RESEARCH ARTICLE OPEN ACCESS

Diagnosis of Genetic White Matter Disorders by Singleton Whole-Exome and Genome Sequencing Using Interactome-Driven Prioritization

Agatha Schlüter, PhD,* Agustí Rodríguez-Palmero, MD,* Edgard Verdura, PhD,* Valentina Vélez-Santamaría, MD, Montserrat Ruiz, PhD, Stéphane Fourcade, PhD, Laura Planas-Serra, MSc, Juan José Martínez, MSc, Cristina Guilera, MSc, Marisa Girós, PhD, Rafael Artuch, MD, María Eugenia Yoldi, MD, Mar O'Callaghan, MD, PhD, Angels García-Cazorla, MD, PhD, Judith Armstrong, PhD, Itxaso Marti, MD, PhD, Elisabet Mondragón Rezola, MD, PhD, Claire Redin, PhD, Jean Louis Mandel, MD, PhD, David Conejo, MD, Concepción Sierra-Córcoles, MD, Sergi Beltrán, PhD, Marta Gut, PhD, Elida Vázquez, MD, Mireia del Toro, MD, PhD, Mónica Troncoso, MD, PhD, Luis A. Pérez-Jurado, MD, PhD, Luis G. Gutiérrez-Solana, MD, PhD, Adolfo López de Munain, MD, PhD, Carlos Casasnovas, MD, PhD, Sergio Aguilera-Albesa, MD, PhD, Alfons Macaya, MD, PhD, and Aurora Pujol, MD, PhD, on behalf of the GWMD working group

Correspondence
Dr. Pujol
apujol@idibell.cat

Neurology® 2022;98:e912-e923. doi:10.1212/WNL.0000000000013278

Abstract

Background and Objectives

Genetic white matter disorders (GWMD) are of heterogeneous origin, with >100 causal genes identified to date. Classic targeted approaches achieve a molecular diagnosis in only half of all patients. We aimed to determine the clinical utility of singleton whole-exome sequencing and whole-genome sequencing (sWES-WGS) interpreted with a phenotype- and interactome-driven prioritization algorithm to diagnose GWMD while identifying novel phenotypes and candidate genes.

Methods

A case series of patients of all ages with undiagnosed GWMD despite extensive standard-of-care paraclinical studies were recruited between April 2017 and December 2019 in a collaborative study at the Bellvitge Biomedical Research Institute (IDIBELL) and neurology units of tertiary Spanish hospitals. We ran sWES and WGS and applied our interactome-prioritization algorithm based on the network expansion of a seed group of GWMD-related genes derived from the Human Phenotype Ontology terms of each patient.

Results

We evaluated 126 patients (101 children and 25 adults) with ages ranging from 1 month to 74 years. We obtained a first molecular diagnosis by singleton WES in 59% of cases, which increased to 68% after annual reanalysis, and reached 72% after WGS was performed in 16 of

*These authors contributed equally to this work.

From the Neurometabolic Diseases Laboratory (A.S., A.R.-P., E. Verdura, V.V.-S., M.R., S.F., L.P.-S., J.J.M., C.G., C.C., A.P.), Bellvitge Biomedical Research Institute (IDIBELL); Instituto de Salud Carlos III (ISCIII) (A.S., A.R.-P., E. Verdura, M.R., S.F., L.P.-S., J.J.M., C.G., R.A., M.O., A.G.-C., J.A., M.d.T., L.A.P.-J., A.M., A.P.) and Secció d'Errors Congènits del Metabolisme–IBC, Servei de Bioquímica i Genètica Molecular, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) (M. Girós), Center for Biomedical Research on Rare Diseases (CIBERER); Pediatric Neurology Unit, Department of Pediatrics, Hospital Universitari Germans Trias i Pujol (A.R.-P.), and Pediatric Neurology Research Group, Vall d'Hebron Research Institute (A.M.), and Pediatric Neurology Department, Vall d'Hebron University Hospital (M.d.T., A.M.), Universitat Autònoma de Barcelona; Neuromuscular Unit, Neurology Department (V.V.-S., C.C.), Hospital Universitari de Bellvitge and Hospitalet de Llobregat, Universitat de Barcelona; Institut de Recerca Pediàtrica (R.A., M.O., A.G.-C.) and Molecular and Genetics Medicine Section (J.A.), Hospital Sant Joan de Déu (IRP-HSJD), Barcelona; Pediatric Neurology Unit, Department of Pediatrics (M.E.Y., S.A.-A.), Navarra Health Service, Navarrabiomed Research Foundation; Departments of Neuropediatrics (I.M.) and Neurology (E.M.R., A.L.d.M.), Hospital Universitario Donostia; Biodonostia Health Research Institute (Biodonostia HRI) (I.M., E.M.R., A.L.d.M.); University of the Basque Country (UPV-EHU) (I.M., A.L.d.M.), San Sebastian; Centro de Investigación Biomédica en Red para Enfermedades Neurodegenerativas (CIBERNED) (I.M., E.M.R., A.L.d.M.), Carlos III Health Institute, Madrid, Spain; Département de Médecine Translationnelle et Neurogénétique (C.R., J.L.M.), IGBMC, CNRS UMR 7104/INSERM U964/Université de Strasbourg, Illkirch; Laboratoire de Diagnostic Génétique (J.L.M.), Hôpitaux Universitaires de Strasbourg; Chaire de Génétique Humaine (J.L.M.), Collège de France, Illkirch; Complejo Asistencial Universitario de Burgos (D.C.); Department of Paediatric Neurology (C.S.-C.), Complejo Hospitalario Jaén; CNAG-CRG, Centre for Genomic Regulation (CRG) (S.B., M. Gut), Barcelona Institute of Science and Technology (BIST); Department of Pediatric Radiology (E. Vázquez), Hospital Materno-Infantil Vall d'Hebrón, Barcelona, Spain; Pediatric Neurology (M.T.), Hospital Clínico San Borja Arriarán, Central Campus Universidad de Chile; Genetics Service (L.A.P.-J.), Hospital del Mar Research Institute (IMIM); Department of Experimental and Health Sciences (L.A.P.-J.), Universitat Pompeu Fabra, Barcelona; Department of Paediatric Neurology (L.G.G.-S.), Children's University Hospital Niño Jesús, Madrid; and Catalan Institution of Research and Advanced Studies (ICREA) (A.P.), Barcelona, Spain.

GWMD working group coinvestigators are listed in Appendix 2 at the end of the article.

Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

The Article Processing Charge was funded by the authors.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Glossary

aCGH = array comparative genomic hybridization; **ACMG** = American College of Medical Genetics and Genomics; **CNV** = copy number variant; **GO** = Gene Ontology; **GWMD** = genetic white matter disorders; **HPO** = Human Phenotype Ontology; **NGS** = next-generation sequencing; **PBMC** = peripheral blood mononuclear cells; **sWES** = singleton whole-exome sequencing; **VUS** = variants of uncertain significance; **WES** = whole-exome sequencing; **WGS** = whole-genome sequencing.

the remaining negative cases. We identified variants in 57 different genes among 91 diagnosed cases, with the most frequent being *RNASEH2B*, *EIF2B5*, *POLR3A*, and *PLP1*, and a dual diagnosis underlying complex phenotypes in 6 families, underscoring the importance of genomic analysis to solve these cases. We discovered 9 candidate genes causing novel diseases and propose additional putative novel candidate genes for yet-to-be discovered GWMD.

Discussion

Our strategy enables a high diagnostic yield and is a good alternative to trio WES/WGS for GWMD. It shortens the time to diagnosis compared to the classical targeted approach, thus optimizing appropriate management. Furthermore, the interactome-driven prioritization pipeline enables the discovery of novel disease-causing genes and phenotypes, and predicts novel putative candidate genes, shedding light on etiopathogenic mechanisms that are pivotal for myelin generation and maintenance.

The advent of next-generation sequencing (NGS) in clinical applications (especially targeted sequencing panels and whole-exome sequencing [WES]) has increased the diagnostic yield of hereditary neurologic diseases with high genetic heterogeneity and low mutational burden.[1-4] Genetic white matter disorders (GWMD) are a heterogeneous group of diseases with an MRI pattern suggestive of a genetic etiology, encompassing both leukodystrophies and genetic leukoencephalopathies.[5,6] The classic combined MRI, biochemical, and target gene-based approach leaves approximately half of patients with GWMD without a genetic diagnosis.[7-10] In these undiagnosed cases, trio WES followed by whole-genome sequencing (WGS) allowed a diagnosis in 62% of the cases in a recent study on a cohort of 71 pediatric patients.[4,11]

Despite continuous advances, the analysis of NGS data poses the challenge of variant selection and interpretation, which is especially relevant for singleton exomes, or when there is no possibility to perform family cosegregation/linkage studies. WES genotypes yield approximately 500–1,000 variants per individual, after filtering by frequency below 1% and deleteriousness. Hence, establishing a prioritization system based on the patient's phenotype[12,13] or gene interaction networks[14-17] may prove useful to improve rapid selection of candidate variants.

We describe 126 families with patients displaying GWMD analyzed by singleton WES–WGS (sWES-WGS). We interpret genetic data by integrating standardized phenotypic data in Human Phenotype Ontology (HPO) terms, as well as interaction and functional network information to facilitate the identification of causal genes and enable novel disease-gene discovery.

Methods

Patient Recruitment

Study participants were identified at child and adult neurology units from several tertiary hospitals around Spain from April 2017 to December 2019. They were pediatric and adult patients with clinical and MRI patterns consistent with a GWMD defined as symmetrical, confluent white matter involvement, in absence of perinatal or vascular complications or suggestive of an autoimmune process. A molecular diagnosis could not be established by the referring physicians despite applying standard-of-care paraclinical studies (including mainly MRI, metabolic, neurophysiologic, and genetic studies such as array comparative genomic hybridization [aCGH], targeted Sanger sequencing, or NGS gene panels). Clinical information, MRIs, and samples were collected by the Neurometabolic Diseases laboratory of Bellvitge Biomedical Research Institute (IDIBELL) and although strict filtering of cases by a neuroradiologist focused on leukodystrophies was not performed, re-evaluation by a team of experienced child and adult neurologists and neuroradiologists under the URD-Cat initiative for neurologic undiagnosed disorders was made before inclusion. This clinical team was driving the diagnostic process and exchanged information with the referring clinicians when required, both pre- and postvariant calling. MRI pattern was classified according to previous published articles.[18,19]

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent for genetic testing and publication was obtained by the parents or legal guardians of each patient at each site. The ethics committee of IDIBELL approved the study with CEIC PR076/14.

See Supplemental Methods for NGS, variant calling and classification, functional validation, and the interactome-driven gene prioritization method.

Data Availability

Data not provided in the article because of space limitations may be shared (anonymized) at the request of any qualified investigator for purposes of replicating procedures and results.

Results

Clinical Data

We recruited 126 families with an undiagnosed GWMD. Based on cranial MRI findings, 86 cases (68%) were classified as non-hypomyelinating, whereas 40 of the cases showed a hypomyelinating picture. The index cases included 50 female and 76 male patients, with ages ranging from 1 month to 74 years (median 10.3 years). The age at onset ranged from the first month of life to 72 years (median 1 year); age was lower than 18 years in 101 cases (80%) and higher in 25. The median evolution of disease before WES testing was 6.3 years (1 month–34 years), and it was longer than 10 years in 37% of patients. Consanguinity was reported in 18 families (14%). Clinical characteristics, MRI patterns, studies performed, and sWES-WGS results of every patient are summarized in Table 1 and eTable 1, links.lww.com/WNL/B741.

Diagnostic Yield of WES and WGS in a Cohort of Patients With GWMD

All the patients were initially studied by WES. The first diagnostic rate was 74/126 (59%), which increased to 86/126 cases (68%) after a subsequent reanalysis 12–24 months later. The reasons for this increase in yield were attributed to variants not initially identified because of filtering issues (3 cases); variants located in noncoding regions (3 cases); pipeline update/technical issues (2 cases); and newly reported disease-causing genes (3 cases). Next, we performed WGS in 16 of the remaining 38 negative cases, prioritized by availability of DNA from proband and parents, and solved 5 more cases involving intronic variants or 3′ UTR variants.

This approach allowed us to identify 9 novel candidate genes, for which we gathered additional patients with very similar phenotypes through collaboration with international Leukodystrophy Reference Centers and the platform GeneMatcher.[20] We functionally validated and reported 2 novel disease genes (*DEGS1*[21] and *PI4KA*[22]) in 2 families each, whereas the other 5 validated cases are in preparation. Two more candidate genes are awaiting additional patients while functional studies are ongoing.

Overall, we obtained a positive genetic diagnosis in 91 out of 126 GWMD cases (72%) (Figure 1, eFigure 1, and eTables 1 and 2, links.lww.com/WNL/B741). The diagnostic rates by age group were 77% in those with onset before 3 years, 73% in those with onset between 3 and 18 years, and 60% in the adult-onset group (Figure 1D). Considering the MRI pattern, the diagnostic rate was 57/86 (66%) in the non-hypomyelinating group and 34/40 (85%) in those with hypomyelination. Following the classification proposed in Vanderver et al.,[5] 46 (51%) of the diagnosed families had variants in genes associated with "canonical or classic leukodystrophies," and the remaining 45 (49%) had variants in genes associated with "genetic leukoencephalopathies."

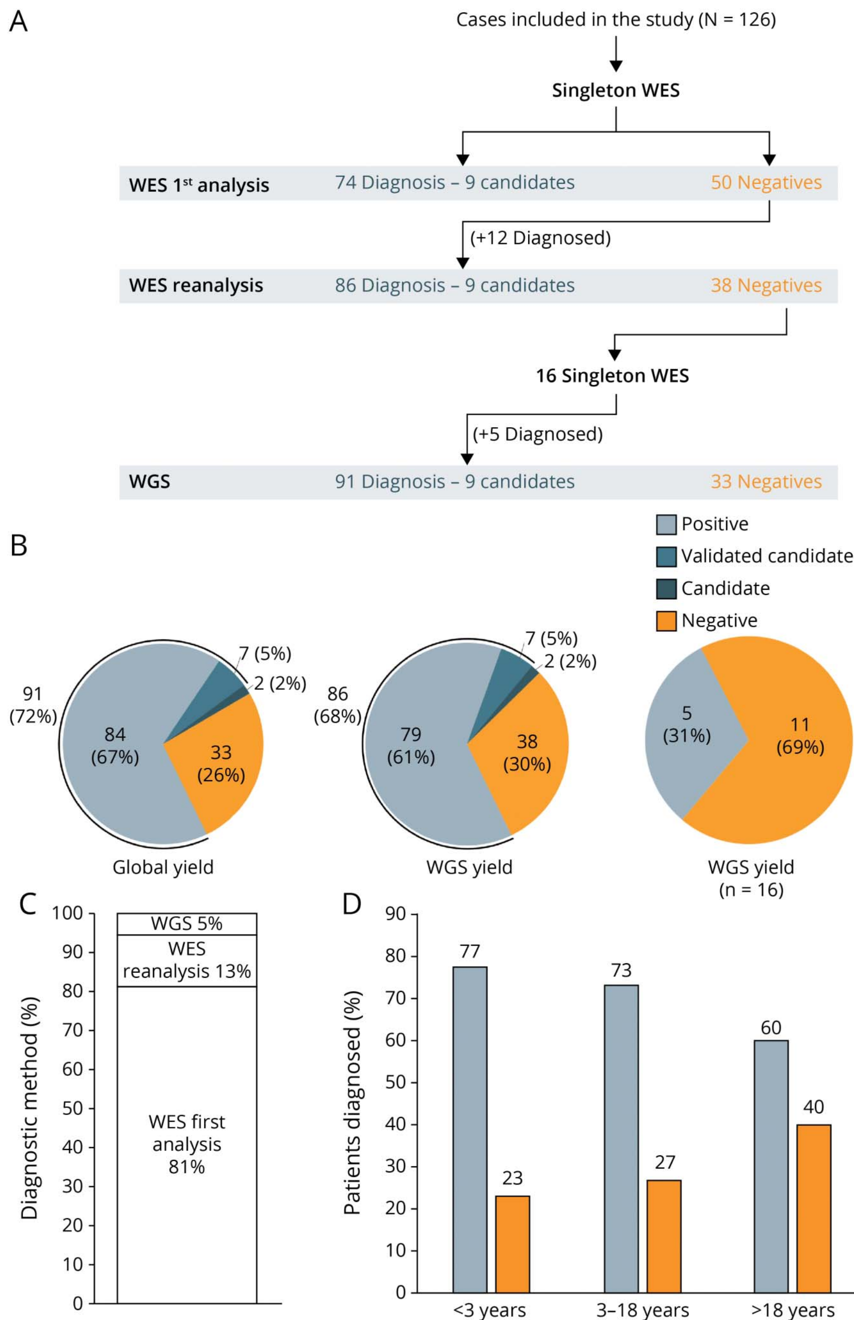
Table 1 Main Clinical Features of the 126 Index Cases

	N	%
Sex		
Female	50	40
Male	76	60
Age at onset, y		
<3	86	68
3–18	15	12
>18	25	20
Consanguinity	18	14
Main clinical features		
Motor symptoms		
Pyramidal	94	74
Extrapyramidal	34	27
Hypotonia	15	12
GDD/ID/cognitive decline	91	72
ASD/behavior/psychiatric manifestations	21	16
Cerebellar	42	33
Epilepsy	36	28
Ophthalmologic	55	43
Predominant MRI pattern		
Hypomyelination	40	31
Nonhypomyelination		
Periventricular	49	39
Diffuse	19	15
Frontal	12	9
Multifocal	3	2
Parieto-occipital	2	1
Cerebellar	2	1
Complementary examinations		
Metabolic studies	116	92
Neurophysiologic studies	99	78
Karyotype/aCGH/NGS panel	65	51
Targeted genetic studies	65	51
Total cases	126	

Abbreviations: aCGH = array comparative genomic hybridization; ASD = autism spectrum disorder; GDD = global developmental delay; ID = intellectual disability; NGS = next-generation sequencing.

For the 33 cases that remained undiagnosed after WES/WGS, we noted a trend towards adulthood onset (30% of unsolved cases were adults vs 16% of adults in solved cases), cystic lesions

Figure 1 Diagnostic Process Diagram and Diagnostic Yield



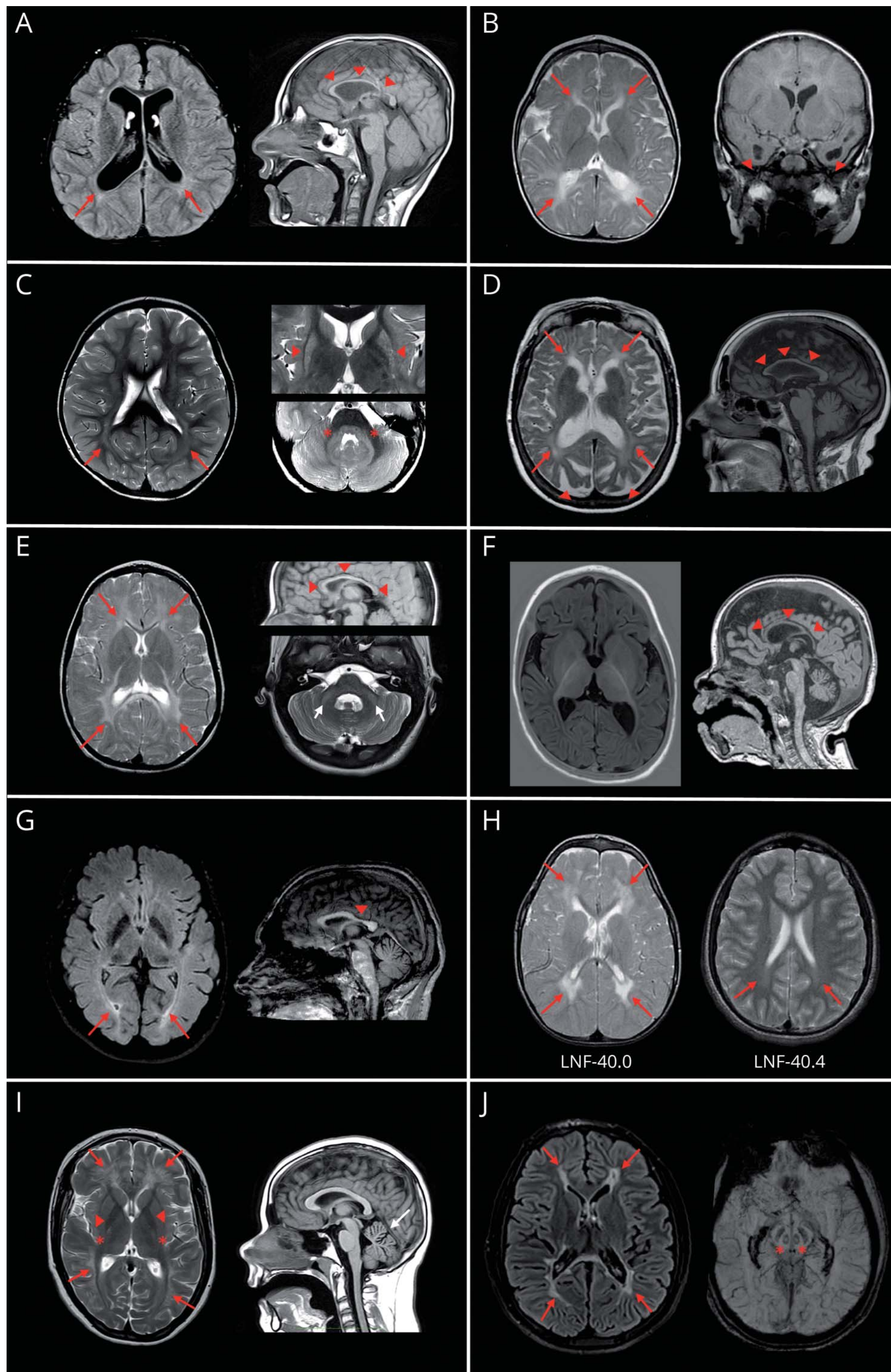
(A) Number of cases included in the study and diagnostic process. (B) Global, whole-exome sequencing (WES), and whole-genome sequencing (WGS) diagnostic yield. (C) Percentage of diagnosis in the first WES analysis, obtained by WES reanalysis and by WGS. (D) Diagnostic percentage according to age.

on MRI (12% of undiagnosed cases vs 5% in solved cases), and absence of consanguinity (97% nonconsanguineous in unsolved vs 82% in solved cases).

Although genetic heterogeneity in our cohort was very high, some genes were found to be more frequently mutated, including *EIF2B5*, *POLR3A*, and *RNASEH2B*, in 6 families each, and *PLP1* variants in 5 families (eTable 3, links.lww.com/WNL/B741). New phenotypes were identified in 2 cases, atypical forms of presentation in 7, and 6 more cases were complex, blended phenotypes with variants in more than 1 gene (see Figure 2, eTable 4, and eResults for clinical summaries). Moreover, several cases with variants in the classical spastic paraplegia genes *SPG11* and *CYP2U1* presented clear white matter involvement, as shown in Figure 3.

According to the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology guidelines,[23-25] 86 out of the 91 diagnosed cases were classified as definitively diagnosed with pathogenic or likely pathogenic variants. In 14 of these 86 cases, the functional validation converted variants of uncertain significance (VUS)

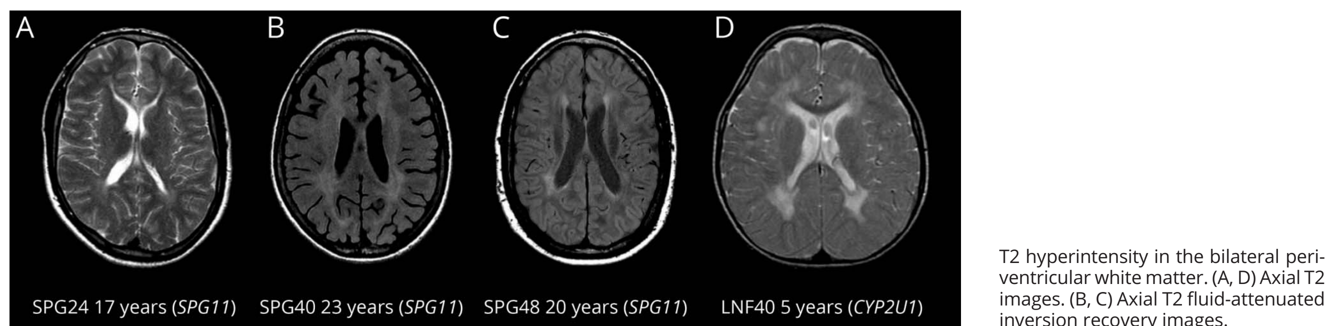
Figure 2 MRI Findings in Patients With New/Atypical and Blended Phenotypes



(A) LNF-48, 5 years. *PARS2*; p.Arg186Gly/p.Lys187Arg (COMP HTZ). Periatrial white matter (WM) hyperintensity (red arrows) with frontal-parietal atrophy, ventriculomegaly, and thin corpus callosum (arrowheads) (axial T2 fluid-attenuated inversion recovery [FLAIR], sagittal T1-weighted images). (B) LNF-29, 10 months. *PNPT1*; p.Ala507Ser (HMZ). Bilateral periatrial and temporal anterior subcortical WM hyperintensities (red arrows) with temporal cystic lesions (arrowheads) (axial T2 and coronal T2 FLAIR-weighted images). (C) LNF-47, 2 years. POLR3A; c.1771-7C > G/p.Leu1129 (COMP HTZ). Optic radiation mild WM hyperintensity (red arrows), striatal atrophy and hyperintensity (arrowheads), and superior cerebellar peduncles hyperintense signal (asterisks) (axial T2 images). (D) LNF-85, 48 years. *PSEN1*; p.Thr350Ile (HTZ). MRI showed diffuse WM hyperintensities (red arrows) with corpus callosum and cortical atrophy (arrowheads) (axial T2 and sagittal T1 FLAIR images). (E) LNF-88, 13 years. *GFPT1*; p.Asp296Val (HMZ). Axial T2 hyperintensities involving deep cerebral WM (red arrows), cerebellar peduncles (white arrows), and middle blade of corpus callosum (arrowheads), sparing subcortical WM (axial T2 and sagittal T1-weighted images). (F) LNF-114, 5 months. *SCN8A*; p.Val409Met (HTZ). Important myelination delay, thin corpus callosum and signs of cerebral and cerebellar atrophy (axial and sagittal T1-weighted images). (G) SPG-25, 44 years. *SOX10*; p.Tyr83Asp (HTZ). Periventricular WM signal abnormality, sparing U fibers (red arrows), and thin isthmus of the corpus callosum (arrowhead) (axial T2-FLAIR and sagittal T1 weighted images). (H) LNF-40.0, 13 years. *CYP2U1*; p.Arg178Thr (HMZ) and LNF-40.4, 15 years. *PAH*; p.Thr380Met (HMZ). Periventricular WM hyperintensities (red arrows) (axial T2 weighted images). (I) LNF-56, 15 years. *POLR3A*; p.Cys724Tyr/p.Pro705Ala (COMP HTZ) and *CACNA1A*; p.Tyr546Ter (HTZ). Periventricular symmetric heterogeneous WM hyperintensities (red arrows) and hypointensity in globus pallidus (arrowheads), thalamic anterolateral nuclei (asterisks), optic radiations, and pyramidal tracts, with mild atrophy of the cerebellar superior vermis (white arrow) (axial T2 and sagittal T1-weighted images). (J) LNF-89.3, 15 years. *CP*; p.Gly868GlufsTer26 (HMZ)/*NDUFS1*; p.Ser701Asn (HTZ). Periventricular symmetric T2 hyperintensity with cystic degeneration and pyramidal tract involvement (red arrows) and corpus callosum atrophy. Accumulation of paramagnetic material in the substantia nigra (asterisks) (axial T2-FLAIR and axial susceptibility-weighted imaging).

into pathogenic or likely pathogenic variants. We analyzed the effect of 8 variants on splicing using cDNA sequencing (from RNA derived from peripheral blood mononuclear cells [PBMC] or fibroblasts) or minigene splicing assay[26] (n = 3). The minigene assays were instrumental to confirm the pathogenic role of an intronic variant in *MLC1* (c.597 + 37C > G), a gene not expressed in PBMC or fibroblasts, and another intronic variant in EIF2B5 (c.1156 + 13 G > A), which led to a mild form of ovarioleukodystrophy.[26] We also performed targeted lipidomics, which proved a pathogenic role for variants in genes related to lipid metabolism such as *ACER3, DEGS1*,[21] and *PI4KA*,[22] together with qRT-PCR, Western blots, or immunofluorescence as required (eTable 5, links.lww.com/WNL/B741). In other cases that were not amenable to experimental

Figure 3 MRI of Selected Cases With Variants in Hereditary Spastic Paraparesis Genes and White Matter Involvement



T2 hyperintensity in the bilateral periventricular white matter. (A, D) Axial T2 images. (B, C) Axial T2 fluid-attenuated inversion recovery images.

validation (5 remaining until 91), we reported out VUS highly compatible with the clinical and MRI picture and segregation and were considered solved by expert assessment.

Among the 91 cases diagnosed, 60 harbored biallelic mutations (31 homozygous; 16 of them in consanguineous families), 22 in an autosomal dominant mode (12 de novo), and 7 X-linked (5 of them de novo), whereas 2 cases had mutations in more than 1 gene with different inheritance patterns (1 with autosomal dominant and autosomal recessive inheritance; 1 autosomal dominant and X-linked) (eFigure 1, links.lww.com/WNL/B741). Segregation by Sanger was performed in all but 8 patients due to the unavailability of parental samples. We found several variants more than once in our patients: the *RNASEH2B* p.Ala177Thr variant in 6 independent families (frequency: 0.001306 in gnomAD [v2.1.1])[27]; the *EIF2B5* p.Leu106Phe variant (frequency: 0.00004943 in gnomAD [v2.1.1]) in 2 independent families and the *EIF2B5* p.Arg113His variant (frequency: 0.00001647 in gnomAD [v2.1.1])[28] in 5 families; and the *SPG11* frameshift variant p.Met245Valfs*2 twice independently (frequency: 0.0001071 in gnomAD [v2.1.1]).[29]

In addition to single-nucleotide variants and indels, we detected a pathogenic copy number variant (CNV) in 4 cases (4.4%) by WES (eTable 6, links.lww.com/WNL/B741): a 6930 Kb 1p36 heterozygous deletion in LNF-36 and validated by aCGH (eFigure 2), a 117 Kb duplication in 5q including *HNRNPH1* and *RUFY1* genes in LNF-105,[30] a 60.4 Kb duplication containing *LMNB1* in LNF-34, and a 21.3 Kb homozygous deletion encompassing *TANG O 2* in LNF-97.[31] We validated the last 3 CNVs by Q-PCR (eTable 6). We also identified a uniparental disomy of maternal origin of chromosome 6 in LNF-68, harboring a loss-of-function homozygous variant in a novel candidate gene that was highly ranked by our prioritization method (in preparation).

An added value of our study is that 73 of the 123 identified variants had not been previously reported in the literature, Human Gene Mutation Database (public access), or ClinVar databases (eTable 7, links.lww.com/WNL/B741).

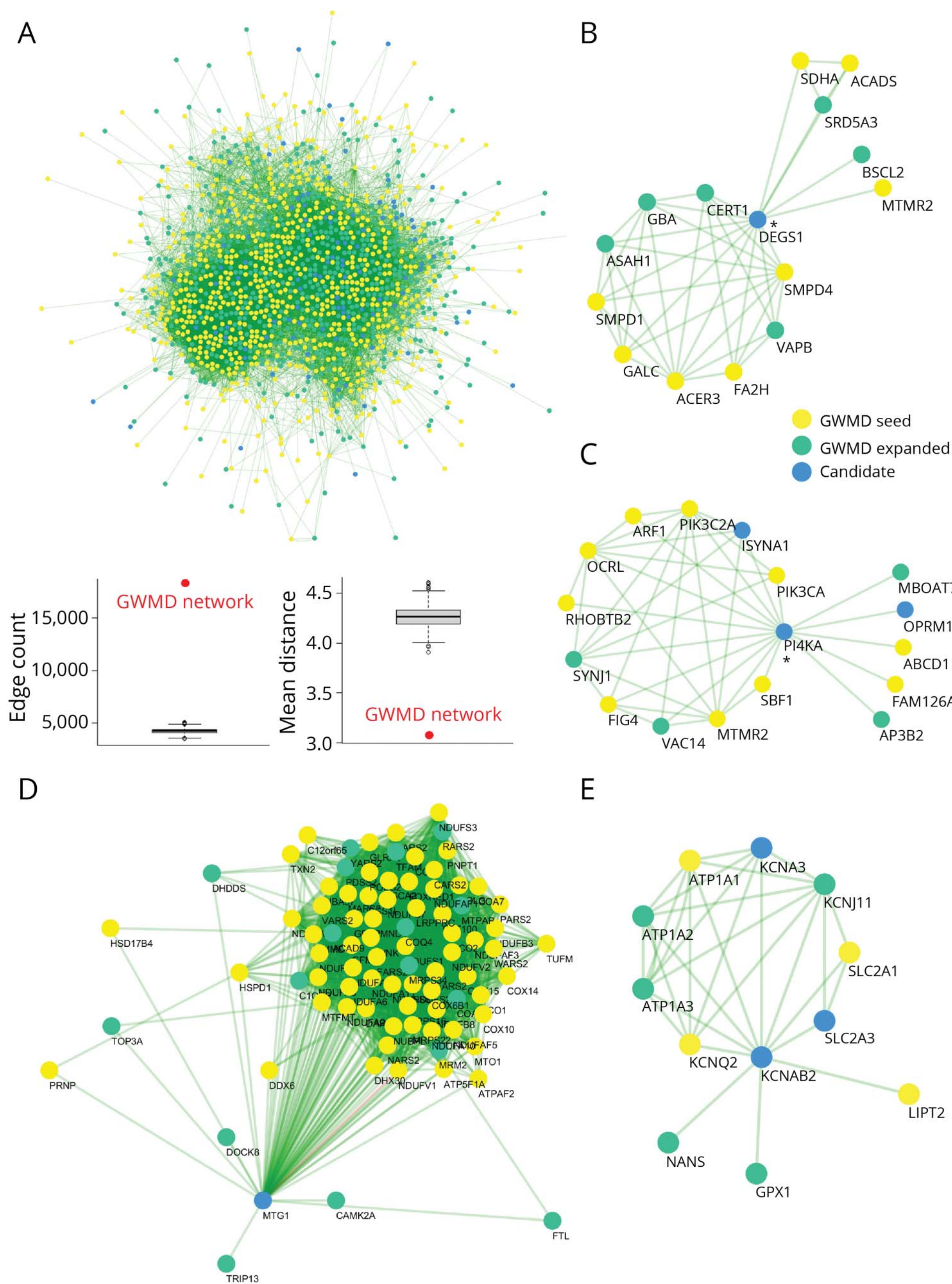
Management Implications of a Positive Diagnosis

Diagnosis allowed us to improve clinical management in 29 cases (eTable 1, links.lww.com/WNL/B741). In 22 of them, it led to the consideration of a specific treatment option for the disease, such as hematopoietic stem cell transplant for Krabbe disease (LNF-18, SPG-72) and hereditary diffuse leukoencephalopathy with spheroids (LNF-6, LNF-16, LNF-70, LMSR), dietary management for phenylketonuria (LNF-40.4), or pyridostigmine for myasthenic syndrome caused by *GFPT1* (LNF-88). In other cases, diagnosis led to an improvement in patient follow-up, such as screening for the appearance of tumors in *PTEN* (LNF-109) or preventative measures for head trauma and infections in patients with vanishing white matter disease. Finally, we identified and reported incidental findings (according to Kalia et al.[32]) in 2 patients: a pathogenic variant in the *MYBPC3* gene (p.Trp792ValfsTer41) in SPG-14 and in *SMAD3* (Loeys-Dietz syndrome) (p.Val363ThrfsTer3) in LNF-48. In both cases, cardiologic follow-up will ensue, with cranial magnetic resonance angiography and orthopedic controls in the second case.

GWMD Expanded Network

Starting with a seed list of 843 genes that are causative or associated with GWMD according to OMIM, we built a protein interactome network based on the principle that physical and functional interacting genes may account for related biological processes and cause similar diseases. We developed a prioritization method that identifies the most likely disease-causing genes associated with each patient's phenotype (standardized in HPO[33] terms) using a global protein human interactome network built with functional and physical interactions, represented by 20.146 genes (see Supplemental Methods and Results [eTables 8–11, links.lww.com/WNL/B741]).[14] We applied this prioritization tool to the respective clinical description in HPOs of the 843 proteins associated with GWMD to build a GWMD interactome or expanded network, resulting in 1,530 proteins and 18,288 interactions (Figure 4). To evaluate the functional signature of these 1,530 proteins, we performed an enrichment analysis

Figure 4 GWMD Expanded Interactome



(A) The genetic white matter disorder (GWMD) seeds + expanded network was generated by the network prioritization tool, resulting in 1,530 proteins. The seed genes known to be mutated in GWMD are shown in yellow circles, disease genes not previously associated with GWMD are shown in green, and new GWMD candidates are shown in blue. Comparison of statistical connectivity strength of the GWMD expanded network with 1,000 permutations of randomly selected proteins from the global human network. Red dots denote the value of the metric on the GWMD expanded network constituted by 1,530 proteins. Box and whisker plots denote matched null distributions (i.e., 1,000 permutations). (D, left) Within-group edge count (i.e., number of edges between members of the query set). (D, right) distance is the average path length in the network obtained by calculating the shortest paths between all pairs of proteins. (B–E) Zoom in the network for specific putative candidates as illustrative example of the GWMD expanded network potentiality. (B) Delta 4-desaturase, sphingolipid 1 (*DEGS1*); (C) phosphatidylinositol 4-kinase alpha (*PI4KA*); (D) mitochondrial ribosome-associated GTPase 1 (*MTG1*); and (E) potassium voltage-gated channel subfamily A regulatory beta subunit 2 (*KCNAB2*) protein. *Recently associated with leukodystrophy. White matter expanded network available in NDEx repository at public.ndexbio.org/#/network/fd5fc166-9ecc-11eb-9e72-0ac135e8bacf?accesskey=a75ac048b59aca2c9310c04a6f1d96ea34052231d9204f284c5e1d420fc2ca26

of the Gene Ontology (GO) terms (eTables 8–10). In line with the hypothesis that genes associated with similar diseases may converge towards specific biological pathways, major modules emerged, which are involved in the pathophysiology of GWMD abnormalities: (1) the mitochondrial oxidative phosphorylation (OXPHOS) system (e.g., NADH-ubiquinone oxidoreductase Fe-S protein 1; *NDUFS1*), (2) the lysosome (e.g., the galactosylceramidase enzyme; *GALC*), (3) the peroxisome (peroxins) (e.g., peroxin 6; *PEX6*), (4) the metabolism of ribonucleotides (e.g., ribonuclease H2 subunit B; *RNASEH2B*), and (5) the purine metabolism pathway with RNA polymerases I and III (e.g., RNA polymerase III subunit A; *POLR3A*). Among the 1,530 proteins, we identified (besides the 843 GWMD seed proteins) (1) 587 proteins associated with disease but not yet with GWMD and (2) 100 novel candidates that were not previously associated with GWMD or any disease (eTable 11). Of particular interest among these last 100 proteins, we highlight (1) the delta 4-desaturase sphingolipid 1 (DEGS1) in patients with LNF-41 and LNF-42 (Figure 4B), causing hypomyelinating leukodystrophy 18 (HLD18, OMIM #615843),[21] (2) the phosphatidylinositol 4-kinase alpha (PI4KA) recently associated

with leukodystrophy and identified in patients LNF-107 and VH-3[22,34] (Figure 4C), (3) the mitochondrial ribosome-associated GTPase 1 (*MTG1*) that plays a role in the regulation of mitochondrial ribosome assembly and translational activity (Figure 4D), and (4) the potassium voltage-gated channel subfamily A regulatory beta subunit 2 protein (*KCNAB2*) (Figure 4E). While Genematcher was key to find additional cases for *DEGS1* and *PI4KA* deficiencies, matches for putative candidates such as *MTG1* and *KCNAB2* are yet to be found.

Discussion

This is the largest series of patients of GWMD studied by WES/WGS reported to date and the first one including patients of all ages offering a global vision of the GWMD diagnosis throughout life. We have proven the utility of sWES-WGS combined with a phenotypic and interactome-driven prioritization method, reaching a diagnostic yield of 72%. These results are superior to those recently reported by a reference genetic diagnostics company on 541 cases, with a WES diagnostic yield for leukodystrophies of 32% (including trio and singleton cases) and 22.6% when considering proband-only cases.[35] In another report including 100 patients with adult-onset leukodystrophy, the diagnostic rate was 26%.[36] Our results are slightly better than those reported in another study including 71 pediatric cases.[4,11] In a report by Vanderver et al.,[4] a first trio WES allowed a definite diagnosis in 42% of cases, while in a second phase of the study[11] including the 41 negative cases, a molecular diagnosis was established in 9 more cases by reanalysis and in 5 cases using WGS, representing 17% and 12%, respectively. We were able to increase diagnostic yield 24% (12/50) by WES reanalysis and 31% (5/16) by singleton WGS. However, in the referred study, previous expert filtering of cases led to a lower proportion of well-known or canonical leukodystrophy genes in their cohort[4] in comparison to ours (36% vs 51% in our cohort), which may have an effect on our higher diagnostic yield. Comparison between the results of these cohorts is difficult because of different study protocols and target population, which comprised 20% adult GWMD in our case vs a pediatric-only population in Vanderver et al.[4] It is likely that the use of trio WES/WGS would have improved our diagnostic yields, and certainly would have ameliorated turnaround times. Because of the very late implantation of clinical exomes (instead of WES) in our health care system and limited research funding resources, we chose to apply singleton WES to help as many families as possible, as trio studies may cost double[37,38] to 3 times higher in our health care system. The use of trio WES/WGS is recommended when urgent diagnosis is required in intensive care unit settings.[39] Thus, the decision to use a singleton or trio sequencing strategy should depend on the clinical urgency, the entities under study that determine the proportion of dominant de novo expected inheritances, the family characteristics and availability of DNA, and funding or structural resources needed to optimize the cost–benefit ratio in every setting.[37]

Our study enabled identification of disorders caused by genes rarely associated with white matter involvement (*PTEN*, *GFPT1*,[40] *CAPN1*[41]), the diagnosis of certain cases with atypical presentation (*SCN8A*,[42] *SOX10*,[43] *POLR3A*[44]), the characterization of families harboring variants in more than 1 causative gene with blended phenotypes, the identification of genes only recently associated with disease (i.e., *PYCR2*[45] or *TMEM63A*[46]), and the discovery of novel disease entities and candidate genes, which constitute important advantages over disease-specific panels or clinical exomes (see Figure 2, eTables 1 and 4, and eResults for clinical summaries, links.lww.com/WNL/B741). Furthermore, in 9 families (10%), we identified variants in genes associated primarily with hereditary spastic paraplegia (*SPG11*, *SPG7*, *SPAST*, *DDHD2*, *CAPN1*, *CYP2U1*) (Figure 3), underscoring the notion of a continuum of clinical spectrum, similarly to X-adrenoleukodystrophy, PMD/SPG2, metachromatic dystrophy, or Alexander disease.[47] Moreover, half of the genes identified in this cohort are linked to genetic leukoencephalopathies, not classically considered leukodystrophy genes. Because many of these genes are not included in multigene panels, the WES/WGS-derived diagnostic yield would be expected to be superior. As an example, the diagnostic yield of a leukodystrophies disease gene panel containing 134 genes was 46% in a recent study.[48]

Our report also exemplifies the genetic heterogeneity of GWMD (57 different genes among the 91 diagnosed cases), which supports that WES/WGS should be considered a first-tier diagnostic test when the clinical presentation and MRI pattern do not point to a specific diagnosis, in agreement with the recent randomized clinical trial on pediatric patients with GWMD.[6] This would allow for gaining time, which is fundamental to establish appropriate genetic counseling and specific treatment when available, usually indicated only in the early stages of these very severe diseases. On average, our patients reached a positive diagnosis at 6 months after study inclusion, which stood in sharp contrast with the previous diagnostic delay of 10 years of disease evolution on average. Hence, reducing multiple unnecessary examinations with a low cost–benefit ratio, as is the case for some metabolic studies in the context of nonspecific neuroimaging, would entail substantial economic savings for the health care system, which together with the continued lowering of WES/WGS costs makes a clear case for the adoption of at least WES if not WGS as a first-tier test for undiagnosed GWMD. However, first-line metabolic tests that may identify potentially treatable cases should always be considered, prior to or in parallel with WES/WGS.

Our study protocol has certain limitations. Paraclinical studies preceding inclusion are heterogeneous and depend on the availability of resources in the different participating centers. In addition, we reported as diagnosed 5 cases harboring VUS using technically strict ACMG criteria, as these variants could

not be functionally validated. However, these cases with VUS were carefully reviewed by expert clinicians and considered to explain the phenotypic presentation with very high probability, and were thus considered solved by expert assessment. Finally, WGS studies were prioritized in only 16 of the remaining 38 negative cases (42%) because of limited DNA availability of parents to perform segregation and funding resources.

We provide evidence of the effectiveness of sWES-WGS analysis based on a phenotype- and interactome-driven prioritization algorithm to diagnose GWMD and to identify new phenotypes and novel disease genes. We also provide a white matter expanded interactome composed of known and putative new GWMD genes with the potential to aid in the validation of private mutations in genes found in single families and the identification of novel candidate genes. The utilization of advanced computational methods together with the integration of a functional genomics laboratory capable of experimental validation of VUS and candidate genes together with the direct implication of adult and pediatric neurologists in the process are determining factors for this high diagnostic yield.

Acknowledgment

The authors thank the patients and families for their collaboration and the European Leukodystrophy Association (ELA-Spain) and the CERCA Program/Generalitat de Catalunya for support.

Study Funding

URDCat program (PERIS SLT002/16/00174) from the Autonomous Government of Catalonia, Centre for Biomedical Research on Rare Diseases (CIBERER, ACCI19-759), The Hesperia Foundation (Royal House of Spain), and CNAG's call "300 exomes to elucidate rare diseases" (A.P.); the Instituto de Salud Carlos III and "Fondo Europeo de Desarrollo Regional (FEDER), Unión Europea, una manera de hacer Europa" (FIS PI20/00758) (C.C.); "La Marató de TV3" Foundation (202006-30) (A.P., C.C.); AWS Cloud Credits for Research program (A.S.); Instituto de Salud Carlos III through the program Miguel Servet (CPII16/00016) (S.F.); Sara Borrell (CD19/00221) (E.Verdura); Rio Hortega, CM18/00145, co-funded by the European Social Fund (V.V.-S.); Center for Biomedical Research on Rare Diseases, an initiative of the Instituto de Salud Carlos III (M.R.); and European Reference Network for Rare Neurologic Diseases: Project ID 739510 (A.M., M.d.T.).

Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

Publication History

Received by *Neurology* May 1, 2021. Accepted in final form December 21, 2021.

Appendix 1 Authors

Name	Location	Contribution
Agatha Schlüter, PhD	Neurometabolic Diseases Laboratory, Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Barcelona, Spain; Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
Agustí Rodríguez-Palmero, MD	Neurometabolic Diseases Laboratory, Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Barcelona, Spain; Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain; Pediatric Neurology Unit, Department of Pediatrics. Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Spain	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
Edgard Verdura, PhD	Neurometabolic Diseases Laboratory, Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Barcelona, Spain; Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
Valentina Vélez-Santamaría, MD	Neurometabolic Diseases Laboratory, Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Barcelona, Spain; Neuromuscular Unit, Neurology Department, Hospital Universitari de Bellvitge, Universitat de Barcelona, Hospitalet de Llobregat, Spain	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data
Montserrat Ruiz, PhD	Neurometabolic Diseases Laboratory, Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Barcelona, Spain; Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Stéphane Fourcade, PhD	Neurometabolic Diseases Laboratory, Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Barcelona, Spain; Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain	Drafting/revision of the manuscript for content, including medical writing for content
Laura Planas-Serra, MSc	Neurometabolic Diseases Laboratory, Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Barcelona, Spain; Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain	Major role in the acquisition of data

Appendix 1 *(continued)*

Name	Location	Contribution
Juan José Martínez, MSc	Neurometabolic Diseases Laboratory, Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Barcelona, Spain; Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain	Major role in the acquisition of data
Cristina Guilera, MSc	Neurometabolic Diseases Laboratory, Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Barcelona, Spain; Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain	Major role in the acquisition of data
Marisa Girós, PhD	Secció d'Errors Congènits del Metabolisme-IBC, Servei de Bioquímica i Genètica Molecular, Hospital Clínic, IDIBAPS, CIBERER, Barcelona, Spain	Major role in the acquisition of data
Rafael Artuch, MD	Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain; Institut de Recerca Pediàtrica-Hospital Sant Joan de Déu (IRP-HSJD), Barcelona, Spain	Major role in the acquisition of data
María Eugenia Yoldi, MD	Pediatric Neurology Unit, Department of Pediatrics, Navarra Health Service, Navarrabiomed Research Foundation, Pamplona, Spain	Major role in the acquisition of data
Mar O'Callaghan, MD, PhD	Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain; Institut de Recerca Pediàtrica-Hospital Sant Joan de Déu (IRP-HSJD), Barcelona, Spain	Major role in the acquisition of data
Angels García-Cazorla, MD, PhD	Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain; Institut de Recerca Pediàtrica-Hospital Sant Joan de Déu (IRP-HSJD), Barcelona, Spain	Major role in the acquisition of data; Analysis or interpretation of data
Judith Armstrong, PhD	Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain; Molecular and Genetics Medicine Section, Hospital Sant Joan de Déu, Barcelona, Spain	Major role in the acquisition of data
Itxaso Marti, MD, PhD	Department of Neuropediatrics, Hospital Universitario Donostia, San Sebastián, Spain; Biodonostia Health Research Institute (Biodonostia HRI), San Sebastián, Spain; University of the Basque Country (UPV-EHU), San Sebastian, Spain; Centro de Investigación Biomédica en Red para Enfermedades Neurodegenerativas (CIBERNED), Carlos III Health Institute, Madrid, Spain	Major role in the acquisition of data

Appendix 1 *(continued)*

Name	Location	Contribution
Elisabet Mondragón Rezola, MD, PhD	Biodonostia Health Research Institute (Biodonostia HRI), San Sebastián, Spain; Centro de Investigación Biomédica en Red para Enfermedades Neurodegenerativas (CIBERNED), Carlos III Health Institute, Madrid, Spain; Department of Neurology, Hospital Universitario Donostia, San Sebastián, Spain	Major role in the acquisition of data
Claire Redin, PhD	Département de Médecine translationnelle et Neurogénétique, IGBMC, CNRS UMR 7104/INSERM U964/Université de Strasbourg, Illkirch, France	Major role in the acquisition of data; Analysis or interpretation of data
Jean Louis Mandel, MD, PhD	Département de Médecine translationnelle et Neurogénétique, IGBMC, CNRS UMR 7104/INSERM U964/Université de Strasbourg, Illkirch, France; Laboratoire de Diagnostic Génétique, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; Chaire de Génétique Humaine, Collège de France, Illkirch, France	Major role in the acquisition of data; Analysis or interpretation of data
David Conejo, MD	Complejo asistencial universitario de Burgos, Burgos, Spain	Major role in the acquisition of data
Concepción Sierra-Córcoles, MD	Department of Paediatric Neurology, Complejo Hospitalario Jaén, Jaén, Spain	Major role in the acquisition of data
Sergi Beltran, PhD	CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain	Major role in the acquisition of data; Analysis or interpretation of data
Marta Gut, PhD	CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain	Major role in the acquisition of data
Elida Vázquez, MD	Department of Pediatric Radiology, Hospital Materno-Infantil Vall d'Hebrón, Barcelona, Spain	Major role in the acquisition of data
Mireia del Toro, MD, PhD	Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain; Pediatric Neurology Department, Vall d'Hebron University Hospital, Universitat Autónoma de Barcelona, Spain	Major role in the acquisition of data
Mónica Troncoso, MD, PhD	Pediatric Neurology, Hospital Clínico San Borja Arriarán, Central Campus Universidad de Chile, Chile	Major role in the acquisition of data

Continued

Appendix 1 *(continued)*

Name	Location	Contribution
Luis A. Pérez-Jurado, MD, PhD	Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain; Genetics Service, Hospital del Mar Research Institute (IMIM), Barcelona, Spain; Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain	Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data
Luis G. Gutiérrez-Solana, MD, PhD	Department of Paediatric Neurology, Children's University Hospital Niño Jesús, Madrid, Spain	Major role in the acquisition of data; Analysis or interpretation of data
Adolfo López de Munain, MD, PhD	Biodonostia Health Research Institute (Biodonostia HRI), San Sebastián, Spain; University of the Basque Country (UPV-EHU), San Sebastian, Spain; Centro de Investigación Biomédica en Red para Enfermedades Neurodegenerativas (CIBERNED), Carlos III Health Institute, Madrid, Spain; Department of Neurology, Hospital Universitario Donostia, San Sebastián, Spain	Major role in the acquisition of data; Analysis or interpretation of data
Carlos Casasnovas, MD, PhD	Neurometabolic Diseases Laboratory, Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Barcelona, Spain; Neuromuscular Unit, Neurology Department, Hospital Universitari de Bellvitge, Universitat de Barcelona, Hospitalet de Llobregat, Spain	Major role in the acquisition of data; Analysis or interpretation of data
Sergio Aguilera-Albesa, MD, PhD	Pediatric Neurology Unit, Department of Pediatrics, Navarra Health Service, Navarrabiomed Research Foundation, Pamplona, Spain	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data
Alfons Macaya, MD, PhD	Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain; Pediatric Neurology Department, Vall d'Hebron University Hospital, Universitat Autónoma de Barcelona, Spain; Pediatric Neurology Research Group, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
Aurora Pujol, MD, PhD	Neurometabolic Diseases Laboratory, Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Barcelona, Spain; Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain; Catalan Institution of Research and Advanced Studies (ICREA), Barcelona, Catalonia, Spain	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

Appendix 2 Coinvestigators

Name	Location	Contribution
Hugo A. Arroyo, MD	H. Garrahan, Argentina	Major role in the acquisition of data
Andrés Barrios, MD	H. San Borja Arriarán, Chile	Major role in the acquisition of data
Andrea Campo, MD	H. Virgen Macarena, Sevilla, Spain	Major role in the acquisition of data
Tamara Castillo, MD	H. Donostia, Spain	Major role in the acquisition of data
Rosario Cazorla, MD	H. Puerta de Hierro, Madrid, Spain	Major role in the acquisition of data
María Asunción García, MD	H. Alcorcón, Madrid, Spain	Major role in the acquisition of data
Ainhoa García, MD	H. Cruces, Bilbao, Spain	Major role in the acquisition of data
Antonio Hedrera, MD	H. Central de Asturias, Oviedo, Spain	Major role in the acquisition of data
Juan Hernández, MD	H. Universitario de Guadalajara, Spain	Major role in the acquisition of data
Nathalie Launay, MD	IDIBELL, Barcelona, Spain	Major role in the acquisition of data
Maria Lorenzo, MD	H. Infanta Cristina, Madrid, Spain	Major role in the acquisition of data
Concepción Miranda, MD	H. Gregorio Marañón, Madrid, Spain	Major role in the acquisition of data
Fermín Moreno, MD	H. Donostia, Spain	Major role in the acquisition of data
Amaia Muñoz, MD	H. Donostia, Donostia, Spain	Major role in the acquisition of data
Juan Narbona, MD	Clínica U. Navarra, Pamplona, Spain	Major role in the acquisition of data
Mª Socorro Pérez, MD	H. Marqués de Valdecilla, Santander, Spain	Major role in the acquisition of data
Maria Antonia Ramos, MD	H. Virgen del Camino, Pamplona, Spain	Major role in the acquisition of data
Miquel Raspall-Chaure, MD	H. Vall d'Hebron, Barcelona, Spain	Major role in the acquisition of data
Manel Roig-Quilis, MD	H. Vall d'Hebron, Barcelona, Spain	Major role in the acquisition of data
Miguel Ángel Urtasun, MD	H. Donostia, Spain	Major role in the acquisition of data
María Esther Vázquez, MD	H. Lucus Augusti, Lugo, Spain	Major role in the acquisition of data
Juan Francisco Vázquez, MD	H. La Fe, Valencia, Spain	Major role in the acquisition of data

References

1. Fogel BL, Lee H, Deignan JL, et al. Exome sequencing in the clinical diagnosis of sporadic or familial cerebellar ataxia. *JAMA Neurol.* 2014;71(10):1237-1246.
2. Gonzaga-Jauregui C, Harel T, Gambin T, et al. Exome sequence analysis suggests that genetic burden contributes to phenotypic variability and complex neuropathy. *Cell Rep.* 2015;12(7):1169-1183.
3. van de Warrenburg BP, Schouten MI, de Bot ST, et al. Clinical exome sequencing for cerebellar ataxia and spastic paraplegia uncovers novel gene-disease associations and unanticipated rare disorders. *Eur J Hum Genet.* 2016;24(10):1460-1466.

4. Vanderver A, Simons C, Helman G, et al. Whole exome sequencing in patients with white matter abnormalities. *Ann Neurol.* 2016;79(6):1031-1037.
5. Vanderver A, Prust M, Tonduti D, et al. Case definition and classification of leukodystrophies and leukoencephalopathies. *Mol Genet Metab.* 2015;114(4):494-500.
6. 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.
7. van der Knaap MS, Breiter SN, Naidu S, Hart AA, Valk J. Defining and categorizing leukoencephalopathies of unknown origin: MR imaging approach. *Radiology.* 1999; 213(1):121-133.
8. Köhler W, Curiel J, Vanderver A. Adulthood leukodystrophies. *Nat Rev Neurol.* 2018; 14(2):94-105.
9. van der Knaap MS, Schiffmann R, Mochel F, Wolf NI. Diagnosis, prognosis, and treatment of leukodystrophies. *Lancet Neurol.* 2019;4422(19):962-972.
10. 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.
11. Helman G, Lajoie BR, Crawford J, et al. Genome sequencing in persistently unsolved white matter disorders. *Ann Clin Transl Neurol.* 2020;7(1):144-152.
12. Köhler S, Vasilevsky NA, Engelstad M, et al. The human phenotype ontology in 2017. *Nucleic Acids Res.* 2017;45(D1):D865–D876.
13. Boudellioua I, Kulmanov M, Schofield PN, Gkoutos GV, Hoehndorf R. DeepPVP: phenotype-based prioritization of causative variants using deep learning. *BMC Bioinformatics.* 2019;20(1):65.
14. Novarino G, Fenstermaker AG, Zaki MS, et al. Exome sequencing links corticospinal motor neuron disease to common neurodegenerative disorders. *Science.* 2014; 343(6170):506-511.
15. Cornish AJ, David A, Sternberg MJE. PhenoRank: reducing study bias in gene prioritization through simulation. *Bioinformatics.* 2018;34(12):2087-2095.
16. Vanunu O, Magger O, Ruppin E, Shlomi T, Sharan R. Associating genes and protein complexes with disease via network propagation. *PLoS Comput Biol.* 2010;6(1):e1000641.
17. Yang H, Robinson PN, Wang K. Phenolyzer: phenotype-based prioritization of candidate genes for human diseases. *Nat Methods.* 2015;12(9):841-843.
18. Schiffmann R. An MRI-based approach to the diagnosis of white matter disorders. *Neurology.* 2009;72:750-759.
19. 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.
20. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. *Hum Mutat.* 2015;36(10):928-930.
21. Pant DC, Dorboz I, Schluter A, et al. Loss of the sphingolipid desaturase DEGS1 causes hypomyelinating leukodystrophy. *J Clin Invest.* 2019;129(3):1240-1256.
22. Verdura E, Rodríguez-Palmero A, Vélez-Santamaria V, et al. Biallelic PI4KA variants cause a novel neurodevelopmental syndrome with hypomyelinating leukodystrophy. *Brain.* 2021;144(9):2659-2669.
23. 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.
24. Amendola LM, Jarvik GP, Leo MC, et al. Performance of ACMG-AMP variant-interpretation guidelines among nine laboratories in the clinical sequencing exploratory research consortium. *Am J Hum Genet.* 2016;98(6):1067-1076.
25. Brandt T, Sack LM, Arjona D, et al. Adapting ACMG/AMP sequence variant classification guidelines for single-gene copy number variants. *Genet Med.* 2020;22(2):336-344.
26. Rodríguez-Palmero A, Schlüter A, Verdura E, et al. A novel hypomorphic splice variant in EIF2B5 gene is associated with mild ovarioleukodystrophy. *Ann Clin Transl Neurol.* 2020;7(9):1574-1579.
27. Crow YJ, Leitch A, Hayward BE, et al. Mutations in genes encoding ribonuclease H2 subunits cause Aicardi-Goutières syndrome and mimic congenital viral brain infection. *Nat Genet.* 2006;38(8):910-916.
28. Turón-Viñas E, Pineda M, Cusí V, et al. Vanishing white matter disease in a Spanish population. *J Cent Nerv Syst Dis.* 2014;6:59-68.
29. Stevanin G, Azzedine H, Denora P, et al. Mutations in SPG11 are frequent in autosomal recessive spastic paraplegia with thin corpus callosum, cognitive decline and lower motor neuron degeneration. *Brain.* 2008;131(pt 3):772-784.
30. Reichert SC, Li R, Turner S, et al. HNRNPH1-related syndromic intellectual disability: seven additional cases suggestive of a distinct syndromic neurodevelopmental syndrome. *Clin Genet.* 2020;98(1):91-98.
31. Mingirulli N, Pyle A, Hathazi D, et al. Clinical presentation and proteomic signature of patients with TANGO2 mutations. *J Inherit Metab Dis.* 2020;43(2):297-308.
32. Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med.* 2017;19(2):249-255.
33. Köhler S, Doelken SC, Mungall CJ, et al. The Human Phenotype Ontology project: linking molecular biology and disease through phenotype data. *Nucleic Acids Res.* 2014;42(Database issue):D966-D974.
34. Salter CG, Cai Y, Lo B, et al. Biallelic PI4KA variants cause neurological, intestinal and immunological disease. *Brain.* Epub 2021 awab313.
35. Zou F, Zuck T, Pickersgill CD, et al. A comprehensive and dynamic approach for genetic testing for patient with leukodystrophy demonstrates a genetic etiology in 33% of cases (P4.6-054). *Neurology.* 2019;92(15 suppl):P46-P54.
36. Lynch DS, Rodrigues Brandão de Paiva A, Zhang WJ, et al. Clinical and genetic characterization of leukoencephalopathies in adults. *Brain.* 2017;140(5): 1204-1211.
37. Tan TY, Lunke S, Chong B, et al. A head-to-head evaluation of the diagnostic efficacy and costs of trio versus singleton exome sequencing analysis. *Eur J Hum Genet.* 2019; 27(12):1791-1799.
38. Richards J, Korgenski EK, Taft RJ, Vanderver A, Bonkowsky JL. Targeted leukodystrophy diagnosis based on charges and yields for testing. *Am J Med Genet A.* 2015; 167A(11):2541-2543.
39. Kingsmore SF, Cakici JA, Clark MM, et al. A randomized, controlled trial of the analytic and diagnostic performance of singleton and trio, rapid genome and exome sequencing in ill infants. *Am J Hum Genet.* 2019;105(4):719-733.
40. Senderek J, Müller JS, Dusl M, et al. Hexosamine biosynthetic pathway mutations cause neuromuscular transmission defect. *Am J Hum Genet.* 2011;88(2):162-172.
41. Gan-Or Z, Bouslam N, Birouk N, et al. Mutations in CAPN1 cause autosomal-recessive hereditary spastic paraplegia. *Am J Hum Genet.* 2016;98(5):1038-1046.
42. Gardella E, Møller RS. Phenotypic and genetic spectrum of SCN8A-related disorders, treatment options, and outcomes. *Epilepsia.* 2019;60(suppl 3):S77–S85.
43. Bondurand N, Dastot-Le Moal F, Stanchina L, et al. Deletions at the SOX10 gene locus cause Waardenburg syndrome types 2 and 4. *Am J Hum Genet.* 2007;81(6): 1169-1185.
44. Harting I, Al-Saady M, Krägeloh-Mann I, et al. POLR3A variants with striatal involvement and extrapyramidal movement disorder. *Neurogenetics.* 2020;21(2): 121-133.
45. Nakayama T, Al-Maawali A, El-Quessny M, et al. Mutations in PYCR2, encoding pyrroline-5-carboxylate reductase 2, cause microcephaly and hypomyelination. *Am J Hum Genet.* 2015;96(5):709-719.
46. Yan H, Helman G, Murthy SE, et al. Heterozygous variants in the mechanosensitive ion channel TMEM63A result in transient hypomyelination during infancy. *Am J Hum Genet.* 2019;105(5):996-1004.
47. Müller vom Hagen J, Karle KN, Schüle R, Krägeloh-Mann I, Schöls L. Leukodystrophies underlying cryptic spastic paraparesis: frequency and phenotype in 76 patients. *Eur J Neurol.* 2014;21(7):983-988.
48. Cohen L, Manín A, Medina N, et al. Argentinian clinical genomics in a leukodystrophies and genetic leukoencephalopathies cohort: diagnostic yield in our first 9 years. *Ann Hum Genet.* 2020;84(1):11-28.

Neurology®

Diagnosis of Genetic White Matter Disorders by Singleton Whole-Exome and Genome Sequencing Using Interactome-Driven Prioritization

Agatha Schlüter, Agustí Rodríguez-Palmero, Edgard Verdura, et al.

Neurology 2022;98;e912-e923 Published Online before print January 10, 2022
DOI 10.1212/WNL.0000000000013278

This information is current as of January 10, 2022

Updated Information & Services	including high resolution figures, can be found at: http://n.neurology.org/content/98/9/e912.full
References	This article cites 47 articles, 3 of which you can access for free at: http://n.neurology.org/content/98/9/e912.full#ref-list-1
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): **Leukodystrophies** http://n.neurology.org/cgi/collection/leukodystrophies
Permissions & Licensing	Information about reproducing this article in parts (figures,tables) or in its entirety can be found online at: http://www.neurology.org/about/about_the_journal#permissions
Reprints	Information about ordering reprints can be found online: http://n.neurology.org/subscribers/advertise

Neurology ® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.. All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.