Neurology Publish Ahead of Print
DOI: 10.1212/WNL.00000000000013278

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

Author(s):

Agatha Schlüter, PhD^{1,2}; Agustí Rodríguez-Palmero, MD^{1,2,3}; Edgard Verdura, PhD^{1,2}; Valentina Vélez-Santamaría, MD^{1,4}; Montserrat Ruiz, PhD^{1,2}; Stéphane Fourcade, PhD^{1,2}; Laura Planas-Serra, MSc^{1,2}; Juan José Martínez, MSc^{1,2}; Cristina Guiler, MSc^{1,2}; Marisa Girós, PhD⁵; Rafael Artuch, MD^{2,6}; María Eugenia Yoldi, MD⁷; Mar O'Callaghan, MD, PhD^{2,6}; Angels García-Cazorla, MD, PhD^{2,6}; Judith Armstrong, PhD^{2,8}; Itxaso Marti, MD, PhD^{9,10,11,12}; Elisabet Mondragón Rezola, MD, PhD^{10,12,13}; Claire Redin, PhD¹⁴; Jean Louis Mandel, MD, PhD^{14,15,16}; David Conejo, MD¹⁷; Concepción Sierra-Córcoles, MD¹⁸; Sergi Beltran, PhD¹⁹; Marta Gut, PhD¹⁹; Elida Vázquez, MD²⁰; Mireia del Toro, MD, PhD^{2,21}; Mónica Troncoso, MD, PhD²²; Luis A. Pérez-Jurado, MD, PhD^{2,23,24}; Luis G. Gutiérrez-Solana, MD, PhD²⁵; Adolfo López de Munain, MD, PhD^{10,11,12,13}; Carlos Casasnovas, MD, PhD^{1,4}; Sergio Aguilera-Albesa, MD, PhD⁷; Alfons Macaya, MD, PhD^{2,21,26}; Aurora Pujol, MD, PhD^{1,2,27} on behalf of GWMD working group

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.

Neurology® Published Ahead of Print articles have been peer reviewed and accepted for publication. This manuscript will be published in its final form after copyediting, page composition, and review of proofs. Errors that could affect the content may be corrected during these processes.

Equal Author Contributions:

These authors contributed equally to this work: Agatha Schlüter, Agustí Rodríguez-Palmero, Edgard Verdura

Corresponding Author:

Aurora Pujol
apujol@idibell.cat

Affiliation Information for All Authors: 1. Neurometabolic Diseases Laboratory, Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat, Barcelona, Spain; 2. Center for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Spain; 3. Pediatric Neurology Unit, Department of Pediatrics, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Spain; 4. Neuromuscular Unit, Neurology Department, Hospital Universitari de Bellvitge, Universitat de Barcelona, Hospitalet de Llobregat, Spain; 5. Secció d'Errors Congènits del Metabolisme-IBC, Servei de Bioquímica i Genètica Molecular, Hospital Clínic, IDIBAPS, CIBERER, Barcelona, Spain; 6. Institut de Recerca Pediàtrica-Hospital Sant Joan de Déu (IRP-HSID), Barcelona, Spain; 7. Pediatric Neurology Unit, Department of Pediatrics, Navarra Health Service, Navarrabiomed Research Foundation, Pamplona, Spain; 8. Molecular and Genetics Medicine Section, Hospital Sant Joan de Déu, Barcelona, Spain; 9. Department of Neuropediatrics, Hospital Universitario Donostia, San Sebastián, Spain; 10. Biodonostia Health Research Institute (Biodonostia HRI), San Sebastián, Spain; 11. University of the Basque Country (UPV-EHU), San Sebastian, Spain; 12. Centro de Investigación Biomédica en Red para Enfermedades Neurodegenerativas (CIBERNED), Carlos III Health Institute, Madrid, Spain; 13. Department of Neurology, Hospital Universitario Donostia, San Sebastián, Spain; 14. Département de Médecine translationnelle et Neurogénétique, IGBMC, CNRS UMR 7104/INSERM U964/Université de Strasbourg, Illkirch, France; 15. Laboratoire de Diagnostic Génétique, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; 16. Chaire de Génétique Humaine, Collège de France, Illkirch, France; 17. Complejo asistencial universitario de Burgos, Burgos, Spain; 18. Department of Paediatric Neurology, Complejo Hospitalario Jaén, Jaén, Spain; 19. CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain; 20. Department of Pediatric Radiology, Hospital Materno-Infantil Vall d'Hebrón, Barcelona, Spain; 21. Pediatric Neurology Department, Vall d'Hebron University Hospital, Universitat Autònoma de Barcelona, Spain; 22. Pediatric Neurology, Hospital Clínico San Borja Arriarán, Central Campus Universidad de Chile, Chile; 23. Genetics Service, Hospital del Mar Research Institute (IMIM), Barcelona, Spain; 24. Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain; 25. Department of Paediatric Neurology, Children's University Hospital Niño Jesús, Madrid, Spain; 26. Pediatric Neurology Research Group, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain; 27. Catalan Institution of Research and Advanced Studies (ICREA), Barcelona, Catalonia, Spain.

Contributions:

Agatha Schlüter: 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: 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: 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: 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: Drafting/revision of the manuscript for content, including medical writing for content;
Major role in the acquisition of data
Stéphane Fourcade: Drafting/revision of the manuscript for content, including medical writing for
content
Laura Planas-Serra: Major role in the acquisition of data
Juan José Martínez: Major role in the acquisition of data
Cristina Guilera: Major role in the acquisition of data
Marisa Girós: Major role in the acquisition of data
Rafael Artuch: Major role in the acquisition of data
María Eugenia Yoldi: Major role in the acquisition of data
Mar O'Callaghan: Major role in the acquisition of data
Angels García-Cazorla: Major role in the acquisition of data; Analysis or interpretation of data
Judith Armstrong: Major role in the acquisition of data
Itxaso Marti: Major role in the acquisition of data
Elisabet Mondragón Rezola: Major role in the acquisition of data
Claire Redin: Major role in the acquisition of data; Analysis or interpretation of data
Jean Louis Mandel: Major role in the acquisition of data; Analysis or interpretation of data
David Conejo: Major role in the acquisition of data
Concepción Sierra-Córcoles: Major role in the acquisition of data
Sergi Beltran: Major role in the acquisition of data; Analysis or interpretation of data
Marta Gut: Major role in the acquisition of data
Elida Vázquez: Major role in the acquisition of data
Mireia del Toro: Major role in the acquisition of data
Mónica Troncoso: Major role in the acquisition of data
Luis A. Pérez-Jurado: Drafting/revision of the manuscript for content, including medical writing for
content; Analysis or interpretation of data
Luis G. Gutiérrez-Solana: Major role in the acquisition of data; Analysis or interpretation of data
Adolfo López de Munain: Major role in the acquisition of data; Analysis or interpretation of data
Carlos Casasnovas: Major role in the acquisition of data; Analysis or interpretation of data
Sergio Aguilera-Albesa: 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: 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: 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

Number of characters in title: 130

Abstract Word count: 324

Word count of main text: 3161

References: 48

Figures: 4

Tables: 1

Supplemental: CARE Checklists

Search Terms: [155] Leukodystrophies

Acknowledgements: We thank patients and families for their collaboration, and the European Leukodystrophy Association ELA-Spain for its support. This study was funded by the 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" to AP, the Instituto de Salud Carlos III and Fondo Europeo de Desarrollo Regional (FEDER), Unión Europea, una manera de hacer Europa (FIS PI20/00758) to CC, La Marató de TV3 Foundation (202006-30) to CC and AP, and the AWS Cloud Credits for Research program to AS. This study has also been funded by Instituto de Salud Carlos III through the programs Miguel Servet (CPII16/00016) to SF, Sara Borrell (CD19/00221) to EV, and Rio Hortega, CM18/00145 to VV co-funded by the European Social Fund (ESF, investing in your future). MR was funded by the Center for Biomedical Research on Rare Diseases, an initiative of the Instituto de Salud Carlos III. Several authors of this publication are members of the European Reference Network for Rare Neurological Diseases - Project ID No 739510: AM, MdT. We thank the CERCA Program/Generalitat de Catalunya for institutional 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) CNAG's call "300 exomes to elucidate rare diseases" the Instituto de Salud Carlos III and Fondo Europeo de Desarrollo Regional (FEDER), Unión Europea, una manera de hacer Europa (FIS PI20/00758) La Marató de TV3 Foundation (202006-30) AWS Cloud Credits for Research program. Instituto de Salud Carlos III through the programs Miguel Servet (CPII16/00016) to SF Sara Borrell (CD19/00221) to EV, Rio Hortega, CM18/00145 to VV co-funded by the European Social Fund (ESF, investing in your future). MR was funded by the Center for Biomedical Research on Rare Diseases, an initiative of the Instituto de Salud Carlos III. European Reference Network for Rare Neurological Diseases - Project ID No 739510: AM, MdT.

Disclosures: The authors report no disclosures relevant to the manuscript.

ABSTRACT

Background and Objectives: Genetic white matter disorders (GWMD) are of heterogeneous origin, with more than a hundred causal genes identified to date. Classical targeted approaches achieve a molecular diagnosis in only half of all patients. Here we aim 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 patients, 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 HPO terms of each patient.

Results: We received 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 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 six families, underscoring the importance of genomic analysis to solve these cases. Finally, 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.

INTRODUCTION

The advent of next-generation sequencing (NGS) in clinical applications (especially targeted sequencing panels and whole-exome sequencing) has increased the diagnostic yield of hereditary neurological diseases with high genetic heterogeneity and low mutational burden¹⁻⁴. 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 classical combined MRI, biochemical, and target gene-based approach leaves approximately half of GWMD patients without a genetic diagnosis⁷⁻¹⁰. In these undiagnosed cases, trio WES (whole-exome sequencing) followed by WGS (whole-genome sequencing) 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 networks¹⁴⁻¹⁷ may prove useful to improve rapid selection of candidate variants.

Here, 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 HPO (Human Phenotype Ontology) terms, as well as interaction and functional network information to facilitate the identification of causal genes and enable novel disease-gene discovery.

MATERIALS AND 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, neurophysiological and genetic studies such as 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 neurological undiagnosed disorders, was made before inclusion. This clinical team was driving the diagnostic process and exchanged information with the referring clinicians when required, both prior and post-variant 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 n. 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 were 50 females and 76 males, 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 one 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**.

Diagnostic Yield of WES and WGS in a cohort of GWMD patients

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 i) variants not initially identified because

of filtering issues (3 cases); ii) variants located in noncoding regions (3 cases); iii) pipeline update/technical issues (2 cases); and iv) 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.

Furthermore, 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 GeneMatcher²⁰. We functionally validated and reported two novel disease genes (*DEGSI*²¹ and *PI4KA*²²) in two families each, whereas the other 5 validated cases are currently in preparation. Two more candidate genes are awaiting additional patients while functional studies are on-going.

Overall, we obtained a positive genetic diagnosis in 91 out of 126 GWMD cases (72%) (**Figure 1, eFigure 1, eTables 1 and 2**). 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".

For the 33 cases that remained undiagnosed after WES/WGS, we noted a trend towards adulthood onset (30% of unsolved cases were adults *versus* 16% of adults in solved cases), cystic lesions on MRI (12% of undiagnosed cases versus 5% in solved cases), and absence of consanguinity (97% non consanguineous in unsolved versus 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 six families each,

and *PLP1* variants in five families (**eTable 3**). New phenotypes were identified in two cases, atypical forms of presentation in seven, and six more cases were complex, blended phenotypes with variants in more than one gene (**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 ACMG/AMP 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 VUS variants into pathogenic or likely pathogenic variants. We analysed the impact of 8 variants on splicing using either cDNA sequencing (from RNA derived from PBMC or fibroblasts), and/or minigene splicing assay²⁶ (n=3). The minigene assays were instrumental to confirm the pathogenic role of an intronic variant in *MLC1* (c.597+37C>G), gene not expressed in PBMC nor fibroblasts, and another intronic variant in *EIF2B5* (c.1156+13G>A), which lead to a mild form of ovarioleukodystrophy²⁶. Importantly, 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**). In other cases which were not amenable to experimental validation (5 remaining until 91), we reported out VUSs highly compatible with the clinical, 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 two cases had mutations in more than one gene with different inheritance patterns (one with AD and AR inheritance; one AD and X-linked) (**eFigure 1**). 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: i) the *RNASEH2B* p.Ala177Thr variant in 6 independent families (frequency: 0.001306 in gnomAD

(v2.1.1))²⁷; ii) 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 iii) the *SPG11* frameshift variant p.Met245Valfs*2 twice independently (frequency: 0.0001071 in gnomAD (v2.1.1))²⁹.

Of note, in addition to SNVs and indels, we detected a pathogenic copy number variant (CNV) in four cases (4.4%) by WES (**eTable 6**): a 6930 Kb 1p36 heterozygous deletion in LNF-36 and validated by aCGH (eFigure 2), a 117 Kb duplication in 5q including *HNRNP1* and *RUFY1* genes in LNF-105³⁰, a 60.4 Kb duplication containing *LMNB1* in LNF-34, and a 21.3 Kb homozygous deletion encompassing *TANGO2* in LNF-97³¹. We validated the last three 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 (HGMD, public access), or ClinVar databases (**eTable 7**).

Management implications of a positive diagnosis

Importantly, diagnosis allowed us to improve the clinical management in 29 cases (**eTable 1**). In 22 of them, it led to the consideration of a specific treatment option for the disease, such as hematopoietic stem cell transplant (HSCT) 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 SS *et al.*³²) in two 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, cardiological 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 of 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 Human Phenotype Ontology (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)**)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 1530 proteins and 18288 interactions (**Figure 4**). To evaluate the functional signature of these 1530 proteins, we performed an enrichment analysis 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: i) the mitochondrial oxidative phosphorylation (OXPHOS) system (e.g., NADH-ubiquinone oxidoreductase Fe-S protein 1, *NDUFS1*), ii) the lysosome (e.g., the galactosylceramidase enzyme, *GALC*), iii) the peroxisome (peroxins) (e.g., peroxin 6, *PEX6*), iv) the metabolism of ribonucleotides (e.g., ribonuclease H2 subunit B, *RNASEH2B*), and v) the purine metabolism pathway with RNA polymerases I and III (e.g., RNA polymerase III subunit A, *POLR3A*). Among the 1530 proteins we identified (beside the 843 GWMD seed proteins): i) 587 proteins associated with disease but not yet with GWMD and ii) 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: i) the delta 4-desaturase sphingolipid 1 (DEGS1) in patients LNF-41 and LNF-42 (**Figure 4B**), causing Hypomyelinating Leukodystrophy 18 (HLD18, OMIM #615843)²¹ ii) the phosphatidylinositol 4-kinase alpha (PI4KA) recently associated with leukodystrophy and identified in patient LNF-107 and VH-3^{22,34} (**Figure 4C**), iii) the mitochondrial ribosome-associated GTPase 1 (*MTG1*) that plays a role in the regulation of mitochondrial ribosome assembly and translational activity (**Figure 4D**), and iv) 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 GWMD patients 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. Here, 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 cases³⁵. In another report including 100 adult-onset leukodystrophy patients, the diagnostic rate was 26%³⁶. Our results are slightly better than those reported in another study including 71 pediatric cases^{4,11}. In Vanderver's report, a first trio WES allowed a definite diagnosis in 42% of cases⁴, while in a second phase of the study including the 41 negative cases, a molecular diagnosis was established in nine more cases by reanalysis and in five cases using WGS, representing 17% and 12%, respectively¹¹. 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 impact on our higher diagnostic yield. Comparison between the results of these cohorts is difficult because of different study protocols and target population, which comprised a 20% of adult GWMD in our case versus a pediatric population-only in Vanderver's case ⁴. It is likely that the use of trio WES/WGS would have improved our diagnostic yields, and most certainly would have ameliorated turn-around-times. Because of the very late implantation of clinical exomes (instead of WES) in our healthcare system and limited research funding resources we chose to apply singleton WES to help as many families as possible, since trio studies may cost double^{37,38} to three times higher in our healthcare system. The use of trio WES/WGS is however recommended when urgent diagnosis is required in ICU settings³⁹. Thus in our opinion, 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 setting³⁷.

Our study enabled identification of disorders caused by genes rarely associated with white matter involvement (*PTEN*, *GFPT1*⁴⁰ and *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 one 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 (**Figure 2, eTables 1 and 4, eResults for clinical summaries**). Further, in nine families (10%), we identified variants in genes associated primarily with hereditary spastic paraplegia (*SPG11*, *SPG7*, *SPAST*, *DDHD2*, *CAPN1* and *CYP2U1*) (Figure 3), underscoring the notion of a continuum of clinical spectrum, similarly to X-adrenoleukodystrophy, PMD/SPG2, metachromatic dystrophy (MLD) or Alexander disease⁴⁷. Moreover, half of the genes identified in this cohort are linked to genetic leukoencephalopathies, not classically considered

“leukodystrophy genes”. Since 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 study⁴⁸.

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 GWMD patients⁶. This would allow for gaining precious 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 odyssey” of 10 years of disease evolution on average. Hence, reducing multiple unnecessary examinations with a low cost-benefit ratio, as it is the case for some metabolic studies in the context of a nonspecific neuroimaging, would entail significant economic savings for the healthcare 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 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. Additionally, we reported as diagnosed 5 cases harboring VUS using technically strict ACMG criteria, since 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.

In summary, we provide evidence of the effectiveness of sWES-WGS analysis based on a phenotype- and interactome-driven prioritization algorithm to diagnose patients with GWMD and to identify new phenotypes and novel disease genes. Finally, we also provide a WM 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. We believe that the utilization of advanced computational methods together with the integration of a functional genomics laboratory capable of experimental validation of VUSs and candidate genes, together with the direct implication of adult and pediatric neurologists in the process has been determining factors for this high diagnostic yield.

Supplement-<http://links.lww.com/WNL/B741>

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. doi:10.1212/WNL.0b013e3181eee46b
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. Boudelloua I, Kulmanov M, Schofield PN, Gkoutos G V., 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* (80-). 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. doi:10.1212/01.wnl.0000343049.00540.c8
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.
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 D, 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*. Published online August 20, 2021. doi:10.1093/BRAIN/AWAB124
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:JCNSD.S13540.
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:D966-74.
34. Salter CG, Cai Y, Lo B, et al. Biallelic PI4KA variants cause neurological, intestinal

and immunological disease. *Brain*. Published online August 20, 2021. doi:10.1093/brain/awab313/6355377

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 Supplement):P4.6-054.
36. Lynch DS, Rodrigues Brandao 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 Part 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-

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. doi:10.1016/j.ajhg.2015.03.003
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.

TABLE AND FIGURE LEGENDS

Table 1. Main clinical features of the 126 index cases.

	n° cases	(%)
Sex		
Female	50	40
Male	76	60
Age at onset		
<3 years	86	68
3-18 years	15	12
>18 years	25	20
Consanguinity		
Yes	18	14
Main clinical features		
Motor symptoms		
Pyramidal	94	74
Extrapyramidal	34	27
Hypotonia	15	12
GDD/ID/cognitive decline	91	72
ASD/behaviour/psychiatric manif.	21	16
Cerebellar	42	33
Epilepsy	36	28
Ophthalmologic	55	43
Predominant MRI pattern		
Hypomyelination	40	31
Non-hypomyelination		
Periventricular	49	39
Diffuse	19	15
Frontal	12	9
Multifocal	3	2
Parieto-Occipital	2	1
Cerebellar	2	1
Complementary exams		
Metabolic studies	116	92
Neurophysiologic studies	99	78
Karyotype/aCGH/NGS panel	65	51
Targeted genetic studies	65	51
TOTAL cases	126	

Figure 1. Diagnostic process diagram and diagnostic yield (A) Number of cases included in the study and diagnostic process. **(B)** Global, WES and 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.

Figure 2. MRI findings in patients with new/atypical and blended phenotypes. (A) LNF-48, 5 years. *PARS2*; p.Arg186Gly /p.Lys187Arg (COMP HTZ). Peritrial WM hyperintensity (red arrows) with frontal-parietal atrophy, ventriculomegaly and thin corpus callosum (arrowheads) (axial T2-FLAIR, sagittal T1-weighted images). **(B)** LNF-29, 10 months. *PNPT1*; p.Ala507Ser (HMZ). Bilateral peritrial 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 SWI).

Figure 3. MRI images of selected cases with variants in hereditary spastic paraparesis (HSP) genes and white matter involvement. T2 hyperintensity in the bilateral periventricular white matter (axial T2 images (A and D); axial T2-FLAIR images (B and C)).

Figure 4. GWMD expanded interactome. (A.a) The GWMD seeds + expanded network was generated by the network prioritization tool, resulting in 1530 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 525 of the GWMD expanded network with 1000 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 1530 proteins. Box and whisker plots denote matched null distributions (i.e., 1000 permutations). (A.b) Within-group edge count (i.e., number of edges between members of the query set). (A.c) Mean distance is the average path length in the network obtained by calculating the shortest paths between all pairs of proteins. (B, C, D and E) Zoom in the network for specific putative candidates as illustrative example of the GWMD expanded network potentiality: (A) delta 4-desaturase, sphingolipid 1 (*DEGSI*); (B) 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. * indicates recently associated with leukodystrophy.

White Matter expanded network. Available in NDEx repository at <https://public.ndexbio.org/#/network/fd5fc166-9ecc-11eb-9e72-0ac135e8bacf?accesskey=a75ac048b59aca2c9310c04a6f1d96ea34052231d9204f284c5e1d420fc2ca26>

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, 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, 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, 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
M ^a 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, 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

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 published online January 10, 2022

DOI 10.1212/WNL.00000000000013278

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/early/2022/01/10/WNL.00000000000013278.full
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.