RESEARCH ARTICLE OPEN ACCESS

Spectrum of Leukodystrophy and Genetic Leukoencephalopathy in Indian Population Diagnosed by Clinical Exome Sequencing and Clinical Utility

Karamala Yalapalli Manisha, DM, Alfiya Fasaludeen, MPhil, Prashanth Poulose, DM, Ramshekhar Menon, DM, Bejoy Thomas, MD, Sruthi S. Nair, DM, Ajith Cherian, DM, Kalikavil Puthanveedu Divya, DM, and Soumya Sundaram, DM

Correspondence
Dr. Sundaram
drsoumya@sctimst.ac.in

Neurol Genet 2024;10:e200190. doi:10.1212/NXG.0000000000200190

Abstract

Background and Objectives

Next-generation sequencing (NGS) has expedited the diagnostic process and unearthed many rare disorders in leukodystrophy (LD) and genetic leukoencephalopathy (gLE). Despite the progress in genomics, there is a paucity of data on the distribution of genetic white matter disorders (WMDs) and the diagnostic utility of NGS-based assays in a clinical setting. This study was initiated to explore the clinical, radiologic, and genetic spectrum of LD and gLE in the Indian population and also to estimate the diagnostic yield of clinical exome sequencing (CES).

Methods

This is a retrospective descriptive analysis of patients with a diagnosis of genetic WMDs from a single tertiary referral center who had CES performed as part of the diagnostic evaluation between January 2016 and December 2021. The demographic, clinical, radiologic, and genetic data were collected. The variants were classified using the American College of Medical Genetics and Genomics criteria. Pathogenic and likely pathogenic variants were included in the calculation of the diagnostic yield.

Results

In the study period, 138 patients were clinically diagnosed with either LD or gLE, of which 86 patients underwent CES. Pathogenic variants, likely pathogenic variants, and variants of uncertain significance with phenotype match were seen in 40 (41.8%), 13 (29.1%), and 15 (15.2%) patients, respectively. The mean age at onset in these 68 patients was 6.35 years (range 1 month–39 years), and 38 (55.9%) were male. LDs and gLE were diagnosed in 31 and 37 patients, respectively. 56 patients (71.8%) had autosomal recessive inheritance. The common clinical presentations were developmental delay (23.5%), psychomotor regression (20.6%), progressive myoclonic epilepsy syndrome (19.1%), and spastic ataxia (14.7%). Myelin disorders (48.5%) and leuko-axonopathies (41.2%) were the commonest type of disorders. The most frequently identified genes were *ARSA*, *CLN5*, *ABCD1*, *CLN6*, *TPP1*, *HEXA*, and *L2HGDH*. The diagnostic yield of the study was 61.6% (53/86), which increased to 79.1% when VUS with phenotype match were included.

Discussion

This study demonstrated a high diagnostic yield from proband-only CES in the evaluation of genetic WMDs and should be considered as a first-line investigation for genetic diagnosis.

Classification of Evidence

This study provides Class IV evidence that proband-only clinical exome sequencing is a useful "first-line investigation" for patients with genetic white matter disorders.

MORE ONLINE

Class of Evidence
Criteria for rating therapeutic and diagnostic studies
NPub.org/coe

From the Department of Neurology (K.Y.M., A.F., P.P., S.S.N., A.C., K.P.D.); Pediatric Neurology and Neurodevelopmental Disorders (R.M., S.S.); and Department of Imaging Sciences and Intervention Radiology (B.T.), Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Thiruvananthapuram, Kerala, India.

Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

Glossary

AD = autosomal dominant; **AGS** = Aicardi-Goutières syndrome; **ALD** = adrenoleukodystrophy; **ALSP** = adult-onset leukoencephalopathy with axonal spheroids and pigmented glia; **AR** = autosomal recessive; **C.Het** = compound heterozygous; **CMT** = Charcot-Marie-Tooth disease; **CMTX** = Charcot-Marie-Tooth disease X-linked dominant; **CNV** = copy number variation; **CTX** = cerebrotendinous xanthomatosis; **HABC** = hypomyelinating leukodystrophy with atrophy of basal ganglia and cerebellum; **Hemi** = hemizygous; **Het** = heterozygous; **HLD** = hypomyelinating leukodystrophy; **Hom** = homozygous; **Hp** = heteroplasmy; **LD** = leukodystrophy; **MELAS** = mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes; **MLC** = megalencephalic leukoencephalopathy with subcortical cysts; **MLD** = metachromatic leukodystrophy; **MMDS** = multiple mitochondrial dysfunctions syndrome; **MPS** = mucopolysaccharidosis; **Mt** = mitochondrial; **NCL** = neuronal ceroid lipofuscinosis; **PMD** = Pelizaeus-Merzbacher disease; **PMLD** = Pelizaeus-Merzbacher-like disease; **SLS** = Sjogren-Larsson syndrome; **VWMD** = vanishing white matter disease; **XLD** = X-linked dominant; **XLR** = X-linked recessive.

Introduction

Leukodystrophy (LD) is a group of rare heritable disorders primarily affecting the white matter of the CNS with or without involvement of the peripheral nervous system.[1,2] Genetic disorders where primary neuronal involvement or systemic manifestations are at the forefront over and above white matter abnormalities are labeled as genetic leukoencephalopathy (gLE).[1] MRI of the brain has a crucial role in recognizing and categorizing these white matter disorders (WMDs); however, the exact diagnosis depends on genetic confirmation.[3,4] Up until a few years ago, genetic testing was not widely available except in developed countries and very few specialized centers in other regions and was limited by the high cost. The traditional step-by-step diagnostic approach of biochemical and enzymatic assays and targeted gene sequencing was tiresome and time consuming.[5] Our understanding of these conditions has tremendously improved in the past 2 decades with the advent of next-generation sequencing (NGS) for genetic diagnosis, which has also expedited the diagnostic odyssey. The discovery of newer genes and novel variants causing LDs and gLE by whole-exome sequencing (WES) and whole-genome sequencing (WGS) was instrumental in reducing undiagnosed genetic WMDs from 50% to 30%.[5-7] A chance for disease-specific treatment or enrolment in clinical trials is not possible without an early genetic diagnosis.[8-10] Moreover, molecular genetic analysis would also help to streamline genetic counseling, family screening, and reproductive decision making.[2]

The most prevalent and well-studied LDs worldwide, including in India, are metachromatic leukodystrophy (MLD), Pelizaeus-Merzbacher disease (PMD), adrenoleukodystrophy (ALD), Krabbe disease, and megalencephalic leukoencephalopathy with subcortical cysts (MLC).[6,10-15] The earlier concept of LDs being a disorder of myelin or oligodendrocyte has now become obsolete because it has now been widely recognized that defects in microglia, astrocytes, axons, neurons, and blood vessels can lead to abnormalities in white matter.[3,16] Hence, a newer classification for genetic WMDs was proposed recently, in which disorders of lysosomes, peroxisomes, and mitochondria; aminoacidemias; organic acidemias; DNA repair disorders; defects in ion and water channels; and genetic vasculopathies were all placed under the 5 broad categories.[3] We have limited knowledge of the epidemiology and the genetic spectrum of LDs and gLE in accordance with the latest classification, especially after the discovery of many newer and rarer disorders. Owing to the perceived rarity of these disorders, research into the molecular mechanisms and development of newer therapeutics for these conditions has been lackadaisical. Hence, to foster research, we need more information about the distribution and genetic profile of these disorders in different populations.[1] The number of genetic laboratories offering various genetic testing at an affordable price in the past 7 to 8 years in our region has helped clinicians pursue genetic diagnosis in these disorders. Despite this, only very few studies have explored the applicability and estimated the diagnostic yield of NGS-based technology in a clinical setting.[17-20]

Our primary objectives in this study were to describe the clinical, radiologic, and genetic spectrum of LDs and gLE as per the classification scheme of van der Knaap[3] and to identify the relative proportion of the specific subtypes from a tertiary neurologic referral center. The secondary objective was to explore the diagnostic utility of clinical exome sequencing (CES) in genetic white matter disorders.

Methodology

This is a retrospective descriptive analysis of patients with a diagnosis of LD or gLE from a single tertiary referral center (Paediatric Neurology subdivision and Department of Neurology) who had CES performed as part of the diagnostic evaluation of genetic WMDs between January 2016 and December 2021. We have adhered to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines for the study. Patients were identified from the electronic medical records (EMRs) using the search terms "leukodystrophy," "genetic leukoencephalopathy," and "hypomyelinating disorders" and individual names of the disorders (e.g., MLD and Krabbe disease) mentioned in the recent diagnostic classification for genetic WMDs.[1,3] Patients were included in this study when all the 3 inclusion criteria were satisfied: (1) clinical phenotype suggestive of LD or gLE,

(2) white matter abnormalities in MRI, and (3) genetic evaluation by CES. Exclusion criteria were as follows: (1) no white matter involvement in MRI or if an MRI/radiology report was not available; (2) acquired causes of cerebral white matter lesions such as vasculitis, inflammatory demyelination of the CNS, and vascular WMDs not due to genetic vasculopathies; and (3) genetic diagnosis confirmed by targeted gene sequencing, chromosomal microarray, PCR, or multiplex ligation probe amplification. Individual case files were scrutinized for clinical details and reports of CES by the first author (KYM) and the principal investigator (SS), and MRI scans were reviewed by the principal investigator and the neuroradiologist (BT) for selection of the patients based on inclusion and exclusion criteria. If the MRI scan was not available for review, then the findings from the radiology reports were recorded.

Data Collection

Demographic and clinical data, including the age at onset; developmental aspects; presence of psychomotor regression; pyramidal, extrapyramidal, and cerebellar signs; peripheral neuropathy and seizures; anthropometric measurements; ophthalmologic findings (cherry red spot, retinitis pigmentosa, and optic atrophy); facial and nonfacial dysmorphism; and dermatological manifestations, were recorded. Family history of similar disorders or any other neurologic disorders and consanguinity were noted, and the inheritance pattern was classified into autosomal recessive (AR), autosomal dominant (AD), X-linked, and mitochondrial. Laboratory studies including metabolic and specific enzyme assays and electrophysiologic studies such as electroencephalograms, nerve conduction studies, and visual-evoked potentials were recorded.

The MRI brain sequences reviewed were T1-weighted (T1W), T2W, fluid-attenuated inversion recovery, susceptibility-weighted imaging, diffusion-weighted imaging, apparent diffusion coefficient maps, and magnetic resonance spectroscopy. White matter abnormalities in MRI were described based on the pattern recognition approach provided previously.[2,4] LDs were broadly classified into hypomyelinating LD (HLD) and other white matter pathologies.

Clinical Exome Sequencing

As per the routine protocol at our institute, genetic testing was procured only after informed written consent from the parents of the proband (children), patients, or caregivers (in adult patients unable to provide consent). CES was performed in 2 private laboratories (MedGenome Labs Ltd. and Strand Life Sciences), and reports generated were recorded in the study. The clinical exome panel consisted of approximately 6,000–8,000 genes, which included nuclear genes and mitochondrial DNA analysis in a few patients, depending on the protocol prevalent at that time. The Illumina sequencing platform was used for exome sequencing, and the sequences were aligned to the human reference genome (GRCh37/hg19) using the Sentieon aligner. Single nucleotide variants (SNVs) were annotated using the variant effect predictor (VEP) program.[21] Small indels, delins, and copy number variants (CNVs) were detected from targeted sequence data using the ExomeDepth (v1.1.10) method.[22]

Clinically relevant variants were cross-referenced with the literature evidence (segregation analysis, functional study, allelic data set, and genome-wide association studies) and the disease databases such as ClinVar[23] and Online Mendelian Inheritance in Man (OMIM[24]). The allele frequency was estimated from the population data sets of 1000 Genome Phase 3 (1000 G), Genome Aggregation Database (gnomAD v3.0), dbSNP (v151), and the internal Indian population database. The types of variants were denoted as missense, nonsense, frameshift, indels, start loss, and splice site variants. The computation tools used for functional prediction of the nonsynonymous variants were implemented using sorting intolerant from tolerant (SIFT), PolyPhen-2, MutationTaster2, and combined annotation-dependent depletion (CADD). For splice site variant analysis, SpliceAI was used. Annotation of the clinically relevant variants was performed using American College of Medical Genetics and Genomics (ACMG) criteria, and the variants were classified as pathogenic (P) variants, likely pathogenic (LP) variants, variants of uncertain significance (VUS), and likely benign and benign variants. Null variants (nonsense, frameshift, splice site, initiation codon, single or multiexon deletion) were classified as either pathogenic or likely pathogenic if the loss of function in that gene causes the specific disorder. Missense variants were classified as P/LP only if there was any previous literature or functional evidence for their pathogenicity, or else they were kept as VUS.[25]

Diagnosis of Leukodystrophy and Genetic Leukoencephalopathy

The final diagnosis was arrived at for each patient based on the clinical, neuroimaging, and genetic profile. In the case of VUS, the variant was attributed as disease causing only if there was a genotype-phenotype correlation with supporting evidence from biochemical assays. Patients must have either P/LP variants or VUS with phenotype match for the diagnosis to be confirmed genetically. Patients with genetic diagnosis were broadly categorized as LDs, which include 30 distinct WMDs, and gLE, which has more than 61 disorders.[1] These disorders were again subclassified into myelin disorders (hypomyelination, demyelination, and myelin vacuolization), astrocytopathies, leukoaxonopathies, microgliopathies, and leukovasculopathies.[3] For the calculation of diagnostic yield of CES, only P and LP variants were included.

Statistical Analysis

The data were collated in an Excel sheet, and descriptive statistics were used. Means were compared between the LD and gLE groups with the *t* test and proportions with the Fisher exact test. p Values less than 0.05 were considered as significant.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the institutional ethics committee (IEC) of Sree Chitra Tirunal Institute for Medical Sciences

and Technology. Because this was a retrospective descriptive study, the requirement of consent was waived off by the IEC.

Data Availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

Results

Demographic, Clinical, and Radiologic Profile

A total of 138 patients were identified from the EMR using the keywords, of which 86 had undergone CES. Among them, 40 had pathogenic variants and 13 had likely pathogenic variants. Of 26 patients with VUS, only 15 had phenotype match. Therefore, a genetic diagnosis was established in 68 patients (Figure 1).

The mean age at onset was 6.35 years (range, 1 month to 39 years), and 38 (55.9%) were male. Pediatric onset of symptoms (younger than 18 years) was found in 60 patients (88.2%). 31 (45.6%) and 37 (54.4%) patients were categorized as LD and gLE, respectively. Table 1 summarizes the demographic, clinical, and radiologic features of patients with LD and gLE. In the clinical presentation, spastic ataxia syndrome (p 0.167) was more common in LD but progressive myoclonic epilepsy syndrome was more common in gLE (p 0.004). Myoclonus ($p <$ 0.001), microcephaly (p 0.097), seizures (p 0.153), and visual impairment (p 0.115) were more frequent in gLE while spasticity (p 0.015) and peripheral neuropathy (p 0.400) were more frequent in LD. Background activity slowing or epileptiform discharges were more common in gLE, and the results were significant. In MRI, hypomyelination was more often seen in gLE (p 0.031) while other white matter pathologies were more common in LD (p 0.031). gLE had more frequent occurrence of white matter rarefaction or cystic changes and BG involvement than LD, but the difference did not attain statistical significance.

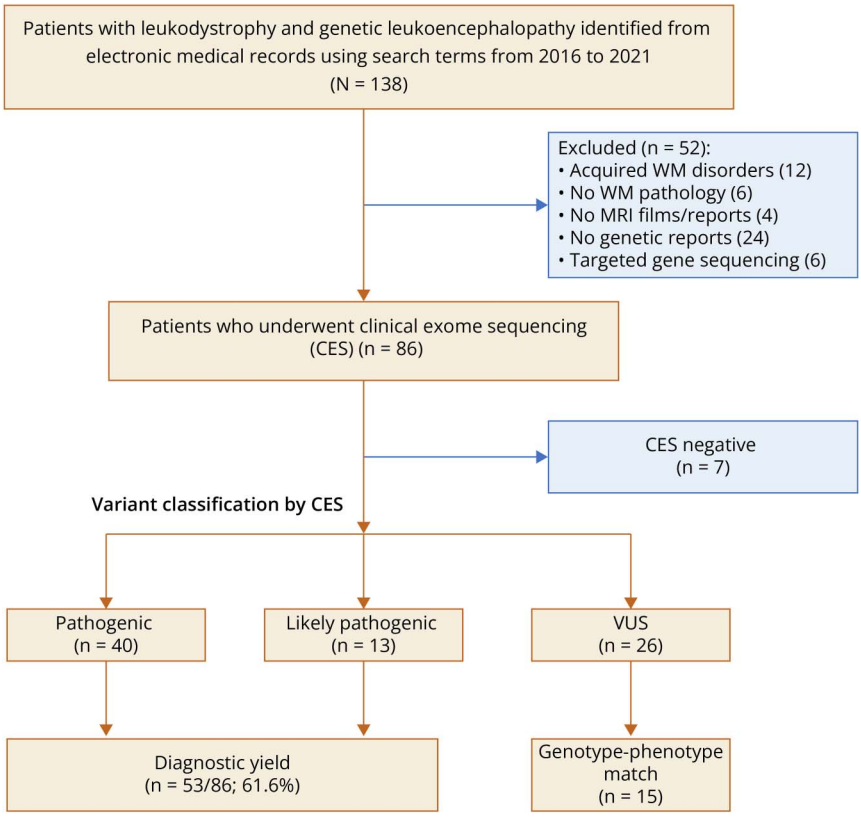
ACMG Variant Classification

Among 86 patients who underwent CES, as per ACMG criteria, pathogenic variants (Table 2 and a more comprehensive description in eTable 1) were reported in 40 patients (46.5%), likely pathogenic variants (Table 3) in 13 (15.2%), and VUS with phenotype match (Table 4) in 15 (17.4%). Detailed information on the clinical phenotypes and MRI findings of individual patients are listed in eTables 2–4. The clinical and radiologic profile of patients with negative results in CES (7 patients) and VUS without phenotype match (11 patients) is given in eTable 5. All patients with P and LP variants had a clinical-radiologic phenotype consistent with the genetic variant identified.

Variant Type

In total, 79 variations were found in 68 patients, of which 22 were novel. In patients with pathogenic variants, 45 variants

Figure 1 Flowchart Depicting Methodologic Workflow



n = number; VUS = variants of uncertain significance; WM = white matter.

Table 1 Demographic, Clinical, and Radiologic Parameters

Variables	Total, n = 68	LD, n = 31 (%)	gLE, n = 37 (%)	*p* Value
Age in y, mean ± SD (range)	6.35 ± 8.67 (1 mo to 39 y)	7.14 ± 9.82 (2 mo to 39 y)	5.7 ± 7.65, (1 mo to 30 y)	0.499
Onset <18 y of age	60 (88.2)	27 (87.1)	33 (89.2)	1.000
Sex, male	38 (55.9)	22 (71)	16 (43.2)	0.029
Neurologic presentation				
Global developmental delay	16 (23.5)	8 (25.8)	8 (21.6)	0.774
Psychomotor regression	14 (20.6)	6 (19.4)	8 (21.6)	1.000
Spastic ataxia syndrome/cerebellar ataxia	10 (14.7)	7 (22.6)	3 (8.1)	0.167
Cognitive decline	7 (10.3)	4 (12.9)	3 (8.1)	0.694
Progressive myoclonic epilepsy syndrome	13 (19.1)	1 (3.2)	12 (32.4)	0.004
Others	8 (11.8)	5 (16.1)	3 (8.1)	1.000
Clinical features				
Microcephaly	10 (14.7)	2 (6.5)	8 (21.6)	0.097
Macrocephaly	4 (5.9)	1 (3.2)	3 (8.1)	0.619
Visual impairment	20 (29.4)	6 (8.8)	14 (37.8)	0.115
Hearing loss	4 (5.9)	1 (3.2)	3 (8.1)	0.619
Seizures	33 (48.5)	12 (38.7)	21 (56.8)	0.153
Eye movement abnormalities	21 (30.9)	10 (32.3)	11 (29.7)	1.000
Nystagmus	10 (14.7)	6 (8.8)	4 (10.8)	0.494
Spasticity	37 (54.4)	22 (71)	15 (40.5)	0.015
Cerebellar ataxia	31 (45.6)	12 (38.7)	19 (51.4)	0.336
Dystonia	9 (13.2)	4 (12.9)	5 (13.5)	1.000
Myoclonus	15 (22.1)	1 (3.2)	14 (37.8)	<0.001
Peripheral neuropathy	6 (8.8)	4 (12.9)	2 (5.4)	0.400
Ophthalmologic features				
Optic atrophy	8 (11.8)	1 (3.2)	7 (18.9)	0.063
Retinitis pigmentosa	6 (8.8)	2 (6.5)	4 (10.8)	0.681
Cherry red spot	2 (2.9)	0 (0)	2 (5.4)	0.497
EEG, n	60	27	33	
Slow background activity	21 (35)	4 (14.8)	17 (51.5)	0.006
IEDs	25 (41.7)	6 (22.2)	19 (57.6)	0.008
MRI findings				
Hypomyelination	32 (47.1)	10 (32.3)	22 (59.5)	0.031
Other WM pathologies	36 (52.9)	21 (67.7)	15 (40.5)	0.031
Diffuse cerebral	13 (36.1)	7 (33.3)	6 (40)	0.549
Cystic/rarefaction	11 (30.1)	4 (19.1)	7 (46.7)	0.742
Basal ganglia	10 (14.7)	3 (4.4)	7 (18.9)	0.326
Thalamus	5 (7.4)	3 (4.4)	2 (5.4)	0.653
Posterior fossa involvement	24 (35.3)	10 (32.3)	14 (37.8)	0.799

Abbreviations: gLE = genetic leukoencephalopathy; IEDs = interictal epileptiform discharges; LD = leukodystrophy; mo = months; WM = white matter; y = years

Table 2 Genetic Spectrum of Patients With Pathogenic Variants

Pt ID	Gene	Variant	AA change	Variant type	Zygosity	In silico predictions
2	*GJC2*	c.508delG	E170Rfs*40	Fs	Hom	—
5	*LYRM7*	c.2T>C	M1?	Start loss	Hom	SIFT (0), PP2 (0.978), CADD (23.8)
6	*L2HGDH*	c.293A>G	H98R	Mis	Hom	SIFT (0), PP2 (1), CADD (27.5)
11	*L2HGDH*	c.829C>T	R277*	Non	Hom	CADD (39)
12	*CYP27A1*	c.646+1G>A	Intronic	SS	Hom	SpliceAI (0.96)
13	*CSF1R*	c.2381T>C	I794T	Mis	Het	SIFT (0), PP2 (1), CADD (28.3)
14	*FOL1R*	c.428G>A	W143*	Non	Hom	MT (1)
16	*MT-TL1*	n.14A>G	—	Mis	Hp	MitoTip (13.35)
17	*PLP1*	c.354_355delAG	G120Pfs*83	Fs	Hemi	—
18	*ALDH3A2*	c.798+1del/c.1094C>T	Intronic/S365L	SS/mis	C.Het	SIFT (-/0), PP2 (-/0.98), CADD (-/24.4)
21	*PLP1*	Exons 3–8 del*	—	CNV	Hemi	—
22	*CLN5*	c.595C>T	R199*	Non	Hom	CADD (37), MT (1)
24	*TPP1*	c.89+1G>A	Intronic	5′ SS	Hom	CADD (33), MT (1)
30	*SLC17A5*	c.116G>A	R39H	Mis	Hom	SIFT (0), PP2 (1), CADD (25.5)
31	*GALC*	c.956A>G/c.236G>A	Y319C/R79H	Mis/Mis	C.Het	SIFT (0.01/0.002), PP2 (0.98/1), CADD (24.8/29.5)
33	*MT-ND3*	n.1019T>C	S45P	Mis	Hp	—
37	*CLN3*	Exons 11–14 del‡	—	CNV	Hom	—
38	*CLN5*	c.595C>T	R199*	Non	Hom	MT (1)
39	*MFSD8*	c.590dupG	V198Cfs*7	Fs	Hom	—
40	*TPP1*	c.565C>T	Q189*	Non	Hom	MT (1), CADD (35)
44	*ARSA*	c.465+1G>A	Intronic	5′ SS	Hom	SpliceAI (0.86), CADD (33)
45	*ABCD1*	c.612delT	F204Lfs*12	Fs	Hemi	—
46	*ERCC8*	c.78_79delAG	R26Sfs*8	Fs	Hom	SpliceAI (0.98)
47	*CLN5*	c.433C>T	R145*	Non	Hom	—
50	*SUOX*	c.144_145insC/c.1096dupC	N49Qfs*28/R366Pfs*50	Fs/Fs	C.Het	—
53	*ARSA*	c.883G>A	G295S	Mis	Hom	SIFT (0), PP2 (1), CADD (31)
54	*L2HGDH*	c.829C>T	R277*	Non	Hom	MT (1), CADD (37)
55	*MLC1*	c.353C>T	T118M	Mis	Hom	SIFT (0.01), PP2 (1), CADD (26.7)
60	*RNASEH2C*	c.205C>T	R69W	Mis	Hom	SIFT (0), PP2 (0.997), CADD (28.8)
61	*CLCN2*	c.2068G>T	E690*	Non	Hom	MT (1), CADD (39)
62	*CLN6*	Exons 3 to 5 del†	—	CNV	Hom	—
64	*MT-TL1*	n.14A>G	—	Mis	Hp	MitoTip (13.35)
65	*MLC1*	c.135dup	C46Lfs*34	Fs	Hom	—
66	*HEXA*	c.509G>A	R170Q	Mis	Hom	SIFT (0), PP2 (1), CADD (33)
67	*CLN5*	c.565+1G>A	—	SS	Hom	MT (1), CADD (34)
70	*HEXA*	c.821C>T	T274I	Mis	Hom	SIFT (0), PP2 (0.123), CADD (29.1)
72	*HEXA*	c.1151G>A/c.1337A>G	W384*/Y446C	Non/Mis	C.Het	SIFT (-/0), CADD (45/27.2), MT (1/1)
73	*FIG4*	c.1601+1G>T	Intronic	5′ SS	Hom	SpliceAI (0.99), CADD (33), MT (1)

Continued

Table 2 Genetic Spectrum of Patients With Pathogenic Variants *(continued)*

Pt ID	Gene	Variant	AA change	Variant type	Zygosity	In silico predictions
76	*POLR3B*	c.2303G>A/c.2980A>C	R768H/T994P	Mis/Mis	C.Het	SIFT (0/0.015), MT (1/1)
77	*ABCD1*	c.1661G>A	R554H	Mis	Hemi	SIFT (0.002), PP2 (1)

Abbreviations: AA = amino acid; CADD = combined annotation-dependent depletion; C.Het = compound heterozygous; CNV = copy number variation; del = deletion; Fs = frameshift; Hemi = hemizygous; Het = heterozygous; Hom = homozygous; Hp = heteroplasmy; Mis = missense; MT = MutationTaster2; Non = nonsense; PP = PolyPhen-2; Pt = patient; SIFT = sorting intolerant from tolerant; SS = splice site.
*chrX:g.(103031928_103040510)_(103045526_?)del; c.(790 + 1_791-1)_(1056 + 1_1057- 1)del;
†chr15:g.(?_68211209)_(68214438_?)del.

were identified in 40 patients, only 3 of which were in the mitochondrial genome. 5 patients had variants in a compound heterozygous state (P18 *ALDH3A2*, P31 *GALC*, P50 *SUOX*, P72 *HEXA*, and P76 *POLR3B*). One of the compound heterozygous variants in P31 (c.956A>G) and P76 (c.2980A>C) was classified as VUS while one in P72 (c.1337A>G) was classified as LP. In 13 patients with likely pathogenic variations, 17 variants were identified, with P10, P57, and P71 having compound heterozygous variants in *ARSA*, *PI4KA*, and *TPP1*, respectively. In them, P10 had 3 missense variants and 2 were VUS (c.129C>A and c.496C>A) and 1 variant in P71 was VUS (c.1231_1233dup). Among 15 patients with VUS, 2 patients had compound heterozygous variants (P8 *PEX1* and P79 *IBA57*) and all 17 variants were missense (Figure 2, A–C).

Zygosity and Inheritance Pattern

AR inheritance was seen in 56 patients (71.8%), followed by XL in 6 (8.8%) and AD and mitochondrial inheritance in 3 (4.4%) each. In patients with pathogenic variants, 32 had AR inheritance, 4 had XL, and 3 had mitochondrial inheritance. In the patients with likely pathogenic variants, 11 had AR and 1 each had XL and AD inheritance patterns. In patients with VUS, 13 had AR inheritance and 1 patient each had XL and AD inheritance.

Spectrum of LD and gLE

LDs and gLE were seen in 31 (Figure 2D) and 37 (Figure 2E) patients, respectively. The most frequently identified disease-causing gene variants in our cohort were in *ARSA* (MLD) and *CLN5* [neuronal ceroid lipofuscinosis (NCL) 5] in 4 patients each, followed by *ABCD1* (ALD), *CLN6* (NCL 6), *TPP1* (NCL 2), *HEXA* (Tay-Sachs disease), and *L2HGDH* (L2-hydroxyglutaric aciduria) in 3 patients each. Next frequent were in *MLC1* (MLC), *GALC* (Krabbe disease), *PLP1* (PMD), *GJC2* [Pelizaeus-Merzbacher-like disease (PMLD)], *POLR3B* [Pol III-related leukodystrophies/hypomyelination, hypodontia, and hypogonadotropic hypogonadism leukodystrophy (4H syndrome)], *MT-TL1* [mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS)], and *CSF1R* [adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP)] in 2 patients each.

Pathogenic Classification of Genetic White Matter Disorders

As per the recent classification (Figure 3),[3] myelin disorders constituted the majority, which was seen in 33 patients (48.5%). Of the myelin disorders, myelin vacuolization, demyelination, and hypomyelination were seen in 17, 10, and 6 patients, respectively. Leuko-axonopathies were the next most common disorder observed in 28 patients (41.2%), followed by astrocytopathies and microgliopathies in 5 (7.4%) and 2 (2.9%) patients, respectively. None of the patients had leukovasculopathies.

Diagnostic Yield

Among the 86 patients who underwent CES, 7 had negative test results and 53 patients had P or LP variants. Hence, the diagnostic yield of CES in LDs and gLE was determined to be 61.6%. When we included the additional 15 patients with VUS who had a genotype-phenotype match, the yield increased to 79.1%.

Discussion

In this study, we elaborated on the clinical, radiologic, and genetic spectrum of 68 patients with genetic WMDs diagnosed by CES in South India. gLE marginally outnumbered LD, as shown in 37 and 31 cases, respectively. Spastic ataxia was more common in LD while progressive myoclonic epilepsy syndrome was more common in gLE, as was the occurrence of myoclonus, microcephaly, and visual impairment. The presence of white matter rarefaction and cystic alterations and involvement of the basal ganglia suggest gLE on MRI. NCL and mitochondrial disorders emerged as the most frequent groups of disorders in gLE. Among the five categories of genetic WMDs, myelin disorders (48.5%) and leuko-axonopathies (41.2%) were the most common. Only a few patients had astrocytopathies (7.4%) and microgliopathies (2.9%), and none had leukovasculopathies. There were 40 patients with pathogenic variants and 13 patients with LP variants, thus estimating a diagnostic yield of 61.6% by CES, which increased to 79.1% by including additional 15 patients who had VUS with genotype-phenotype correlation.

Almost 2 to 3 decades before, the diagnosis of LDs and gLE was often based on the MRI pattern recognition approach and further corroboration from metabolic analysis, enzyme assays, or when possible, pathology. Genetic diagnosis was laborious at that time because the candidate genes were discovered through genetic linkage studies, which required large

Table 3 Genetic Spectrum of Patients With Likely Pathogenic Variants

Pt ID	Gene	Variant	Refseq ID	AA change	Variant type	Inheritance	Zygosity	In silico predictions	AF	ClinVar
7	*TUBB4A*	c731G>A	rs886041010	G244D	Missense	AD	Het	MT (1), CADD (25.7)	NR	NR
10	*ARSA*	c.129C>A/ c.847G>A/ c.496C>A	—/rs199476386/__	H43Q/ D283N/P166T	Missense (all 3)	AR	C.Het	SIFT (0/0/0.005/0), PP2 (0.998/1/0.88), MT (1/1/1)	NR/ NR/ NR	NR/NR/ NR
26	*DEGS1*	c.565A>G	rs771864122	N189D	Missense	AR	Hom	SIFT (0.002), PP2 (1), MT (1)	0.0004	LP
32	*PDSS2*	c.1145C>T	rs118203956	S382L	Missense	AR	Hom	SIFT (0.002), PP2 (0.98), CADD (31)	0.0032	LP
35	*CLN6*	c.2T>G	rs2093262877	M1?	Start loss	AR	Hom	SIFT (0), CADD (25), MT (1)	NR	Novel
52	*ABCD1*	c.509C>A		A170D	Missense	XLR	Hemi	SIFT (0.001), CADD (27.3), MT (1)	NR	Novel
56	*GALC*	c.1073T>C		L358P	Missense	AR	Hom	SIFT (0), CADD (28.7), MT (1)	NR	Novel
57	*PI4KA*	c.5700del/ c.6122T>C	__/rs1601301648	D1901Tfs*96/ M2041T	Frameshift/ missense	AR	C.Het	SIFT (0/-), CADD (-/27.2)	NR/ NR	Novel/ NR
68	*MOCS1*	c.217C>T	rs104893970	R73W	Missense	AR	Hom	SIFT (0), PP (1) CADD (29.7)	0.0068	P
69	*POLR3A*	c.2171G>A	rs267608679	C724Y	Missense	AR	Hom	SIFT (0), PP (0.98) CADD (29.1)	NR	NR
71	*TPP1*	c.729C>G/ c.1231_ 1233dup		F243L/ G411dup	Missense/in frame dup	AR	C. Het	SIFT (0.048/-), MT (1/-)	NR/ NR	Novel/ Novel
74	*ARSA*	c.736C>T	rs74315470	R246C	Missense	AR	Hom	SIFT (0.002), PP2 (1), CADD (26.3)	0.0008	P
75	*CLN6*	c.393G>T		K131N	Missense	AR	Hom	SIFT (0.144), MT (1)	NR	Novel

Abbreviations: AA = amino acid; AD = autosomal dominant; AF = allele frequency in gnomAD database; AR = autosomal recessive; CADD = combined annotation-dependent depletion; C.Het = compound heterozygous; dup = duplication; Hemi = hemizygous; Het = heterozygous; Hom = homozygous; Hp = heteroplasmy; LP = likely pathogenic; MT = MutationTaster2; NR = not reported; P = pathogenic; Pt = patient; PP = PolyPhen-2; SIFT = sorting intolerant from tolerant; XLR = X-linked recessive.

samples.[16] There has been a dramatic change in the genetic landscape of hereditary WMDs after the widespread implementation of NGS-based diagnostic testing. According to the earlier literature, almost 25% of the cases were constituted by MLD, ALD, PMD, and mitochondrial diseases.[6] Later, many disorders were included under the term gLE.[1,2] In a surveillance of children with progressive intellectual and neurologic deterioration with WMDs in the United Kingdom, 349 and 454 children were diagnosed to have LDs and gLE, respectively. Mucopolysaccharidosis, GM1 and GM2 gangliosidoses, and mitochondrial disorders constituted most of the gLE.[10] In 104 Indian families, mitochondrial disorders were foremost, identified in 20 families using various genetic tests.[18] In the recent study using NGS, MLD, Canavan disease, Tay-Sachs disease, and ALD were the most frequent in the Iranian population.[19] In our study also, disorders of myelin were the most common, comprising predominantly MLD, PMD, Krabbe disease, ALD, and PMLD. A notable finding in this study was the emergence of leuko-axonopathies as the second most common group of disorders, contributed by NCL (*CLN5*, *CLN6, CLN8, MFSD8,* and *TPP1),* Tay-Sachs disease (*HEXA*), Pol III-related leukodystrophies (*POLR3B* and *POLR3A*), and hypomyelination with atrophy of the basal ganglia and cerebellum (HABC, *TUBB4A*), which were earlier classified as hypomyelinating LD.[8,13] NCL, a prototype of leuko-axonopathy, typically presents as progressive myoclonic epilepsy (progressive cognitive decline, ataxia, multifocal myoclonus, and seizures), and its high proportion observed in our cohort could be due to the referral bias because ours is a high volume center for refractory epilepsy.[26] Mitochondrial disorders are another group of disorders that were also frequently observed in our study, similar to another study from India.[18] The variability in the genetic spectrum observed in these studies could be attributed to the methodological differences, NGS pipelines, and the referral pattern.

Our cohort had a lower number of astrocytopathies [MLC1, vanishing white matter disease (VWMD), and Aicardi-Goutières syndrome (AGS)] and microgliopathies (*CSF1R*-related disorders) without any leukovasculopathies. 1 patient with Alexander disease was excluded because the diagnosis was confirmed by targeted gene sequencing. Adult-onset LDs were less frequent in this study, hence accounting for a smaller number of microgliopathies and none in the leukovasculopathy group. In a recent study of young-onset dementia due to genetic WMDs, most had pathogenic or novel variants in *NOTCH3*, causing cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy

Table 4 Genetic Spectrum of Patients With Variants of Uncertain Significance With Phenotype Match

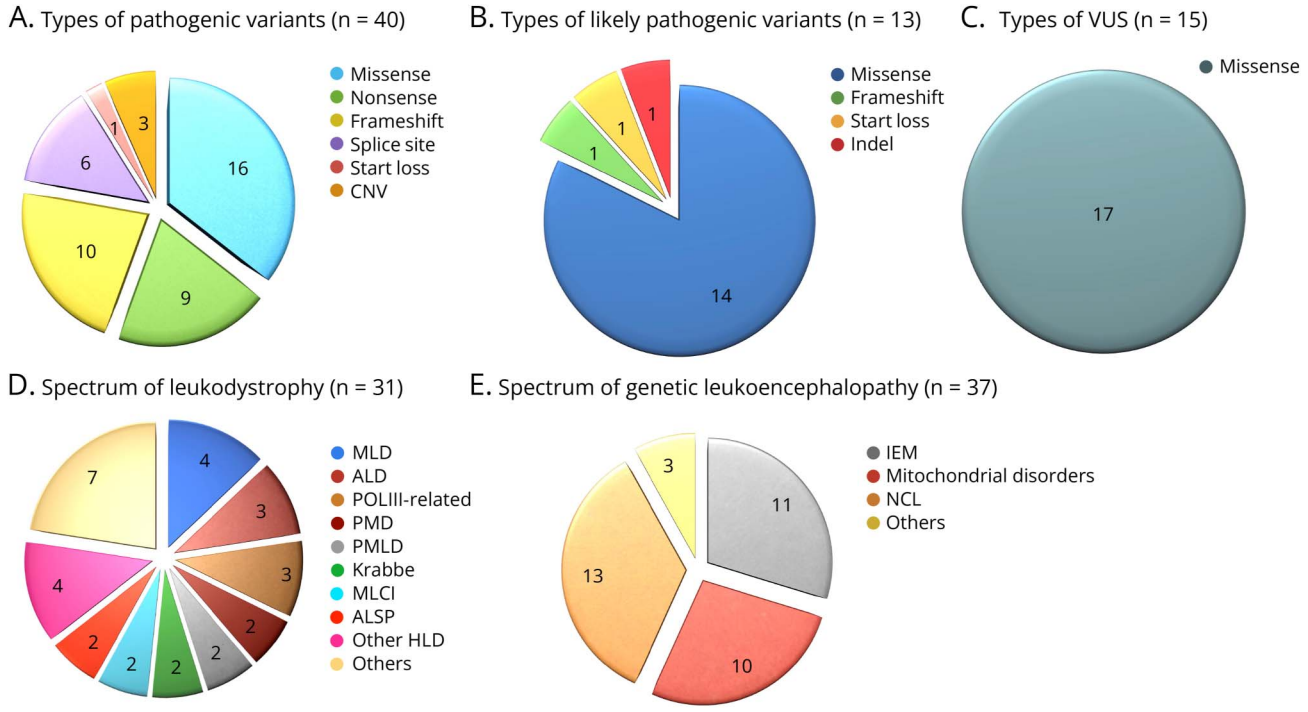
Pt ID	Gene	Variant	Refseq ID	AA change	Variant type	Inheritance	Zygosity	In silico predictions	AF	ClinVar
1	*GJB1*	c.677A>G	rs781312706	N226S	Missense	XLD	Het	SIFT (0.731), PP (0), CADD (10.58)	0.0175	LB
3	*GJC2*	c.251T>G		I84S	Missense	AR	Hom	SIFT (0), CADD (23.7), MT (1)	NR	Novel
4	*COQ2*	c.1039A>G	rs566845170	S347G	Missense	AR	Hom	SIFT (0.014), PP (0.1), CADD (9.78)	0.0055	NR
8	*PEX1*	c.2308C>G/ c.701G>A	rs751076530/__	Q770E/ R234H	Missense/ missense	AR	C.Het	SIFT (0.848/0.32), PP (0.01/0), MT (0.98/0)	0.0107	NR/NR
15	*SGSH*	c.613G>C		G205R	Missense	AR	Hom	CADD (26.7), MT (1)	NR	NR
20	*EIF2B3*	c.1103C>T		S368L	Missense	AR	Hom	SIFT (0.005), CADD (25.1), MT (1)	NR	Novel
25	*OPA1*	c.211C>T	rs368488165	R71C	Missense	AR	Hom	SIFT (0.013), CADD (24.5), MT (1)	0.0047	B
28	*POLR1C*	c.118T>G		W40G	Missense	AR	Hom	SIFT (0), CADD (32), MT (1)	NR	Novel
29	*CSF1R*	c.1852_1854del		L618del	Missense	AD	Het	—	NR	Novel
41	*POLR3B*	c.2458A>G		K820E	Missense	AR	Hom	SIFT (0.287), CADD (23), MT (1)	NR	Novel
42	*IDUA*	c.143C>G	rs1362102709	A48G	Missense	AR	Hom	SIFT (0.029), MT (1)	NR	Novel
58	*ISCA1*	c.244G>T	rs1324359474	V82F	Missense	AR	Hom	SIFT (0), CADD (23.6), MT (1)	0.0004	NR
59	*MSTO1*	c.920G>A	rs751943144	G307D	Missense	AR	Hom	SIFT (0.069), PP (0.98), CADD (23.6), MT (1)	0.1042	NR
63	*CLN8*	c.433G>T	rs753439965	G145W	Missense	AR	Hom	SIFT (0.049), PP (1), CADD (23.1), MT (1)	0.0004	NR
79	*IBA57*	c.802C>T/ c.738C>G		R268C/ N246K	Missense/ missense	AR	C. Het	SIFT (0/0), PP (-/1), CADD (-/25.7), MT (1/1)	NR/ 0.0004	VUS/NR

Abbreviations: AA = amino acid; AD = autosomal dominant; AF = allele frequency in gnomAD database; AR = autosomal recessive; B = benign; CADD = combined annotation-dependent depletion; C.Het = compound heterozygous; del = deletion; Het = heterozygous; Hom = homozygous; LB = likely benign; MT = MutationTaster2; NR = not reported; PP = PolyPhen-2; Pt = patient; SIFT = sorting intolerant from tolerant; VUS = variants of uncertain significance; XLD = X-linked dominant.

(CADASIL), and only 1 patient had *CSF1R*-related disorders.[17] These disorders are inherited in an AD fashion while three-fourths in our study population had AR inherited disorders, which might be accounted by the higher proportion of consanguineous marriages. In addition, acquired causes for white matter pathology such as vascular, primary progressive MS, other inflammatory demyelinating disorders, and neoplasms would be more common, thus decreasing the yield in adult patients.[27-29] Multifocal white matter lesions, calcifications, and cysts are points favoring genetic leukovasculopathy. However, lack of family history, similar clinical and radiologic picture in small vessel disease, and less suspicion of genetic WMDs in adults were the reasons why genetic testing was pursued less often in adults. Due to their rarity, LDs and gLE can be difficult to diagnose, especially in adults and in disorders with atypical presentations.[8]

In our study, two-thirds of patients were diagnosed using CES and the diagnostic yield of 61.6% for proband-only CES was comparable with the results from another Indian study.[20] WES could further improve the diagnosis because the entire protein-coding region is covered, as shown in a recent study.[18] In unresolved genetic WMDs, trios WES was able to achieve a diagnosis in 42%.[7] A similar finding was observed in a small cohort of hypomyelinating LD, wherein a diagnosis was eluded by targeted gene sequencing for the *PLP1* or LD panel covering more than 100 genes, and trios WES could reveal a diagnosis in more than half of the patients.[30] Even with WES, variants in noncoding parts of the gene and noncoding RNAs, and CNVs, are missed.[5] The results could further improve with WGS, where the diagnostic yield was found to be 75% in an Iranian cohort.[19] CES used in our study had a coverage of only 6,000–8,000 genes, thus missing out on ultrarare genetic causes of WMDs. These studies, including ours, support the recommendation of CES/WES as a first-tier investigation in genetic WMD[5] and should be commenced immediately, as shown in a randomized controlled trial wherein the time to achieve a diagnosis was shorter with a much better yield in the genome sequencing compared with the standard approach.[31] Still, one-third of patients may remain undiagnosed, and in

Figure 2 Upper Panel: Types of Variants in the Study Population Lower Panel: Spectrum of Leukodystrophy and Genetic Leukoencephalopathy



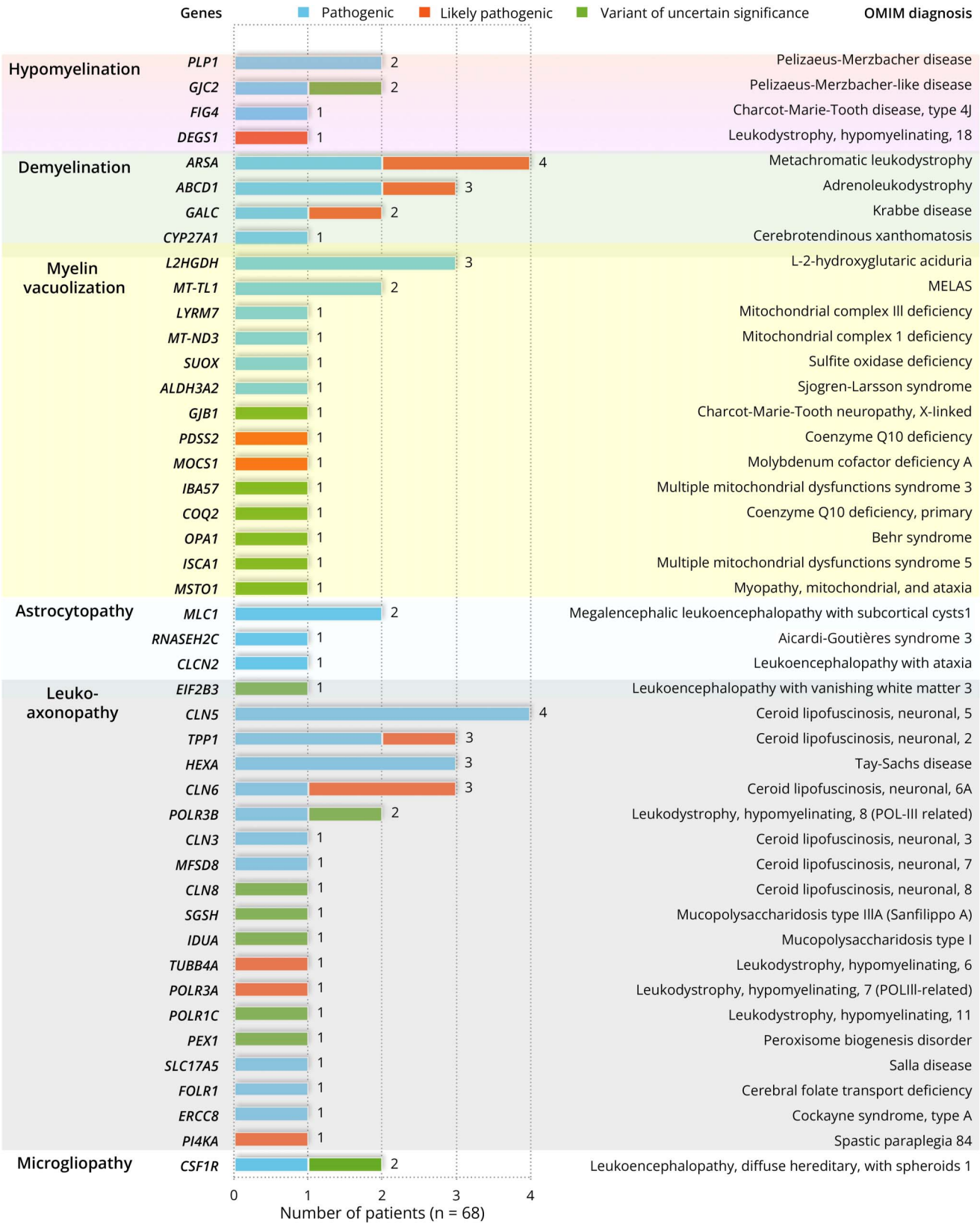
ALD = adrenoleukodystrophy; ALSP = adult-onset leukoencephalopathy with axonal spheroids and pigmented glia; CNV = copy number variant; HLD = hypomyelinating leukodystrophy; IEM = inborn errors of metabolism; MLC = megalencephalic leukoencephalopathy with subcortical cysts; MLD = metachromatic leukodystrophy; n = number; NCL = neuronal ceroid lipofuscinosis; PMLD = Pelizaeus-Merzbacher-like disease; PMD = Pelizaeus-Merzbacher disease; VUS = variants of uncertain significance.

them, WGS in the research setting has been proven useful to decipher ultrarare variants and novel genes.[5,16]

Accurate and early diagnosis has potential therapeutic implications in these devastating disorders—enzyme replacement therapy in Fabry disease and *TPP1* deficiency; hematopoietic stem cell transplantation in the early stages of cerebral ALD, ALSP, and juvenile and adult MLD, and Krabbe disease; and Janus kinase inhibition in AGS.[5,9] These treatments are beneficial only when they are given early; hence, a conventional stepped diagnostic approach, a time-consuming ordeal, is now replaced by NGS in many centers. Devising treatment strategies is an unmet need, and to boost the research, more information is needed on the prevalence of these conditions.[1] Accurate case definitions and classification are extremely important for designing research studies and epidemiologic surveys in LDs and gLE; a lack of adherence leads to variable results. In this study, we have used the latest classification for genetic WMDs.[1,3] Although this categorization looks simple and straightforward, immediate consensus emerged for only 10 of 91 disorders to be classified under LDs, even among experts in this field.[1] When newer concepts on the molecular basis and neuropathologic evidence emerge, the disease classification is bound to change. Some of these disorders have complex biology; hence, exact categorization might be challenging.[3] We were also met with the predicament of categorizing a few disorders—LD related to cerebral folate deficiency (*FOL1R*), Sjogren-Larsson syndrome (*ALDH3A2*), Cockayne syndrome (*ERCC8*), Charcot-Marie-Tooth disease 4J (*FIG4*), and spastic paraplegia 84 (*PI4KA*)—into any specific subcategory because these disorders were not mentioned in the van der Knaap classification.[3] After analyzing the radiologic findings and categorization of the closely related disorders, we assigned them to the most suitable category.

In this study, we were able to achieve a genetic diagnosis in two-thirds of patients with genetic WMDs with proband-only CES. We had diligently looked into neuroimaging patterns and genetic profiles and subclassified various conditions into different categories as per the latest classification, one of the few studies to have adopted this scheme. There are a few limitations in this study. The first and foremost is the retrospective data collection, which does not reflect the actual number of cases because CES was not pursued in all suspected genetic WMDs. We have also excluded patients when an MRI scan was not available for review or when a proper radiology report was unavailable. Sometimes, patients were diagnosed only on the basis of MRI, enzyme assay, pathology, or targeted gene sequencing, and they were excluded. Because of these reasons, the distribution of LDs and gLE does not reflect the actual prevalence in our population but represents the conditions identified by CES. Another limitation is the lack of

Figure 3 Classification and Distribution of Genetic White Matter Disorders



MELAS = mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes; n = number; OMIM = Online Mendelian Inheritance in Man.

Sanger sequencing of parents to ascertain the de novo status of heterozygous variants and the segregation of compound heterozygous variants. Lack of feasibility for functional studies hindered further classification of VUS, which could have predicted a better diagnostic yield. Not all protein-coding regions were sequenced in CES, for which WES was required.

This cohort identified by CES was clinically and genetically diverse, and myelin disorders and leuko-axonopathies topped the list. Neuronal ceroid lipofuscinosis and mitochondrial disorders were the most prominent groups of disorders among genetic leukoencephalopathy. Our study confirms the high diagnostic utility of proband-only CES in the evaluation of genetic WMDs and should be considered as a first-line investigation for genetic diagnosis.

Study Funding

The authors report no targeted funding.

Disclosure

The authors report no relevant disclosures. Go to Neurology.org/NG for full disclosures.

Publication History

Received by *Neurology: Genetics* October 30, 2023. Accepted in final form July 18, 2024. Submitted and externally peer reviewed. The handling editor was Associate Editor Alexandra Durr, MD, PhD.

Appendix Authors

Name	Location	Contribution
Karamala Yalapalli Manisha, DM	Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST)	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data
Alfiya Fasaludeen, MPhil	Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST)	Analysis or interpretation of data
Prashanth Poulose, DM	Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST)	Major role in the acquisition of data
Ramshekhar Menon, DM	Pediatric Neurology and Neurodevelopmental Disorders, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST)	Drafting/revision of the manuscript for content, including medical writing for content
Bejoy Thomas, MD	Department of Imaging Sciences and Intervention Radiology, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST)	Drafting/revision of the manuscript for content, including medical writing for content
Sruthi S. Nair, DM	Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST)	Analysis or interpretation of data
Ajith Cherian, DM	Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST)	Drafting/revision of the manuscript for content, including medical writing for content
Kalikavil Puthanveedu Divya, DM	Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST)	Drafting/revision of the manuscript for content, including medical writing for content
Soumya Sundaram, DM	Pediatric Neurology and Neurodevelopmental Disorders, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST)	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data

References

1. Vanderver A, Prust M, Tonduti D, et al. Case definition and classification of leukodystrophies and leukoencephalopathies. *Mol Genet Metab.* 2015;114(4):494-500. doi:10.1016/j.ymgme.2015.01.006
2. Parikh S, Bernard G, Leventer RJ, et al. A clinical approach to the diagnosis of patients with leukodystrophies and genetic leukoencephelopathies. *Mol Genet Metab.* 2015;114(4):501-515. doi:10.1016/j.ymgme.2014.12.434
3. van der Knaap MS, Bugiani M. Leukodystrophies: a proposed classification system based on pathological changes and pathogenetic mechanisms. *Acta Neuropathol.* 2017;134(3):351-382. doi:10.1007/s00401-017-1739-1
4. Schiffmann R, van der Knaap MS. Invited article: an MRI-based approach to the diagnosis of white matter disorders. *Neurology.* 2009;72(8):750-759. doi:10.1212/01.wnl.0000343049.00540.c8
5. van der Knaap MS, Schiffmann R, Mochel F, Wolf NI. Diagnosis, prognosis, and treatment of leukodystrophies. *Lancet Neurol.* 2019;18(10):962-972. doi:10.1016/S1474-4422(19)30143-7
6. Bonkowsky JL, Nelson C, Kingston JL, Filloux FM, Mundorff MB, Srivastava R. The burden of inherited leukodystrophies in children. *Neurology.* 2010;75(8):718-725. doi:10.1212/WNL.0b013e3181eee46b
7. Vanderver A, Simons C, Helman G, et al. Whole exome sequencing in patients with white matter abnormalities. *Ann Neurol.* 2016;79(6):1031-1037. doi:10.1002/ana.24650
8. Kohlschütter A, Eichler F. Childhood leukodystrophies: a clinical perspective. *Expert Rev Neurother.* 2011;11(10):1485-1496. doi:10.1586/ern.11.135
9. Shukla A, Kaur P, Narayanan DL, do Rosario MC, Kadavigere R, Girisha KM. Genetic disorders with central nervous system white matter abnormalities: an update. *Clin Genet* 2021;99(1):119-132. doi:10.1111/cge.13863
10. Stellitano LA, Winstone AM, van der Knaap MS, Verity CM. Leukodystrophies and genetic leukoencephalopathies in childhood: a national epidemiological study. *Dev Med Child Neurol.* 2016;58(7):680-689. doi:10.1111/dmcn.13027
11. Gulati S, Jain P, Chakrabarty B, Kumar A, Gupta N, Kabra M. The spectrum of leukodystrophies in children: experience at a tertiary care centre from North India. *Ann Indian Acad Neurol.* 2016;19(3):332-338. doi:10.4103/0972-2327.179975
12. Raina A, Nair SS, Nagesh C, Thomas B, Nair M, Sundaram S. Electroneurography and advanced neuroimaging profile in pediatric-onset metachromatic leukodystrophy. *J Pediatr Neurosci.* 2019;14(2):70-75. doi:10.4103/jpn.JPN_155_18
13. Singhal BS. Leukodystrophies: Indian scenario. *Indian J Pediatr.* 2005;72(4):315-318. doi:10.1007/BF02724013
14. Narayanan DL, Matta D, Gupta N, et al. Spectrum of ARSA variations in Asian Indian patients with Arylsulfatase A deficient metachromatic leukodystrophy. *J Hum Genet.* 2019;64(4):323-331. doi:10.1038/s10038-019-0560-1
15. Bindu PS, Mahadevan A, Taly AB, Christopher R, Gayathri N, Shankar SK. Peripheral neuropathy in metachromatic leucodystrophy. A study of 40 cases from south India. *J Neurol Neurosurg Psychiatry.* 2005;76(12):1698-1701. doi:10.1136/jnnp.2005.063776
16. Kevelam SH, Steenweg ME, Srivastava S, et al. Update on leukodystrophies: a historical perspective and adapted definition. *Neuropediatrics* 2016;47(6):349-354. doi:10.1055/s-0036-1588020
17. Chen Z, Tan YJ, Lian MM, et al. High diagnostic utility incorporating a targeted neurodegeneration gene panel with MRI brain diagnostic algorithms in patients with young-onset cognitive impairment with leukodystrophy. *Front Neurol.* 2021;12:631407. doi:10.3389/fneur.2021.631407
18. Kaur P, do Rosario MC, Hebbar M, et al. Clinical and genetic spectrum of 104 Indian families with central nervous system white matter abnormalities. *Clin Genet.* 2021;100(5):542-550. doi:10.1111/cge.14037
19. Mahdieh N, Soveizi M, Tavasoli AR, et al. Genetic testing of leukodystrophies unraveling extensive heterogeneity in a large cohort and report of five common diseases and 38 novel variants. *Sci Rep.* 2021;11(1):3231. doi:10.1038/s41598-021-82778-0
20. Parayil Sankaran B, Nagappa M, Chiplunkar S, et al. Leukodystrophies and genetic leukoencephalopathies in children specified by exome sequencing in an expanded gene panel. *J Child Neurol.* 2020;35(7):433-441. doi:10.1177/0883073820904294

21. Accessed September 23, 2023. grch37.ensembl.org/Homo_sapiens/Tools/VEP
22. Plagnol V, Curtis J, Epstein M, et al. A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. *Bioinformatics*. 2012;28(21):2747-2754. doi:10.1093/bioinformatics/bts526
23. Accessed September 25, 2023. ncbi.nlm.nih.gov/clinvar/
24. Accessed October 17, 2023. omim.org
25. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30
26. Jose M, Poulose P, Sundaram S, Radhakrishnan A, Nampoothiri S, Menon RN. Utility of clinical exome sequencing in progressive myoclonus epilepsy syndromes: an exploratory analysis. *Clin Genet*. 2022;101(2):270-271. doi:10.1111/cge.14090
27. Köhler W. Diagnostic algorithm for the differentiation of leukodystrophies in early MS. *J Neurol*. 2008;255(suppl 6):123-126. doi:10.1007/s00415-008-6023-9
28. Labauge P, Carra-Dalliere C, Menjot de Champfleur N, Ayrignac X, Boespflug-Tanguy O. MRI pattern approach of adult-onset inherited leukoencephalopathies. *Neurol Clin Pract*. 2014;4(4):287-295. doi:10.1212/CPJ.0000000000000047
29. Lynch DS, Wade C, Paiva ARB, et al. Practical approach to the diagnosis of adult-onset leukodystrophies: an updated guide in the genomic era. *J Neurol Neurosurg Psychiatry*. 2019;90(5):543-554. doi:10.1136/jnnp-2018-319481
30. Yan H, Ji H, Kubisiak T, et al. Genetic analysis of 20 patients with hypomyelinating leukodystrophy by trio-based whole-exome sequencing. *J Hum Genet*. 2021;66(8):761-768. doi:10.1038/s10038-020-00896-5
31. Vanderver A, Bernard G, Helman G, et al. Randomized clinical trial of first-line genome sequencing in pediatric white matter disorders. *Ann Neurol*. 2020;88(2):264-273. doi:10.1002/ana.25757