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

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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

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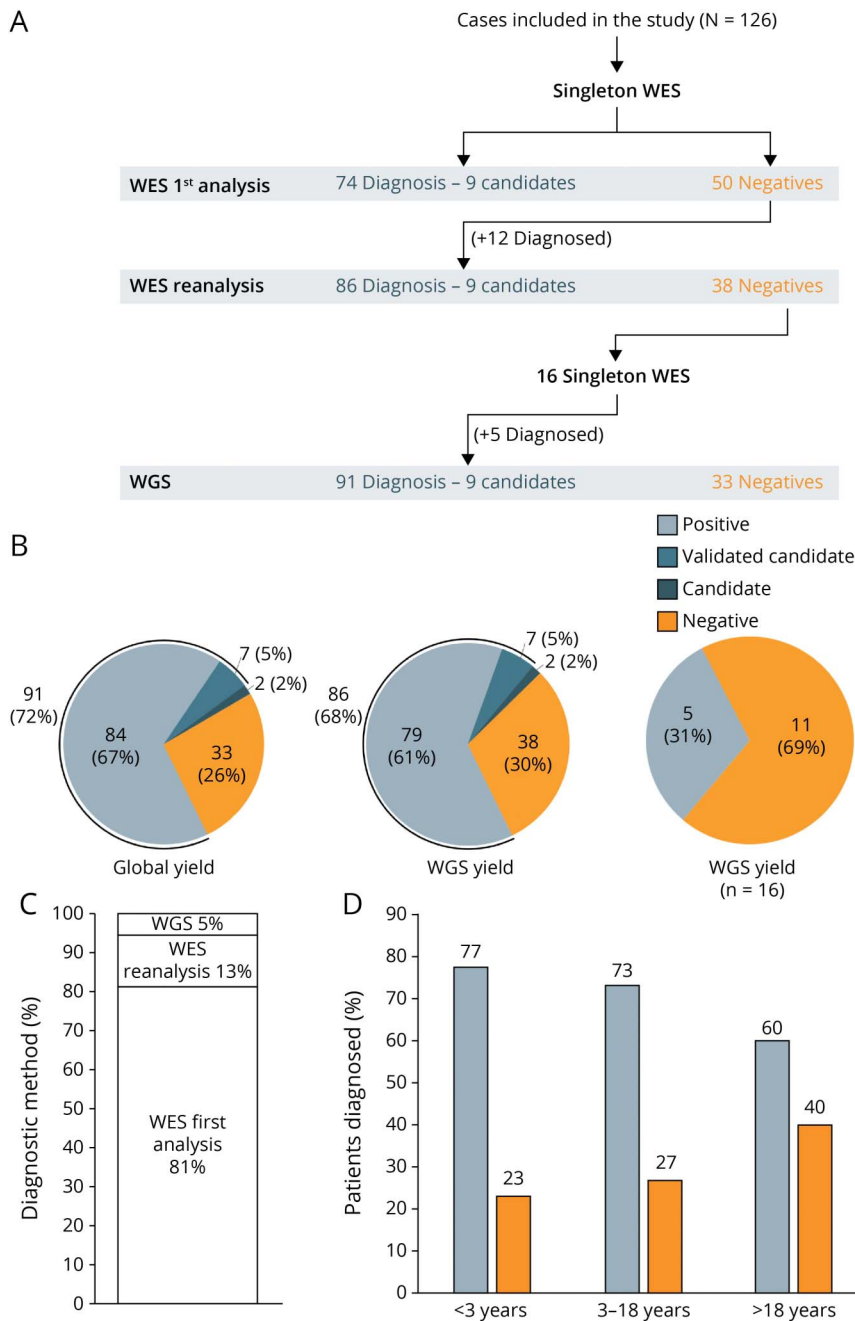
on MRI (12% of undiagnosed cases vs 5% in solved cases), and absence of consanguinity (97% nonconsanguineous in unsolved vs 82% in solved cases).

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)
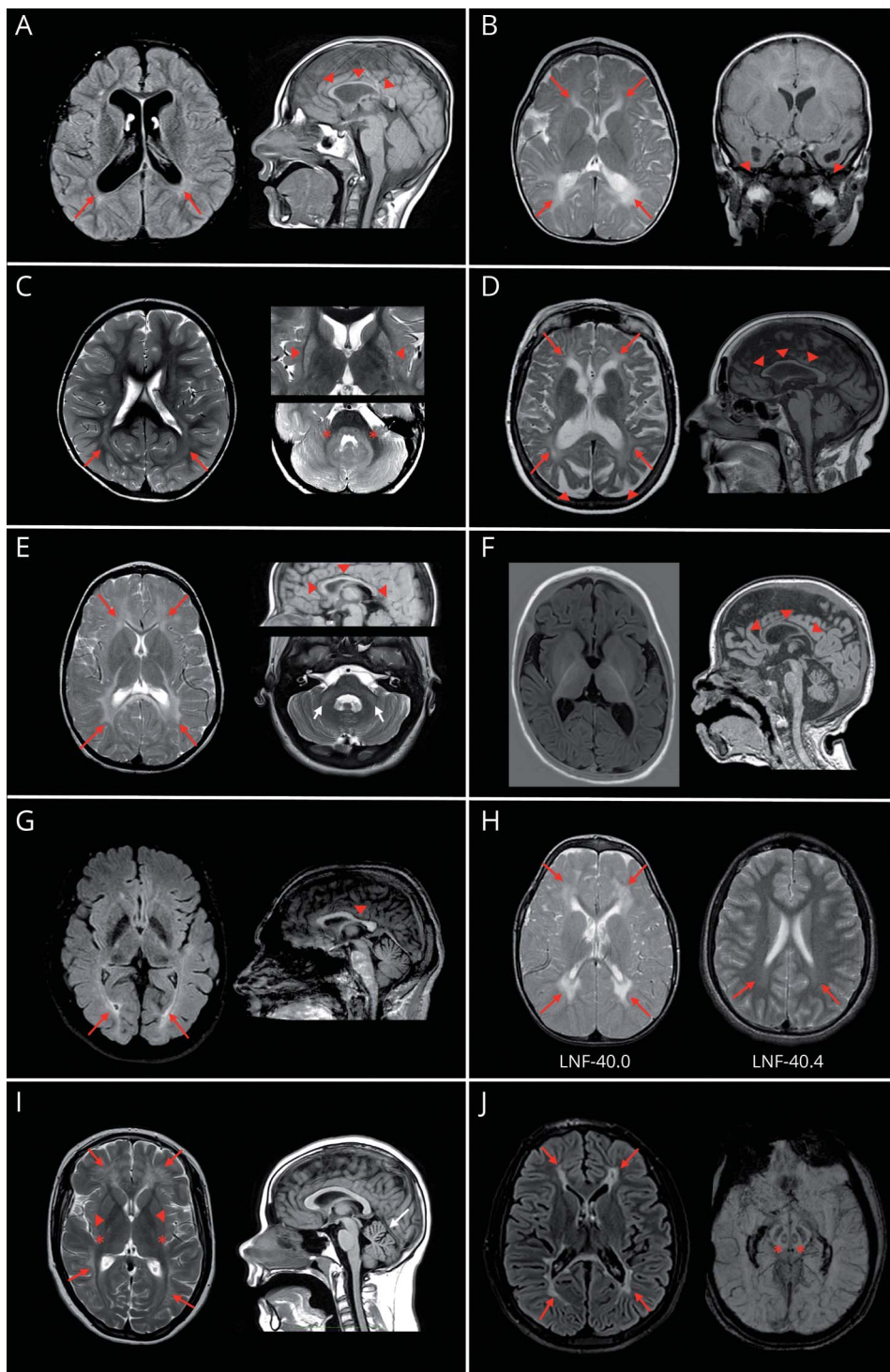
(A) LNF-48, 5 years. *PARS2*; p.Arg186Gly/p.Lys187Arg (COMP HTZ). Periatrial white matter (WM) hyperintensity (red arrows) with frontal-parietal atrophy, ventriculomegaly, and thin corpus callosum (arrowheads) (axial T2 fluid-attenuated inversion recovery [FLAIR], sagittal T1-weighted images). (B) LNF-29, 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 T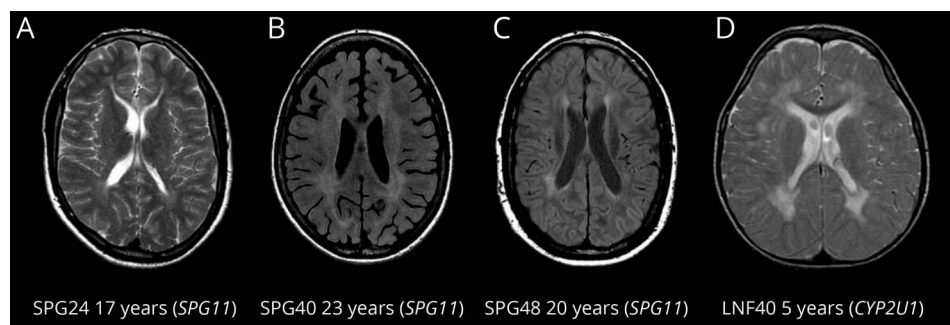1 FLAIR images). (E) LNF-88, 13 years. *GFPT1*; p.Asp296Val (HMZ). Axial T2 hyperintensities involving deep cerebral WM (red arrows), cerebellar peduncles (white arrows), and middle blade of corpus callosum (arrowheads), sparing subcortical WM (axial T2 and sagittal T1-weighted images). (F) LNF-114, 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

SPG24 17 years (*SPG11*)   SPG40 23 years (*SPG11*)   SPG48 20 years (*SPG11*)   LNF40 5 years (*CYP2U1*)

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
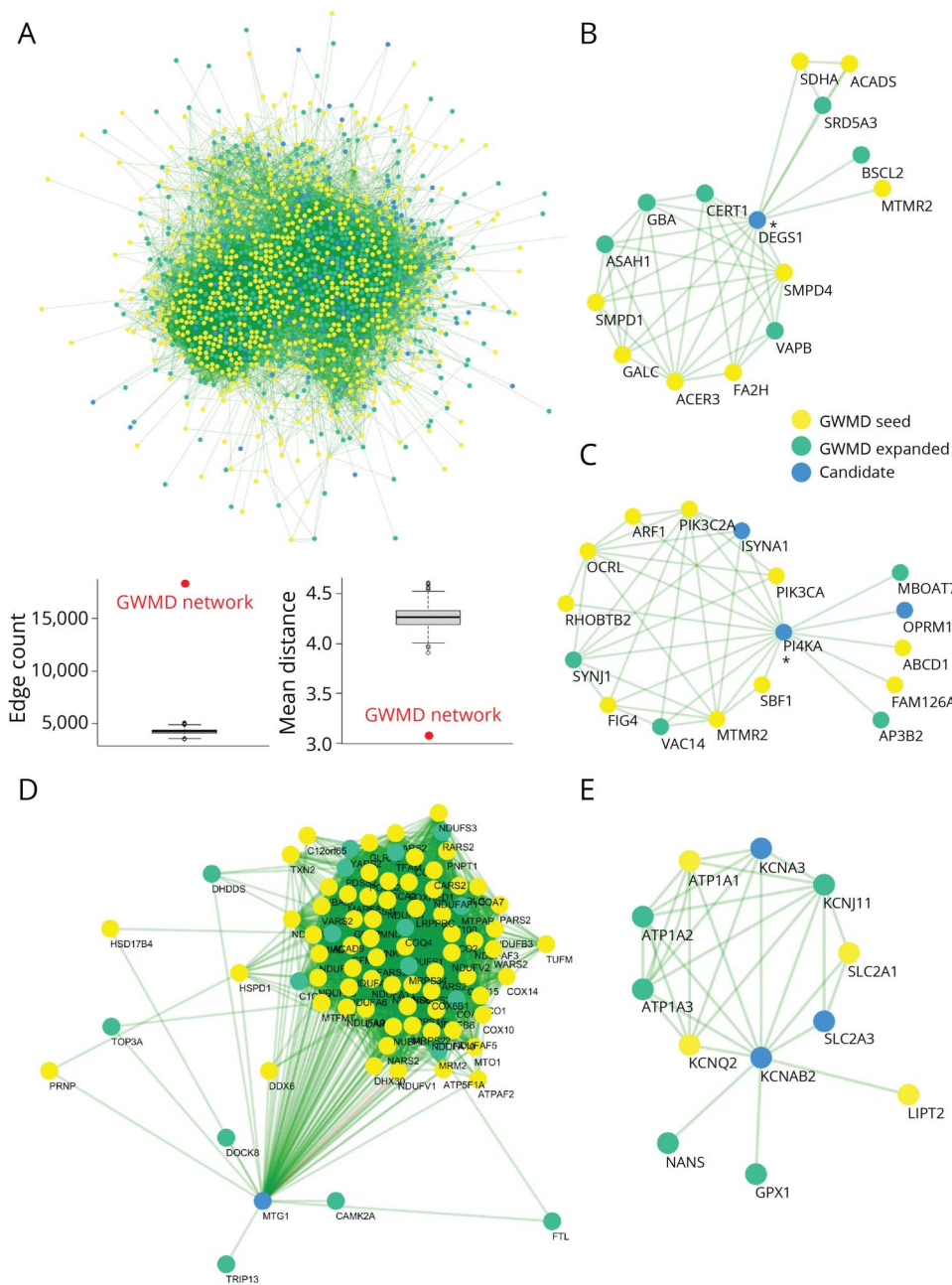
(A) The genetic white matter disorder (GWMD) seeds + expanded network was generated by the network prioritization tool, resulting in 1,530 proteins. The seed genes known to be mutated in GWMD are shown in yellow circles, disease genes not previously associated with GWMD are shown in green, and new GWMD 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?

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.

Disclosure

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

Appendix 1 Authors

Name	Location	Contribution
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