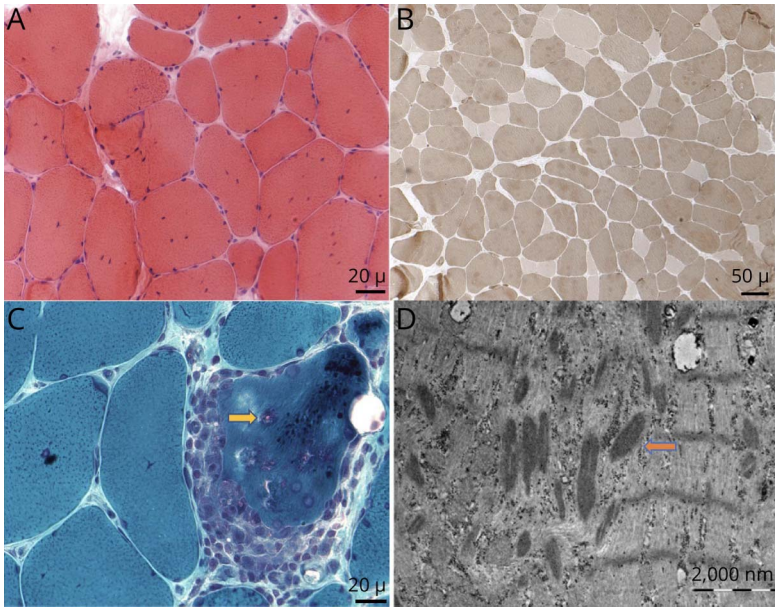
Table Clinical and Laboratory Features of Patients With *ADSSL1* Myopathy

	Families/patients					
Characteristics	**I-1**	**I-2**	**II**	**III**	**IV**	**V**
Sex	F	M	F	M	F	F
Age at onset (y)	7	10	13	17	1	13
Age at presentation (y)	19	15	24	27	3	18
Presenting symptoms	LL proximal weakness	LL proximal weakness	LL proximal weakness and tripping	LL proximal weakness and tripping	LL proximal weakness	LL proximal weakness and tripping
Limb fatigue	+	+	+	−	−	+
Ptosis	−	+	+ (along with divergent squint)	+	−	−
Dysphagia/chewing difficulty	−	+	+	−	−	−
Facial weakness	−	−	+	+	−	+
Muscle power (MRC)						
Proximal UL	5	5	3	4	5	4
Distal UL	5	5	5	5	5	5
Proximal LL	4	4	1	4	4	2
Distal LL	4	4	3	4	5	4
Tendon reflexes	Hypoactive	Hypoactive	Hypoactive	Hypoactive	Hypoactive	Hypoactive
Serum CK (U/L) (normal: 20–200)	150	123	129	1,564	932	221
RNS	Positive	Positive	Negative	Negative	Not done	Negative
MRI muscle	Not done	Fatty infiltration of gluteus maximus and gastrocsoleus	Not done	Fatty infiltration of gluteus maximus and semimembranosus	Normal	Not done
Muscle biopsy	Not done	Not done	Intracellular lipid vacuoles	Rimmed vacuoles with nemaline rods	Not done	CFTD, intracellular lipid vacuoles, ragged red fibres
Pathogenic variants *ADSSL1* gene	c.781G>A (p.Asp261Asn)	c.781G>A (p.Asp261Asn)	c.794G>A (p.Gly265Glu)	c.781G>A (p.Asp261Asn)	c.781G>A (p.Asp261Asn)	c.781G>A (p.Asp261Asn)

Abbreviations: CFTD = congenital fiber-type disproportion; CK = creatine kinase; F = female; LL = lower limb; M = male; MRC = Medical Research Council; RNS = repetitive nerve stimulation; UL = upper limb.

was noted in 3 patients, proximal upper limb (UL) weakness in 3, and significant limb fatigability in 4, among whom 1 had prominent seasonal fluctuations (worsening of limb weakness during summer) (P-II). Two patients had dysphagia with chewing difficulty. Exertion-induced palpitations were seen in 3 (P I-2, P-III, and P-V). Patient PV had significant dyspnea and orthopnea requiring nocturnal noninvasive ventilation from the age of 15 years. All patients were born of non-consanguineous parentage except P-II. There was no significant family history among other patients. The mean disease duration was 9.3 ± 5.2 years ranging from 2 to 15 years. All patients were ambulant with wheelchair bound state in 1 patient (PV) due to respiratory involvement. Examination showed all with thin slender habitus and eyelid ptosis in 3 patients with divergent squint in 1. Prominent facial weakness was seen in 3. Other features such as distal joint laxity and atrophic scar were seen in 1 patient—PIII (Figure 1). The muscle power was scored as per modified Medical Research

Figure 2 Muscle Histopathology of Patient-III



(A) Hematoxylin and eosin—fiber size variability, internally placed nuclei, and fiber splitting (×400). (B) ATPase pH 9.4—type 2 predominance (×200). (C) Modified Gomori trichrome—inflammatory foci and rimmed vacuoles (yellow arrow) (×400). (D) Electron microscopy—varying degrees of disorganization of myofibrils with loss of their normal striation pattern and nemaline rods (X 37,500) (orange arrow).

Council (MRC) grading: neck flexion was grade 3 (n = 3), proximal UL—3 to 4 (n = 3), and ankle dorsiflexion—3 to 4 (n = 4). One patient showed differential proximal LL weakness with predominant involvement of hip flexors and adductors (PII). All patients had hypoactive tendon reflexes with waddling gait. The major clinical possibilities considered in these patients were congenital myopathy, mitochondrial myopathy, or congenital myasthenic syndromes due to prominent limb fatigue with seasonal fluctuations and presence of ptosis (Table).

Laboratory Findings

The median serum CK level was 185.5 IU/L (range: 123–1,564 IU/L– normal to 7 times elevated). Nerve conduction studies and EMG revealed normal findings except PIII who showed myopathic pattern with fibrillations and positive sharp waves. Repetitive nerve stimulation was performed in 5 patients of whom 2 siblings in the first family showed significant decremental response of 15%–20% in trapezius and facial muscles. Electrocardiography and 2D echocardiography were normal in all except evidence of sinus tachycardia with mild QTc prolongation and biventricular dysfunctioning in P-III. MRI of muscles was performed in 3 patients (P I-2, P-III, and P-IV). Two patients showed fatty infiltration of gluteus maximus (PI-2, P-III), semimembranosus (P-III), and gastrocsoleus (PI-2), while 1 patient had normal findings (PIV) because of young age with minimal symptoms. Muscle biopsy was performed in 3 patients (P-II, P-III, and P-V). P-II showed prominent fiber size variation with positive Oil red-O stained intracellular lipid vacuoles. P-III displayed multiple internalized nuclei, splitting, focal inflammation, few fibers, rimmed vacuoles, and nemaline rods in electron microscopy along with fiber size variation and type 2 fiber predominance (Figure 2). The inflammation observed was focal, and the significance of this in the clinical and pathologic context is uncertain because further screening was not possible owing to inability in retrieving and loss of sample. P-V showed features of congenital fiber-type disproportion, intracellular lipid inclusions, ragged red fibers, and cytochrome c oxidase (COX)–deficient fibers. The ragged red fibers and cytochrome oxidase deficiency were seen in only a few scattered fibers (approximately 5%). P-V also had reduction in respiratory chain enzymes I-IV on mitochondrial assays.

Genetic analysis revealed that patients I-1, I-2, III, IV, and V had a homozygous missense *ADSSL1* variant c.781G>A (p.Asp261Asn). While P-II had a homozygous missense variant c.794G>A (p.Gly265Glu). Both p.Asp261Asn ad p.Gly265Glu have been previously reported in patients with *ADSSL1* myopathy in Korea and Japan[3,4,6] and have been classified as pathogenic according to ACMG classification. Patients I-1 I-2, II, and V were given a trial of tablet pyridostigmine with the dose of 30 mg twice a day in patients I-1 and I-2 and 60 mg twice a day in P-II and P-V, and they showed subtle but definite response.

Discussion

ADSSL1 myopathy was first reported among Korean patients as a rare form of distal myopathy.[6] *ADSSL1* gene resides in the chromosome 14q32.33 and has a strong expression in skeletal muscles.[2] Pathogenic variants in *ADSSL1* results in disordered myocyte metabolism and apoptosis. All our patients had symptom onset in early childhood and were slower than their

peers in motor abilities, which was followed by predominant proximal LL weakness in the first decade of life. This is in contrast to previous studies from Korean cohorts where the initial symptom was distal weakness progressing to proximal muscles.[6] However, a subsequent report describing 2 patients, one each from Turkey and India, has expanded the phenotypic spectrum to include proximal myopathy with joint contractures.[7] A study done in 2020, reported a cohort of 63 Japanese patients with recessive *ADSSL1* pathogenic variants presenting with features of nemaline myopathy with proximodistal phenotype and concluded that *ADSSL1* myopathy is the most frequent form of nemaline myopathy in Japan.[4] Our cohort further reiterates that proximodistal pattern of weakness is most common in *ADSSL1* myopathy. Both Korean and Japanese patients were also slow runners since early childhood similar to the present cohort. We also report a patient who presented at 3 years of age, which is the youngest patient reported till now. In vivo studies in zebra fish have shown that *ADSSL1* pathogenic variants result in congenital myoseptal defects, which can explain very early disease onset in these patients.[3] Another distinctive feature noted in our patients was prominent fatigability with seasonal fluctuations and ptosis with squint. This led to the initial consideration of congenital myasthenic syndrome. Moreover, the siblings from family-1 (patients I-1 and I-2) showed significant decremental response on repetitive nerve stimulation. Two of these patients (P I-1 and P-II) also showed partial but definite response to pyridostigmine, with improvement in fatigability of limbs. Though earlier reports from Japanese cohort[4] have shown nonspecific fatigue, ocular involvement and electrophysiologic evidence of neuromuscular junction impairment have not been reported. While fatigability in *ADSSL1* myopathy has been attributed to the defective purine synthesis and reduced ATP synthesis, the exact mechanism of NMJ dysfunction is unclear.[4] This in turn results in secondary changes in myofibrils and mitochondria, which are seen as myofibrillar disarray and lipid droplets in histopathology.[8] Thus, the partial response with oral pyridostigmine noted in 4 patients needs further dedicated trials. Dysphagia with chewing difficulty was noted only in 1 patient. However, 38% of Japanese cohort showed dysphagia with difficulty in mastication.[4,6] Prominent cardiac symptoms with exertion-induced palpitations were seen in 3 patients with one of them showing ECG changes with QTc prolongation. Though the Japanese cohort[4] have shown ventricular hypertrophy, rhythm disturbances have not been previously reported in *ADSSL1* myopathy. Respiratory involvement in *ADSSL1* myopathy is very rare. Most of the previously reported patients had subclinical restrictive pattern in pulmonary function tests.[4,7] However, one of our patients has shown moderate restrictive pattern requiring nocturnal ventilatory support. CK was mildly elevated in 3 of our patients and ranged from 2 to 7 times above normal limit (range: 123–1,564 U/L). In the Japanese cohort, the serum CK level ranged 20–2006 U/L and showed inverse relation with age. However, this study did not show such relation between age and CK levels because congenital myopathies can have normal to mildly elevated CK values. Muscle MRI showed predominant involvement of semimembranosus and leg muscles. This is in contrast to the Korean and Japanese cohorts who showed predominant vastus lateralis, adductor group, and gastrocsoleus involvement.[4,6] However, MRI muscle of an Indian patient reported by Mroczek et al., showed involvement of both anterior and posterior thigh muscles.[7] Thus, involvement of posterior thigh muscles may be a unique finding in the Indian cohort. Muscle MRI in nemaline myopathy due to *ACTA1* mutation shows predominant involvement of sartorius and adductor magnus in thigh and tibialis anterior/posterior and peronei in legs. Whereas in nemaline myopathy due to *NEB* mutation, vastus intermedius and adductor magnus in thigh and tibialis anterior and gastrocsoleus in legs are involved showing overall sparing of posterior thigh muscles.[9]

Muscle biopsy findings were heterogenous. Similar to previous reports in both Korean and Japanese cohorts, patients with muscle biopsy (PII, PIII, and PV) showed chronic myopathic changes with type 1 fiber predominance.[3,4] The presence of focal inflammatory infiltrate in PIII were also reported in an Indian patient by Mroczek et al.[7] Intracellular lipid inclusions noted in patients PII and PV were also reported in all patients of Japanese cohort.[4] The proportion of fibers with nemaline rods and lipid inclusions noted in *ADSSL1* myopathy is significantly less when compared with genetically confirmed nemaline myopathy and lipid storage myopathy with causative genes, respectively.[4,10,11] In our cohort, 1 patient (PIII) was found to have few nemaline rods, while lipid inclusions were a more common finding in other 2 patients biopsied. Nemaline rod formation in *ADSSL1* myopathy (defective adenine nucleotide biosynthesis) may be due to a different mechanism because defects in thin filament formation result in nemaline rods in other nemaline myopathies.[12] In addition, presence of ragged red fibers with few COX-deficient fibers were not previously reported and may indicate secondary mitochondrial dysfunction.

The most common pathogenic variant c.781G>A (p.Asp261Asn) identified in our patients is also previously reported as the most frequent *ADSSL1* myopathy–associated mutation from Korea and Japan.[4,6] Only 1 patient (PII) had c.794G>A (p.Gly265Glu) pathogenic variant. It was also noted that all patients with recurrent p.Asp261Asn mutation were born of nonconsanguineous parentage. In contrast to homozygous mutations noted in all 4 patients, only 9.5% of Japanese cohort[4] and none of Korean cohort[5] had homozygous mutations. While a possibility of founder haplotype previously suggested due to shared ancestry among Asian populations cannot be ruled out, we hypothesize that it is likely a recurrent mutation due to the presence of CpG island (chr14:104741244-104741847and chr14:104741117-104741243) as per Weizmann Evolutionary CpG Island database (Evo CpG track, UCSC). This is further supported by the observation that p.Asp261Asn has been reported in gnomAD database among African, East Asian, and South Asian populations (8 heterozygotes and no homozygotes), suggesting a more wider geographical prevalence.[4,6,13] However, a detailed haplotype analysis was not performed in the families, and we consider this as a limitation of the study. The c.794G>A (p.Gly265Glu) variant

present as homozygous in our PII has also been previously reported in a Japanese patient as compound heterozygous with another frameshift pathogenic variant.[4] Both p.Asp261Asn and p.Gly265Glu are located in highly conserved sites of adenylsuccinate synthetase domain structurally adjacent to critical IGF2 mRNA-binding proteins binding residues.[4]

This report describes the phenotypic presentations of Indian patients with *ADSSL1* myopathy who manifested with ptosis, proximodistal weakness, prominent limb fatigability with seasonal fluctuations, and cardiac rhythm abnormalities. This further expands the phenotypic spectrum and geographical prevalence of *ADSSL1* myopathy beyond East Asia. The high frequency of c.781G>A (p.Asp261Asn) in our Indian patients similar to Korean and Japanese patients suggests that p.Asp261Asn is probably the most common mutation associated with *ADSSL1* myopathy and can be useful for genetic screening of suspected patients. While *ADSSL1* myopathy currently appears to be limited to Asian populations, further studies might be necessary to determine the global prevalence.

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