Whole Exome Sequencing in Patients with White Matter Abnormalities

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Here we report whole exome sequencing (WES) on a cohort of 71 patients with persistently unresolved white matter abnormalities with a suspected diagnosis of leukodystrophy or genetic leukoencephalopathy. WES analyses were performed on trio, or greater, family groups. Diagnostic pathogenic variants were identified in 35% (25 of 71) of patients. Potentially pathogenic variants were identified in clinically relevant genes in a further 7% (5 of 71) of cases, giving a total yield of clinical diagnoses in 42% of individuals. These findings provide evidence that WES can substantially decrease the number of unresolved white matter cases.

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Patients with white matter abnormalities in the central nervous system may have one of more than 100 genetic disorders, including the leukodystrophies (Supplementary Table 1).[1] Over the past 2 decades, magnetic resonance imaging (MRI) pattern recognition has transformed the diagnosis of leukodystrophies.[2,3] Despite these advances, the breadth of conditions that present as a possible leukodystrophy continues to challenge even the most astute clinician.[4] Nearly half of these patients will remain unresolved, resulting in a prolonged diagnostic odyssey for affected families.[5–7]

A number of recent reports have provided evidence that whole exome sequencing (WES) can resolve previously intractable genetic disorders, with diagnostic yields ranging from 16 to 53%.[8–19] Given the unmet diagnostic need among patients with leukodystrophy, and the potential for agnostic next generation sequencing (NGS) to clarify these

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Additional supporting information can be found in the online version of this article.

Members of the Leukodystrophy Study Group who evaluated the patients clinically and referred patients to the Myelin Disorders Bioregistry Project are available as an online supplementary file.

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TABLE 1. Cases with Pathogenic Variants Identified by Exome Sequencing in Classical Leukodystrophy Genes

Family	Gene	Zygosity	Protein	Classification
LD_0139	*TUBB4A*	Het, de novo	p.Arg391His	Likely pathogenic
LD_0181	*DARS*	Het	p.Arg494Gly	Likely pathogenic
		Het	p.Arg460His	Likely pathogenic
LD_0604	*POLR3B*	Het	p.Glu271fs	Pathogenic
		Het	p.Val523Glu	Pathogenic
LD_0672	*TUBB4A*	Het, de novo	p.Val180Gly	Likely pathogenic
LD_0764	*EIF2B5*	Het	p.Gln562*	Pathogenic
		Het	p.Arg339Trp	Pathogenic
LD_0774	*POLR1C*	Het	p.Lys295del	Likely pathogenic
		Het	p.Cys146Arg	Likely pathogenic
LD_0869	*EIF2B2*	Het	p.Gly200Val	Pathogenic
		Het	p.Glu213Gly	Pathogenic

Het = heterozygous.

cases, we performed WES on a cohort of 71 patients referred to the Myelin Disorders Bioregistry Project (MDBP) for unresolved leukoencephalopathy of presumed genetic etiology. These patients were collected prospectively from August 1, 2009 to July 31, 2013 in the MDBP or the Amsterdam Database of Unclassified Leukoencephalopathies with approval from the institutional review boards at all collaborating institutions, including Children's National Medical Center, the Baylor Neurogenetic Institute, and VU University Medical Center.

Patients and Methods

Cohort Description

A total of 191 persistently unresolved cases were identified during the course of the study. 101 patients were diagnosed using MRI pattern recognition followed by biochemical or other molecular approaches. These testing approaches included lysosomal enzymes, very long chain fatty acids, specific electron transport chain or mitochondrial enzyme assays, urine organic acids, microarray testing of copy number variations, gross chromosomal abnormality testing by karyotype or microarray, plasma amino acids, cerebrospinal neurotransmitters and alpha interferon, urine mucopolysaccharide or sialic acid testing, and targeted molecular testing, for example, for *EIF2B1-5*, *PLP1*, or *GFAP* sequencing based on MRI pattern recognition. Of the 90 persistently unsolved cases, 19 were excluded from WES testing: 9 families obtained access to WES at other facilities, and an additional 10 families were excluded because DNA quality for all members of the trio did not meet minimum quality requirements and attempts to collect additional samples during the course of the study were unsuccessful. Seventy-one families remained for which high-quality samples were available for complete trios. These 30 female and 47 male individuals all had abnormal white matter signal on neuroimaging. Individuals ranged in age from 3 to 26 years at the time of sequencing, but symptom onset ranged from birth to 19 years (see complete Supplementary Case Reports, including a description of the radiologic findings of affected individuals, the phenotypic presentation of affected individuals and their families, and the details of each candidate pathogenic variant identified, available at http://imb.uq.edu.au/download/Vanderver_AON_2016.case_reports.pdf). Ethnicities were varied and included individuals of mixed and northern European descent, as well as African American, Arab, African, Asian, and Latin American origin.

WES sequencing was performed at the Queensland Centre for Medical Genomics. Exomes were captured using either the Nextera Rapid Capture kit (Illumina, San Diego, CA) or the SeqCap EZ Human Exome Library v.3.0 (Roche Diagnostics, Risch-Rotkreuz, Switzerland). Captured libraries were sequenced on an Illumina HiSeq 2000 (2 × 100 nucleotides) or on an Illumina NextSeq 500 (2 × 100 nucleotides). WES sequencing was performed such that a minimum of 80% of targeted bases were sequenced to a read depth of 20× or greater (average = 88%). Reads were aligned to the reference human genome (GRCh37), and pedigree informed variant calling was performed using the Real Time Genomics (Hamilton, New Zealand) integrated analysis tool rtgFamily v3.2.[20] All variants were annotated using SnpEff v3.4[21] and filtered using data from the SnpEff GRCh37.72 database, dbSNP138, and dbNSFP v2.4.

We utilized a custom-built variant annotation and interpretation interface to identify possible causal mutations in each

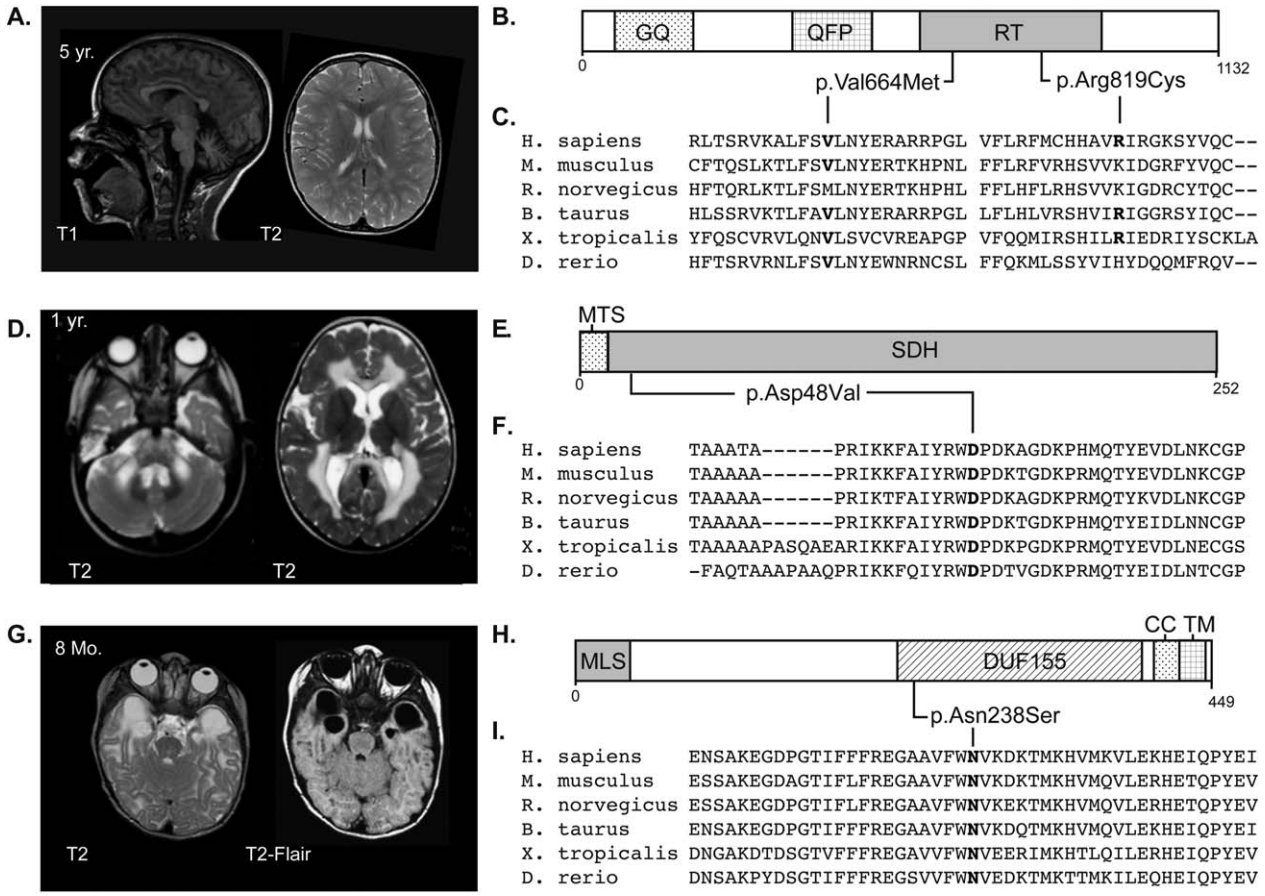

FIGURE 1: Magnetic resonance imaging (MRI) and pathogenic variants for 3 cases. (A) MRI of individual LD_0607.0, a male of mixed European descent with a multisystem disorder characterized by elevated creatine kinase, recurrent infection with hypogammaglobulinemia, dyskeratosis congenita, and mild transaminase abnormalities. MRI revealed moderate cerebellar atrophy and diffuse multifocal white matter changes. Follow-up MRI 1 year later showed unchanged T2 hyperintensities. The variants found in *TERT* in this patient were classified as pathogenic per the American College of Medical Genetics and Genomics guidelines, and the diagnosis was supported by telomere length analysis (data not shown). (B) Schematic of the TERT protein showing heterozygous variants identified in LD_0607.0. Predicted domains: GQ (GQ motif), QFP (QFP motif), RT (reverse transcriptase domain). (C) Clustalo alignment of vertebrate homologs of *TERT* showing conservation of mutated residues. (D) MRI of individual LD_0756.0, a male of Turkish descent in whom motor delays were noted at birth who abruptly decompensated at 7 months of age, with a history of ataxia, hypotonia, and spasticity. MRI at 3 years and 6 months of age was significant for signal abnormality of the supratentorial white matter with sparing of the U fibers, a swollen appearance to the corpus callosum, and involvement of the cerebellar white matter and the brainstem. This pattern has been seen in previously published cases and supports the *SDHB* variant categorization as potentially pathogenic. (E) Schematic of the SDHB protein showing a homozygous variant identified in LD_0756.0. Predicted domains: MTS (mitochondrial targeting signal), SDH (succinate dehydrogenase domain). (F) Clustalo alignment of vertebrate homologs of *SDHB* showing conservation of mutated residues. (G) MRI of individual LD_0286.0B, a male of mixed European descent with leukoencephalopathy and a history of sensorineural hearing loss, developmental delay, and febrile seizures. MRI is significant for bilateral temporal lobe cysts, small corpus callosum, and periatrial white matter abnormalities. Hearing loss and other clinical manifestations were consistent with the phenotype reported for mutations in *RMND1*, and the variant was classified as likely pathogenic based on supporting evidence. (H) Schematic of the RMND1 protein showing heterozygous variants identified in LD_0286. Predicted domains: MLS (mitochondrial localization signal), DUF155 (domain of unknown function 155), CC (coiled-coil domain), TM (transmembrane domain). (I) Clustalo alignment of vertebrate homologs of *RMND1* showing conservation of mutated residues.

case, incorporating evidence including minor allele frequency, conservation, predicted pathogenicity, disease association (in public databases or the published literature), established or predicted biological function, confirmation with Sanger sequencing and familial segregation (Supplementary Tables 2–4).[22] Cases with variants in known disease genes meeting the American College of Medical Genetics and Genomics (ACMG) criteria for pathogenic or likely pathogenic (see Supplementary Case Reports), and whose clinical features were concordant with the established gene–disease relationship, were classified as diagnostically resolved.

Results

In this cohort, we were able to unambiguously resolve 25 cases (Table 1, Supplementary Table 2, and Supplementary Case Reports). In 3 cases, we were able to confirm pathogenicity with downstream biochemical testing. For example,

TABLE 2. Cases with Pathogenic Variants Identified by Whole Exome Sequencing in Genes Not Associated with Leukodystrophy

Family	Gene	Zygosity	Protein	Classification
LD_0106	*GRIN1*	Het, de novo	p.Arg865Cys	Likely pathogenic
LD_0115	*AARS*	Het	p.Arg751Gly	Pathogenic
		Het	p.Lys81Thr	Pathogenic
LD_0119	*KCNT1*	Het, de novo	p.Phe932Ile	Pathogenic
LD_0157	*SZT2*	Het, de novo	p.Pro1833fs	Pathogenic
		Het	p.Gly2306Arg	Likely pathogenic
LD_0158	*CNTNAP1*	Hom	p.Arg388Pro	Likely pathogenic
LD_0232	*MRPS22*	Het	p.Lys248fs	Pathogenic
		Het	p.Arg191Gln	Likely pathogenic
LD_0286	*RMND1*	Hom	p.Asn238Ser	Likely pathogenic
LD_0333	*CNTNAP1*	Het	p.Arg107*	Pathogenic
		Het	p.Cys323Arg	Likely Pathogenic
LD_0358	*STXBP1*	Het, de novo	p.Arg367*	Pathogenic
LD_0366	*GATAD2B*	Het, de novo	p.Gln274*	Pathogenic
LD_0463	*ALS2*	Het	p.Arg1139*	Pathogenic
		Het	p.Gly1083Glu	Pathogenic
LD_0607	*TERT*	Het, de novo	p.Arg819Cys	Pathogenic
		Het	p.Val664Met	Pathogenic
LD_0646[19]	*NDUFS7*	Hom	p.Arg135Cys	Likely pathogenic
LD_0678	*ATM*	Het	p.Leu275fs	Pathogenic
		Het	p.Lys2756*	Pathogenic
LD_0755	*SDHAF1*	Hom	p.Arg55Pro	Pathogenic
LD_0756	*SDHB*	Hom	p.Asp48Val	Pathogenic
LD_0846	*GLB1*	Het	p.Arg196Ser	Pathogenic
		Het	p.Tyr240His	Pathogenic
LD_0857	*AARS*	Hom	p.Arg751Gly	Pathogenic

Het = heterozygous; Hom = homozygous.

we identified a compound heterozygous mutation in *TERT* (Mendelian Inheritance in Man [MIM]: 187270) in a patient who presented with white matter changes, frequent infections, mild developmental delay, and hypogammaglobulinemia, which was validated by flow–fluorescent in situ hybridization telomere length analysis and confirmed a diagnosis of atypical dyskeratosis congenita with hypomyelination (MIM: 613989; Fig 1, Supplementary Case Reports, LD_0607.0).[23] Likewise, an individual with a compound heterozygous mutation in *GLB1* (MIM: 611458) had confirmatory lysosomal enzyme testing (Supplementary Case Reports, LD_0846.0), and an individual with mutations in *ATM* (MIM: 607585) had confirmatory elevated alpha-fetoprotein levels (Supplementary Case Reports, LD_0678.0).

Nine of the 25 cases had mutations in genes associated with disorders classically defined as leukodystrophies[3] (see Table 1 and Supplementary Table 1); 2 patients were identified with *TUBB4A* (MIM: 602662)-related hypomyelination (hypomyelination with atrophy of the basal ganglia and cerebellum [MIM: 612438]),[24] 2 patients were identified with early onset vanishing white matter disease (MIM: 603896; genotype *EIF2B2* [MIM: 606454] and *EIF2B5* [MIM: 603945]),[25,26] 3 families were

TABLE 3. Cases with Potentially Pathogenic Variants Identified with Whole Exome Sequencing

Family	Gene	Zygosity	Protein	Classification
LD_0493	*FLNA*	Hem, de novo	p.Leu2271Arg	VUS
LD_0664	*FUS*	Het	p.Gly500fs	Pathogenic
LD_0673	*AMPD2*	Hom	p.Arg843His	VUS
LD_0675	*AARS2*	Het	p.Gly965Arg	Likely pathogenic
		Het	p.Glu405Lys	VUS
LD_0821	*NDUFA2*	Het	p.Lys45Thr	VUS
		Het	p.Thr75fs	VUS

Het = heterozygous; Hom = homozygous; VUS = variant of uncertain significance.

identified with t-RNA synthetase disorders (*AARS* [MIM: 601065] and *DARS* [MIM: 603084]),[27–29] and 2 families were identified with a POLR3-related disorder (*POLR3B* [MIM: 614366] and *POLR1C* [MIM: 610060]; see Table 1, Supplementary Table 2, and Supplementary Case Reports).[30] The remaining individuals had mutations in genes associated with genetic leukoencephalopathies. In these cases, expert review confirmed that the clinical presentation and MRI were consistent with published phenotypes. These findings are consistent with the estimation that a majority of disorders associated with abnormal white matter on neuroimaging are not classic leukodystrophies.[1] This suggests that testing of leukodystrophy-associated genes on NGS panels may have limited diagnostic efficacy (predicted to be only 13% in this cohort), which may outweigh the perceived cost benefit and limiting exposure to incidental findings.

In a further 4 cases, we identified 1 or more potentially damaging variants of uncertain significance that did not reach the strict burden of proof required to be classified as pathogenic or likely pathogenic. In each of these cases, the neuroradiological findings, clinical features, and familial segregation of the variants in these individuals were consistent with the published phenotype (Table 2, Supplementary Table 3, and Supplementary Case Reports). We therefore classified these variants as potentially pathogenic and considered the cases clinically resolved by expert assessment. This included cases with variants in *AMPD2*, *FLNA*, and *NDUFA2* (see Supplementary Table 4). This also included 1 case (LD_0675) where the individual was reported as part of cohort describing a novel disease due to mutations in *AARS2*.[27]

A final case had a de novo variant in *FUS* (MIM: 205100) classified as pathogenic by ACMG criteria, but because this gene has previously only been associated with juvenile or adult onset amyotrophic lateral sclerosis (ALS), this was not considered an unambiguous resolution (see Table 2, Supplementary Table 3, and Supplementary Case Reports). However, because mutations in other ALS-associated genes are associated with early hypomyelination (including *ALS2* [MIM: 205100] in this cohort), and because the de novo finding segregated in this family, it was classified as a potentially pathogenic variant and a clinically resolved case.

To investigate the burden of actionable incidental findings that may be identified during trio-based WES investigation of rare genetic disorders, the Illumina Clinical Services Laboratory screened all 56 adult and 49

TABLE 4. Incidental Findings Identified in Cohort

Group	Individuals Screened, No.	Individuals with Incidental Findings, No.	Reported Incidental Finding
Unaffected adults	142	3 (2.1%)	*KCNQ1* (NM_000218.2) c.514G>A *KCNQ1* (NM_000218.2) c.1189C>T *SDHB* (NM_003000.2) c.541-2A>G
Affected children	79	3 (3.7%)	As above
Unaffected siblings	10	0	NA

NA = not applicable.

pediatric ACMG-recommended genes for potential incidental variants.[31] This analysis revealed pathogenic or likely pathogenic variants in 3 of the 71 families screened (Table 3). The identified variants were restricted to *KCNQ1* (MIM: 607542) associated with long QT syndrome and *SDHB* (MIM: 115310) associated with hereditary paragangliomas (see Table 2, Supplementary Table 3, and Supplementary Case Reports). Interestingly, mutations in *SDHB* are also now associated with autosomal recessive succinate dehydrogenase deficiency–associated leukoencephalopathy, although the lack of a second mutation in LD_0315 precluded definitive association of this genotype with the patient's phenotype.[32] We found <1 known pathogenic or likely pathogenic variant per 46 adult exomes analyzed, suggesting that the impact of incidental findings is likely to be minimal, especially when weighed against the potential benefits of a successful genetic diagnosis in families with severe, life-threatening neurologic illnesses (Table 4).

Discussion

Using an intention to treat analysis,[33] and if the combined initial cohort of 191 families is considered in which 101 families achieved a diagnosis through standard approaches, the use of the WES approach would result in an ~20% diagnostic increase. This yields an overall rate of diagnosis of ~73% for the combination of standard and WES approaches. Clinical integration of WES (or whole genome sequencing), therefore, may decrease the number of patients with unsolved genetic white matter disorders from 50% to <30%. Taking into consideration the clinical and psychosocial costs of prolonged diagnostic odysseys in these families, this is substantial.

Additionally, although the clinical utility of WES as measured by changes to patient care was not addressed in this study, it should be noted that in several cases the results of WES directly influenced clinical care. For example, patients with *ATM* and *TERT* mutations were both referred to specialist clinics where they now undergo oncologic monitoring, and the patient with a de novo *KCNT1* mutation was treated with a potassium channel anticonvulsant to control refractory epilepsy.[34]

The use of WES in large cohorts enables the identification of sequence variants of varying degrees of clinical certainty. ACMG criteria classify the spectrum of identified variants into 4 tiers; pathogenic, likely pathogenic, variant of unknown significance, or unresolved.[35] However, our study, in which 4 of 30 cases were resolved with MRI pattern recognition[2,3,36,37] and clinical review of the identified variant, suggests that a variant classification system that takes into account clinical context and downstream clinical evaluation and testing (eg, MRI interpretation) should be considered.

In summary, WES has the potential to decrease the number of unsolved cases of leukodystrophy and genetic leukoencephalopathies. Additional research is needed establish the potential value of NGS as a first-line diagnostic tool, and to assess the comparative effectiveness of WES, whole genome sequencing, and targeted panels in this disease population.

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Author Contributions

A.V., C.S., G.H., and R.J.T. designed and managed the project, coordinated the manuscript, and performed literature and case review. C.S., G.H., J.C., A.K., V.R., E.R., S.C., T.H., D.M., K.R., G.J.B., S.M.G., L.C., J.D., N.M., A.T., and R.J.T. acquired the data, performed analysis of the incidental findings, provided

bioinformatics analysis and expertise, and performed laboratory studies. A.V., C.S., G.H., J.C., A.P., N.I.W., G.B., J.L.S., M.B., S.H.E., J.L.P.M., B.L.F., R.S., M.S.v.d.K., and R.J.T. drafted the manuscript and figure. A.V. and C.S. contributed equally to the article.

Potential Conflicts of Interest

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