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Expanding the *DARS* phenotype: Late-adult onset myelopathy and leukoencephalopathy

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Abstract

A significant proportion of adult-onset neurological disorders remain diagnostic odysseys despite extensive evaluation. Hypomyelination with Brainstem and Spinal Cord Involvement and Leg Spasticity (HBSL) is an autosomal recessive disorder caused by mutations in the cytoplasmic aspartyl-tRNA synthetase (*DARS*) gene involved in mRNA translation. Clinical features of patients with *DARS* mutations include developmental delay, leg spasticity, cerebellar dysfunction, cognitive impairment and epilepsy. Most reported cases have been infantile-onset with severe neurological disability and neuroimaging abnormalities. To our knowledge, late- or adult-onset cases have never been reported in the literature. Here, we report for the first time, with video documentation and six-year clinical follow-up, an ethnic Malay patient with onset of spasticity and ataxia in late-adulthood, carrying a pathogenic *DARS* mutation discovered via whole-genome sequencing. His clinical and radiological findings were consistent with HBSL, but this diagnosis was not considered as, up until now, HBSL has only been reported with childhood/adolescent-onset. This case highlights that HBSL/*DARS* mutations should now be considered in the differential diagnosis of adult-onset spastic paraplegia and/or leukoencephalopathy.

Keywords: HBSL, DARS, spastic paraplegia, leukoencephalopathy, ataxia

INTRODUCTION

The causes of spastic paraplegia and leukoencephalopathy are diverse, and many cases remain undiagnosed despite extensive clinical, biochemical and genetic workup.[1-4] One recent discovery was the causative gene for "Hypomyelination with Brainstem and Spinal Cord Involvement and Leg Spasticity" (HBSL), an autosomal recessive disorder caused by mutations in the cytoplasmic aspartyl-tRNA synthetase (*DARS*) gene involved in translation of mRNA into protein.[4,5] Clinical features of these patients include developmental delay, leg spasticity, cerebellar dysfunction, cognitive impairment and epilepsy.[4,5]

Most reported cases were infantile-onset with severe clinical progression and widespread neuroimaging abnormalities.[4,6,7] Adult-onset DARs cases, however, have never been reported. Here, we report, with video documentation and six-years follow-up, an ethnic Malay patient with onset in his late fifties, suggesting that HBSL/*DARS* mutations should be considered in the differential diagnosis in older adults presenting with spastic

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paraplegia and/or leukoencephalopathy.[1,2]

METHODS

Subject

The study was approved by the University of Malaya Medical Centre Medical Research Ethics Committee. Informed written consent was obtained from the patient's family prior to data collection and recording, and for publication of these materials in a scientific medical journal.

Genetic analysis

Whole-genome sequencing (WGS) was conducted using genomic DNA from the patient's blood. Figure 1A illustrates the variant filtering process used to obtain candidate causal variants. WGS data were sequenced to a mean coverage of 31X, with 84% of the genome covered by at least 20 reads. Raw FASTQ files were aligned to the human reference genome (hg19) with BWA[8], and then processed in accordance with the GATK Best Practices workflow.[9] Non-multiallelic variants covered by at least 10 reads and having a quality score of at least 30 were retained. We used slivar (https://github.com/brentp/slivar) to rapidly remove variants with a gnomAD maximum population frequency in excess of 1%, and then retained only variants overlapping a set of 194 genes (Supplementary Table 1) known to be associated with neurodegenerative disorders. These variants were annotated using ANNOVAR. The final set of candidate variants (n=4) were those that passed earlier filtering stages, and in addition were 1) located in exons or splicing regions, and 2) coding variants (i.e., nonsynonymous, stopgain/stoploss, indels, splicing) (Supplementary Table 2).

RESULTS

Case report

An otherwise well 61-year-old Malay man first presented with slight unsteady gait at the age of 55 years. Initial neurological examination was near-normal except for loss of vibration sense at the toes and a positive Romberg test. He was functionally independent and working until retirement at age 57. An older sister was seen elsewhere for gait difficulty in her 50s with a normal spine MRI and was diagnosed with "amyotrophic lateral sclerosis"; she died at age 62. The family reported no parental consanguinity, or other affected members.

His gait difficulty worsened, and he required walking assistance at age 58. He was then noted to be dysarthric, with bilateral limb dysmetria, severe proprioceptive loss and wide-based gait. His Mini-Mental State Examination (MMSE) score was 29/30. The following year, he developed progressive spasticity and weakness in all four limbs (Supplementary videos 1 and 2), becoming wheelchair-bound, with generalized wasting and

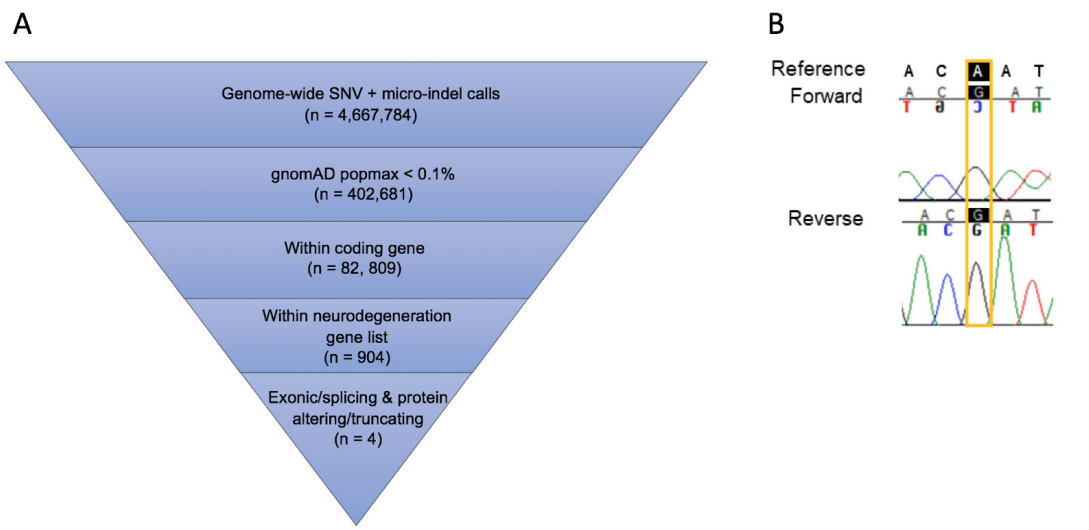

Figure 1. Genetic analysis. (A) Illustration of the variant filtering process used to obtain candidate causal variants; (B) Sanger sequencing chromatogram of rs377510027 DARS in our patient. PCR and Sanger sequencing conducted with forward primer 5'-ATTACCAATGCCTCCACCAG-3' and reverse primer 5'-CCCCACTCCAGACCAAGTAA confirmed the homozygous c.1277T>C (p.Leu426Ser) mutation.

swallowing difficulty. At age 60, he was anarthric and slower in mental response, obeying only simple commands. Eye movements remained normal.

Neuroimaging findings are shown in Figure 2. Cerebrospinal fluid analyses were normal/negative, including lactate and oligoclonal bands. Median and tibial somatosensory evoked potential studies indicated conduction defects in large-fibre sensory pathways bilaterally. Nerve conduction studies and needle electromyography were initially normal; later, changes occurred suggestive of an asymmetrical, axonal polyneuropathy, likely secondary to prolonged immobilization and entrapment neuropathy. Blood tests including paraneoplastic antibodies, connective tissue disease screen, very long chain fatty acids, screening for lysosomal storage disorders, white cell enzymes, and *FMR1* gene test were normal/negative.

Genetic findings

WGS revealed a homozygous c.1277T>C;

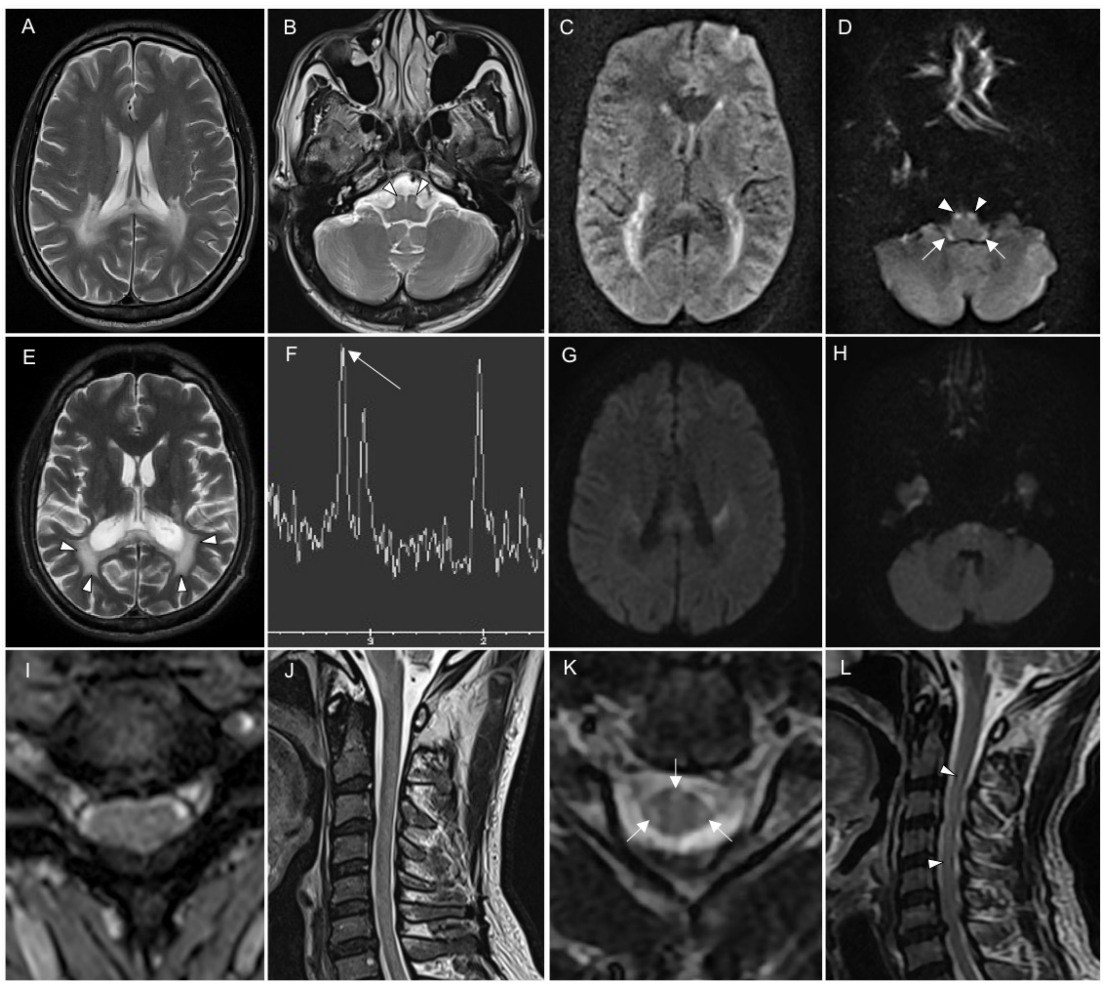

Figure 2. Evolution of brain and spinal MRI findings. MRI at the age of 55 in 2014 showing symmetrical T2-weighted hyperintensities (A, B) and restricted diffusion (C, D) in the posterior cerebral white matter, splenium of the corpus callosum, posterior limbs of the internal capsules, medullary pyramids (arrowheads) and inferior cerebellar peduncles (arrows). Repeat imaging in 2017 demonstrated centrifugal progression of the cerebral white matter changes with involvement of subcortical U-fibres (arrowheads) (E) which demonstrated elevated choline peak (arrow) on MR spectroscopy, possibly indicating increased cellular turnover (F). DWI sequences (G, H) showed a reducing degree of restricted diffusion, now seen only at the anterior edge of the cerebral changes and the anterior brainstem. Initial MRI spine in 2014 demonstrated normal spinal cord (I, J), with subsequent involvement of the anterior and lateral corticospinal tracts (arrows) (K) extending from C2 to C7 (arrowheads) (L) by 2017.

p.Leu426Ser *DARS* mutation (rs377510027), confirmed by Sanger sequencing (Figure 1B). This mutation was previously reported twice in HBSL cases; in homozygous state,[6] and in a compound heterozygous state (ClinVar accession VCV000488394.1, https://www.ncbi.nlm.nih.gov/clinvar/variation/488394/). In genomic Aggregation Database (v2.1.1), the *DARS* p.Leu426Ser variant has a global allele frequency of 1/83,472, (1/15,263 in South Asians, 1/18,372 in East Asians and absent in other populations). In a control dataset comprising 170 Singapore Malay exomes obtained from two studies (SingHEART/Biobank study and Singapore Sequencing Malay Project[10]), three heterozygous carriers were found (allele frequency = 1/113). His asymptomatic elder brother and daughter were found to be single heterozygous carriers of the same *DARS* mutation.

DISCUSSION

Proper diagnosis of spastic paraplegia or leukoencephalopathy are important for clinical management, prognostication and family counseling.[1,2] HBSL demonstrates brain and spinal cord MRI abnormalities which are rarely observed in other leukoencephalopathies (except the closely-related condition "Leukoencephalopathy with Brainstem and Spinal Cord Involvement and Lactate Elevation" [LBSL] caused by *DARS2* mutations)[2]; these include the superior and inferior cerebellar peduncles; brainstem pyramidal tracts and medial lemnisci; and spinal cord lateral corticospinal and dorsal tracts.[4]

In retrospect, our patient had neuroimaging findings that were consistent with HBSL (although the leukoencephalopathy was less extensive compared to classical infantile cases) but was not diagnosed due to its rarity and occurrence - until now - in younger persons only. Interestingly, our patient harbored the same mutation that was found in the oldest patient reported prior to the present case (a 22-year-old man with symptom onset at age 18).[6] Our patient additionally demonstrated prominent restricted diffusion on initial brain MRI. With advancing disease, these signals were reduced while structural changes in the corticospinal tracts progressed to involve the spinal cord. To our knowledge, evolution of MRI abnormalities in *DARS*, including restricted diffusion changes (found to be diagnostically useful in several leukoencephalopathies)[2,3] have not been reported previously.

Anecdotal evidence suggests that certain cases of HBSL may be steroid-responsive.[6] The molecular pathophysiological mechanisms underlying *DARS* gene functions are only beginning to be understood, with possible secondary functions related to potentially steroid-responsive immune responses.[5,6] Unfortunately, by the time of diagnosis, our patient was frail with recurrent sepsis, precluding a trial of steroid therapy.

In conclusion, we report a case of *DARS* mutation causing myelopathy and leukoencephalopathy with late-adult onset and highlight that this condition should be considered in the differential diagnosis of adult-onset spastic paraplegia and/or leukoencephalopathy. Recognition of a characteristic MRI pattern can facilitate earlier diagnosis of this condition.

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DISCLOSURE

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Conflict of interest: None

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SUPPLEMENTARY MATERIALS

Supplementary Video 1 (aged 59 years): The patient is cooperative. There is a moderate dysarthria with spastic and ataxic elements, but speech is still intelligible as he counts (in Malay). Eye movements are intact. There is obvious right arm weakness, and left-sided finger-nose dysmetria, mild-to-moderate in severity. He is wheelchair-bound, unable to support his weight to stand, even with two-person assistance. https://neurology-asia.org/content/28/1/neuroasia-2023-28(1)-185-v1.mp4

Supplementary Video 2 (12 months later): The patient is now anarthric, with a nasogastric tube in situ. There is a mild-to-moderate laterocollis to the left with hypertrophy of the left sternocleidomastoid muscle. Although generally cooperative, he appears to have difficulty sometimes obeying instructions to move his eyes; he is able to respond to simple questions using left arm gestures. There is generalized muscle wasting with no fasciculations. Limb weakness has progressed, with very little movement in the legs (paraplegic), and anti-gravity movements in the left arm. Increased tone is evident in the left leg with ankle clonus, and plantar responses are extensor bilaterally. There is a spastic catch at both elbows. https://neurology-asia.org/content/28/1/neuroasia-2023-28(1)-185-v2.mp4

Supplementary Table 1: List of neurodegeneration-associated genes.

AARS2	CHCHD10	DNAJC6	GALC	ISCA2	NDUFAF1	PEX13	PSEN1	SLC25A12	THAP1
ABCD1	CHCHD2	DNMT1	GBE1	ITM2B	NDUFS1	PEX14	PSEN2	SLC25A4	TOR1A
ACOX1	CHMP2B	DPYD	GCDH	JPH3	NDUFS2	PEX16	PYCR2	SLC30A10	TREM2
ADAR	CIC	EARS2	GCH1	L2HGDH	NDUFS4	PEX19	RARS	SLC39A14	TREX1
AIMP1	CLCN2	EIF2B1	GFAP	LAMB1	NDUFS7	PEX2	RELN	SLC6A3	TUBA1A
ALDH3A2	COL4A1	EIF2B2	GFM1	LMNB1	NDUFS8	PEX26	RNASEH2A	SNCA	TUBB4A
ANO3	COL4A2	EIF2B3	GJA1	LRRK2	NDUFV1	PEX3	RNASEH2B	SNCAIP	TUFM
APP	COQ2	EIF2B4	GJC2	LYRM7	NHLRC1	PEX5	RNASEH2C	SNORD118	TWNK
ARSA	COQ8A	EIF2B5	GLA	MAN2B1	NKX6-2	PEX6	RNASET2	SOX10	TYMP
ASPA	COQ9	EIF4G1	GNAL	MAPT	NOTCH3	PINK1	RRM2B	SPG11	TYROBP
ATN1	COX10	EPM2A	GRN	MCOLN1	NR4A2	PLA2G6	SAMHD1	SPR	UCHL1
ATP13A2	COX15	ERCC6	HEPACAM	MEF2C	NUBPL	PLP1	SCO1	SUCLA2	VCP
ATP1A3	CSF1R	ERCC8	HEXA	MFF	OPA3	POLG	SCO2	SUMF1	VPS13A
ATPAF2	CYP27A1	ETFDH	HSD17B4	MLC1	PAFAH1B1	POLG2	SCP2	SURF1	VPS35
ATXN2	D2HGDH	FAM126A	HSPD1	MPLKIP	PANK2	POLR1C	SDHAF1	SYNJ1	
ATXN3	DARS	FBXO7	HTRA1	MPZ	PARK2	POLR3A	SDHB	TACO1	
AUH	DARS2	FLVCR2	HTRA2	MRPS16	PARK7	POLR3B	SDHD	TARDBP	
BCS1L	DCTN1	FOLR1	HTT	MTFMT	PEX1	PRKRA	SGCE	TBK1	
C19orf12	DGUOK	FTL	IFIH1	MTHFR	PEX10	PRNP	SLC17A5	TBP	
C9orf72	DNAJC5	FUCA1	IPPK	NDE1	PEX12	PSAP	SLC25A1	TH	

Supplementary Table 2. Information of the four candidate variants obtained from the variant filtering process, including the DARS variant.

Gene	Transcript ID cDNA change	Protein change	Reference SNP (rs) ID	SIFT	Polyphen	M-CAP	MutationTaster	gnomAD (ALL)	gnomAD (EAS)
DARS	NM_001349 c.T1277C	L426S	rs377510027	0.149 (Tolerated)	0.998 (Damaging)	0.388 (Damaging)	1.0 (Disease causing)	0.0000159	0.0001000
PEX6	NM_001316313 c.G541A	V181M	rs372248987	0.007 (Damaging)	0.009 (Benign)	0.057 (Damaging)	0.976 (Polymorphism)	0.0000533	0.0003000
LRRK2	NM_198578 c.A5279C	E1760A	-	0.542 (Tolerated)	0.001 (Benign)	0.009 (Tolerated)	0.906 (Polymorphism)	-	-
PLA2G6	NM_003560 c.C1265T	P422L	rs780391923	0.011 (Damaging)	0.421 (Benign)	0.09 (Damaging)	1.0 (Polymorphism)	0.0000041	0