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Curriculum in Clinical Neuroscience, Executive

Clinical and Genetic Characterization of Leukoencephalopathies in Adults

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ABSTRACT

Background In adults, many cases (~30-40%) of leukoencephalopathies (LKENs), i.e. white matter (WM) diseases, are without definitive diagnosis. Patients who remain undiagnosed despite extensive investigations may have atypical forms of known acquired or genetic diseases, or novel diseases more likely genetic in nature. Aims of our work were to explore the efficiency of a systematic approach, including next generation sequencing (NGS), in the diagnosis of a cohort of adult patients with LKEN of unknown cause, and to describe their clinical features.

Patients and Methods In this analytical observational study, we first reviewed the clinical and laboratory features of the adult patients (age ≥ 18 years) with undiagnosed LKEN assessed at the Unit of Rare Neurodegenerative and Neurometabolic Diseases of the Istituto Neurologico "C. Besta", Milan, Italy, from 2012 to 2018. A targeted-gene panel sequencing (TGPS) was subsequently used to investigate 142 genes responsible for genetic LKENs, and a whole-exome sequencing (WES) was performed in one familial case remained undiagnosed.

Results We identified 57 adult patients with LKEN of unknown cause (mean age 43 years, range 18-72; 23 males; 53 with late-adolescence or adult-onset). Thirty of them, henceforward called hypomyelinating leukoencephalopathies (HypoLKENs), presented an MRI pattern suggestive of hypomyelination (mild T2-hyperintensity and normal T1 signal), whereas the remaining 27 (henceforward called demyelinating leukoencephalopathies, DemLKENs) had an MRI pattern suggestive of demyelination (prominent T2-hyperintensity and prominent T1-hypointensity). In 13 HypoLKENs, TGPS identified the disease-causing genes, i.e., *POLR3A* (n = 2), *POLR1C*, *TUBB4A*, *RARS1*, *GJA1*, *PLP1*, *GJC2*, *TBCD*, *CYP7B1*, *SPG11*, *PEX3*, and *PEX13*, while in two further patients, WES led to the identification of a novel disease-causing gene (preliminarily called *GENE_A*). In contrast, TGPS identified the disease-causing gene (i.e., *AUH*) in only one (out of 27) DemLKEN patient affected by methylglutaconic aciduria type 1. In two other DemLKEN patients, the diagnosis was made on the basis of their clinical and MRI features directly by single gene analysis (*PSAP*-related metachromatic leukodystrophy), or by skin biopsy after negative results of TGPS (neuronal intranuclear inclusion disease, NIID). Three patients (one with HypoLKEN and two with DemLKEN) had acquired diseases mimicking a leukodystrophy, i.e., a primary cerebral vasculitis (diagnosed by brain biopsy without genetic analyses) and rare variants of multiple sclerosis, diagnosed after negative results of TGPS. Finally, in eight subjects with an incidentally found DemLKEN who remained without clinical manifestations over a long period of time, no mutation was found by TGPS.

Conclusions In adults, a hypomyelinating pattern characterizes a large number (~50%) of LKENs of unknown cause. HypoLKENs are most commonly due to genes causing severe early-onset hypomyelinating leukodystrophies (HLDs), such as *POLR3A* and *TUBB4A*, or can be due to genes associated with hereditary spastic paraplegias, such as *CYP7B1* and *SPG11*, peroxisomal biogenesis disorders, such as *PEX3* and *PEX13*, or even novel disease-causing genes. Among the DemLKENs of unknown cause, only very few are diagnosed by TGPS if clinical and paraclinical data pointing toward specific diagnoses are lacking. Occasionally, atypical variants of acquired WM diseases can mimic a genetic leukoencephalopathy with demyelinating or hypomyelinating features on MRI. Finally, a subset of DemLKENs characterized by lack of neurological manifestations and no mutation after comprehensive NGS testing may constitute a novel entity we termed subclinical diffuse leukoencephalopathy (SDL).

ABBREVIATIONS

ALDP, Adult Leukoencephalopathy Diagnostic Protocol; **CSF**, cerebrospinal fluid; **DemLKEN**, leukoencephalopathy with demyelinating features; **HLD**, hypomyelinating leukodystrophy; **HSPs**, hereditary spastic paraplegias; **HypoLKEN**, leukoencephalopathy with hypomyelinating features; **LD**, leukodystrophy; **LKEN**, leukoencephalopathy; **MLD**, metachromatic leukodystrophy; **MRI**, magnetic resonance imaging; **MS**, multiple sclerosis; **NGS**, next-generation sequencing; **NIID**, neuronal intranuclear inclusion disease; **PBDs**, peroxisomal biogenesis disorders; **PET**, positron emission tomography; **SDL**, subclinical diffuse leukoencephalopathy; **TGPS**, targeted gene panel sequencing; **WES**, whole-exome sequencing; **WM**, white matter.

INTRODUCTION

The term leukoencephalopathy (LKEN) derives from the combination of the ancient Greek word λευκός (pronunciation lef'kos) meaning "white" or "light in color" (lexical cognates in Latin and English are *lux* and *light*, respectively) with the word "encephalopathy", and indicates any disease affecting the "white" matter (WM) of the central nervous system (CNS) on neuroimaging. Based on this definition, the diagnosis of leukoencephalopathy *in vivo* is imaging-centric (Salsano 2015), and may include a very large number of diseases with very different clinical and pathological characteristics (van der Knaap and Valk 2005; van der Knaap and Bugiani 2017).

LKENs can be grossly dichotomized in acquired and genetic.

In adults, leukoencephalopathies are usually acquired, and include multiple sclerosis (MS) and related disorders (Fox et al. 2021), commonly in young/middle-age people, and degenerative cerebral microangiopathy (DCM), usually in elderly people (Ringelstein and Nabavi 2005). However, WM lesions could be *prima facie* classified as vascular also in young people, as WM lesions attributable to microangiopathy (i.e., small vessel disease, SVD) seem to occur in 5-10% of patients aged 20-40 years (Charil et al. 2006).

In adults, less commonly than in children, leukoencephalopathies can be also genetic in nature. Genetic LKENs include any monogenic disease characterized by prominent, confluent/diffuse WM changes on neuroimaging. They can be further sub-classified on the basis of the cell type which is primarily involved in their pathogenesis. Genetic LKENs primarily involving glial cells, i.e., oligodendrocytes (the myelin-forming cells of the CNS), astrocytes or microglia, can be classified as leukodystrophies (LDs), whereas genetic LKENs primarily involving non-glial cells, such as vascular smooth muscle cells of blood vessels or even neurons, can be classified as genetic LKENs *sensu stricto* (Vanderver et al. 2015). Based on this classification, Pelizaeus-Merzbacher disease (PMD, OMIM # 312080), the prototype of hypomyelinating leukodystrophies (HLDs)

(van der Knaap and Bugiani 2017; Di Bella et al. 2021), and metachromatic leukodystrophy (MLD, OMIM # 250100) and Krabbe disease (KRD, OMIM # 245200), prototypes of demyelinating leukodystrophies (van der Knaap and Bugiani 2017), are examples of oligodendrocyte/myelin-related leukodystrophies; Alexander disease (ALXDRD, OMIM # 203450) is an example of leukodystrophies related to astrocyte (also named astrocytopathies); hereditary diffuse leukoencephalopathy with spheroids 1 (HLDS1, OMIM # 221820) is an example of leukodystrophies related to microglia (also named microgliopathies). In contrast, the autosomal dominant cerebral arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL, OMIM #125310), which is a progressive disorder of the small arterial vessels of the brain, is a genetic LKEN. Likewise, neuronal ceroid lipofuscinoses (NLCs), which are primary gray matter or neuronal disorders, may be classified as genetic LKENs if they are characterized by prominent WM abnormalities due to secondary defects in myelination following primary neuronal/axonal dysfunction (Vanderver et al. 2015). This classification is not clear-cut, but may be of help in clinical practice (Salsano 2015).

As to the age of onset, LKENs can be distinguished in childhood or late-adolescence/adult onset, with genetic diseases more commonly seen in children. Indeed, there are genetic LKENs/LDs, like Canavan disease (OMIM #271900), described *only* in children, and genetic LKENs/LDs, like MLD or KRD, much more commonly seen in children, and with onset rarely in adults (Farina et al. 2000; Benzoni et al. 2021). However, there are also genetic LKENs/LDs with less aggressive variants seen only in adults, such as adrenoleukodystrophy (ALD) / adrenomyeloneuropathy (AMN) (OMIM #300100) (Engelen et al. 2012); genetic LKENs/LDs, like ALXDRD, seen almost equally in children *and* adults, and genetic LKENs/LDs, like HLDS1, polyglucosan body neuropathy, adult form (APBN, OMIM #263570) or autosomal dominant adult-onset demyelinating leukodystrophy (ADLD, OMIM #169500), described *only* in adults

(Ahmed et al. 2014; Köhler, Curiel, and Vanderver 2018). Among the adult cases of genetic LKENs/LDs, however, there are sometimes patients with childhood-onset diseases *persisting* into adulthood, especially when the exact age of onset cannot be confidently established, e.g., because there are overlooked manifestations (e.g., mild learning disabilities or behavioral abnormalities) that could be considered as early symptoms exclusively (or at most) on retrospect.

Despite extensive instrumental and laboratory investigations, including different next-generation sequencing (NGS) strategies for DNA sequencing, many cases (about 30-40%) of adulthood LKENs still remain without definitive diagnosis (Schiffmann and van der Knaap 2009; Lynch et al. 2017; Kunii et al. 2018).

Objectives of our work were: 1) to explore the efficiency of a systematic approach, including targeted sequencing in a comprehensive set of genes associated with genetic LKENs and whole-exome sequencing (WES), in the diagnosis of a cohort of adult patients with LKEN of unknown cause; 2) to describe the clinical features of these disorders.

PATIENS and METHODS

We selected the adult patients (age ≥ 18 years) with a leukoencephalopathy of unknown cause from the adulthood leukodystrophy database at our Unit of Rare Neurodegenerative and Neurometabolic Diseases, Fondazione IRCCS Istituto Neurologico C. Besta, Milano, Italy, and assessed as outpatients, inpatients, or both from September 2012 to December 2018.

All subjects were initially investigated by using our Adult Leukoencephalopathy Diagnostic Protocol (ALDP), i.e., the clinical- and paraclinical-based protocol applied in our Unit for the diagnosis of diffuse leukoencephalopathies. Based on this protocol, the first crucial step was the distinction between leukoencephalopathies with brain MRI findings suggestive of hypomyelination (henceforward hypomyelinating leukoencephalopathies, HypoLKENs) and leukoencephalopathies with brain MRI findings suggestive of demyelination (henceforward demyelinating leukoencephalopathies, DemLKENs) (**Figure 1; appendix Table S1**).

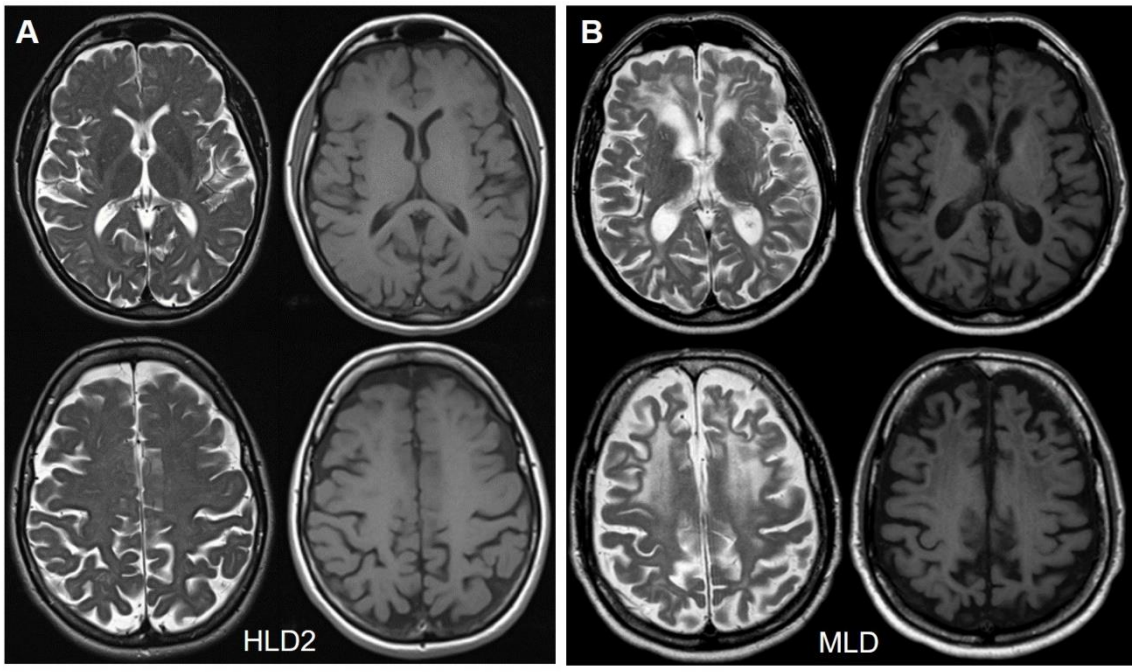

Figure 1. **Differences between MRI patterns suggestive of hypomyelination (A) or demyelination (B).** In **A)**, there is a mild T2-hyperintensity combined with normal T1 signal, as typically observed in hypomyelinating leukodystrophies (HLDs) such as HLD2 (*alias* Pelizaeus-Merzbacher-like disease 1). In **B)**, there is a prominent T2 hyperintensity combined with prominent T1 hypointensity, as observed in demyelinating leukodystrophies such as metachromatic leukodystrophy (MLD).

HypoLKENs are typically considered as monogenic diseases. They are associated to pathogenic variants in a large number of genes, although they remain still undiagnosed in many cases. In more than twenty different genetically-defined forms, the MRI pattern suggestive of hypomyelination is related to defects of myelin formation. These subsets of HypoLKENs *sensu stricto* are called hypomyelinating leukodystrophies, and are identified by the acronym HLD followed by a sequential number (HLD1, HLD2, and so on). Hence, in the presence of an MRI pattern suggestive of hypomyelination, the following diagnostic step should be genetic analyses. At most only two genes, i.e., *PLP1* and *GJC2/Cx47*, were initially analyzed in adult patients, because HLDs have been considered for a long time as childhood diseases with onset (very) early in life, and it is only recently that most of the other associated genes have been identified (Orthmann-Murphy et al. 2009; Pouwels et al. 2014). Therefore, in the present work, we decided to investigate the HypoLKENs of unknown cause, if any, by using a custom targeted gene panel sequencing (TGPS) consisting of 142 genes associated with LKENs (Di Bella et al. 2021); **appendix Table S2**). Further, in one still-undiagnosed familial case, we performed a whole-exome sequencing (WES) followed by functional studies.

Unlike HypoLKENs, DemLKENs in adults are supposed to encompass a larger number of diseases which can be both *acquired* or *genetic* in nature. This distinction is far to be clear-cut. Therefore, in the present work, we decided to initially investigate the DemLKENs of unknown cause by collecting and systematically reviewing the following parameters: presence of other affected family members, parents' consanguinity, age and symptoms at onset, type of onset (gradual, subacute or acute/hyperacute), disease course (progressive/worsening, relapsing-remitting, stationary, improving), clinical manifestations, including extraneurological findings, problems at birth or during the pregnancy of the patient's mother, school performances, results of formal neuropsychological tests, including premorbid IQ assessment, topography of the WM

changes (Schiffmann and van der Knaap 2009), proton MR imaging results, presence of spinal cord abnormalities on neuroimaging, calcification (or micro-calcification) on CT scan, peripheral nerve involvement (based on EMG with nerve conduction studies), evoked potential abnormalities, neuro-ophthalmological assessment with electroretinogram (ERG), pattern ERG, optical coherence tomography (OCT), and, occasionally, fluorescein angiography, cerebral PET results (with ^{18}FDG or other radiotracers), blood examination, including ammonium, B12, folate, homocysteine, and autoimmunity screening (**Table 1**). Non-routine or invasive investigations, including CSF analysis, serum amino acids, urinary organic acids and other specific biochemical analyses (e.g., very long chain fatty acids), muscle and/or peripheral nerve biopsy, respiratory chain enzyme activity in muscle homogenate, cerebral biopsy and cerebral angiography, and bone scintigraphy were performed in selected cases. Regarding the topography of the WM changes, the MRI-based recognition pattern developed by Schiffmann and Van Der Knapp was primarily used for both the distinction between acquired and genetic forms, and the selection of the most appropriate single-gene analyses when a specific genetic leukoencephalopathy was suspected (Schiffmann and van der Knaap 2009; Lynch et al. 2019). Multiple sclerosis (MS) and degenerative cerebral microangiopathy (DCM) were regularly considered in the differential diagnosis of leukoencephalopathies of unknown cause when no better explanation was found (Charil et al. 2006). When the exact diagnosis was not made after the combination of all the previously reported diagnostic procedures, including few (usually one or two) single-gene analyses, the previously described TGPS was used.

Table 1. Laboratory investigations to rule out acquired leukoencephalopathies

Blood analyses	**Cerebrospinal Fluid (CSF) analyses**
• Full Blood Count (FBC) • Erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) • Electrolytes • Liver and thyroid function tests • Serum protein electrophoresis and immunofixation (serum and urine) • Vitamin B12, folate and homocysteine • Ammonia • Lipids • Syphilis serology, HIV serology, Hepatitis B and C serology • Antinuclear antibodies (ANA), extractable nuclear antigen antibodies (ENA), anti-neutrophil cytoplasmic antibodies (ANCA), anti-double stranded DNA antibodies (ds-DNA) • Lupus anticoagulant, anti-cardiolipin antibodies, anti-beta2-glycoprotein • Anti-neuronal antibodies • Antithyroglobulin and antimicrosomal antibodies • Coeliac disease-associated antibodies • Complement components 3 and 4 • Serum chitotriosidase • HLA-B51 testing • Anti-acquaporin-4 (AQP4), anti-myelin oligodendrocyte glycoprotein (MOG) • Anti-Borrelia antibodies	• Protein • Glucose • Cell count and cytology • Link and Reiber indexes • Oligoclonal band analysis • Immunoglobulin free light chain analysis • Flow cytometry for cell immunophenotyping • Immunoglobulin Heavy Chain/T Cell Receptor rearrangement analyses • PCR for JC virus and *Tropheryma whipplei* • Anti-measles antibodies • Anti-Brucella antibodies • Chitotriosidase

RESULTS

A flow diagram synthetizing the results is shown in **Figure 2** on the next page.

We identified 57 adult index cases, 23 males and 34 females. Their mean age was 43 years (range: 18-72). In 53 patients, the onset was in the late-adolescence or adulthood, while the remaining four patients presented a diffuse leukoencephalopathy with onset in childhood and *persisting* into adulthood. Thirty patients had MRI findings suggestive of central hypomyelination (mild T2 hyperintensity combined with (near-)normal T1 signal, HypoLKENs), while the remaining 27 patients had MRI findings suggestive of demyelination (marked T2 hyperintensity combined with T1 hypointensity, DemLKENs).

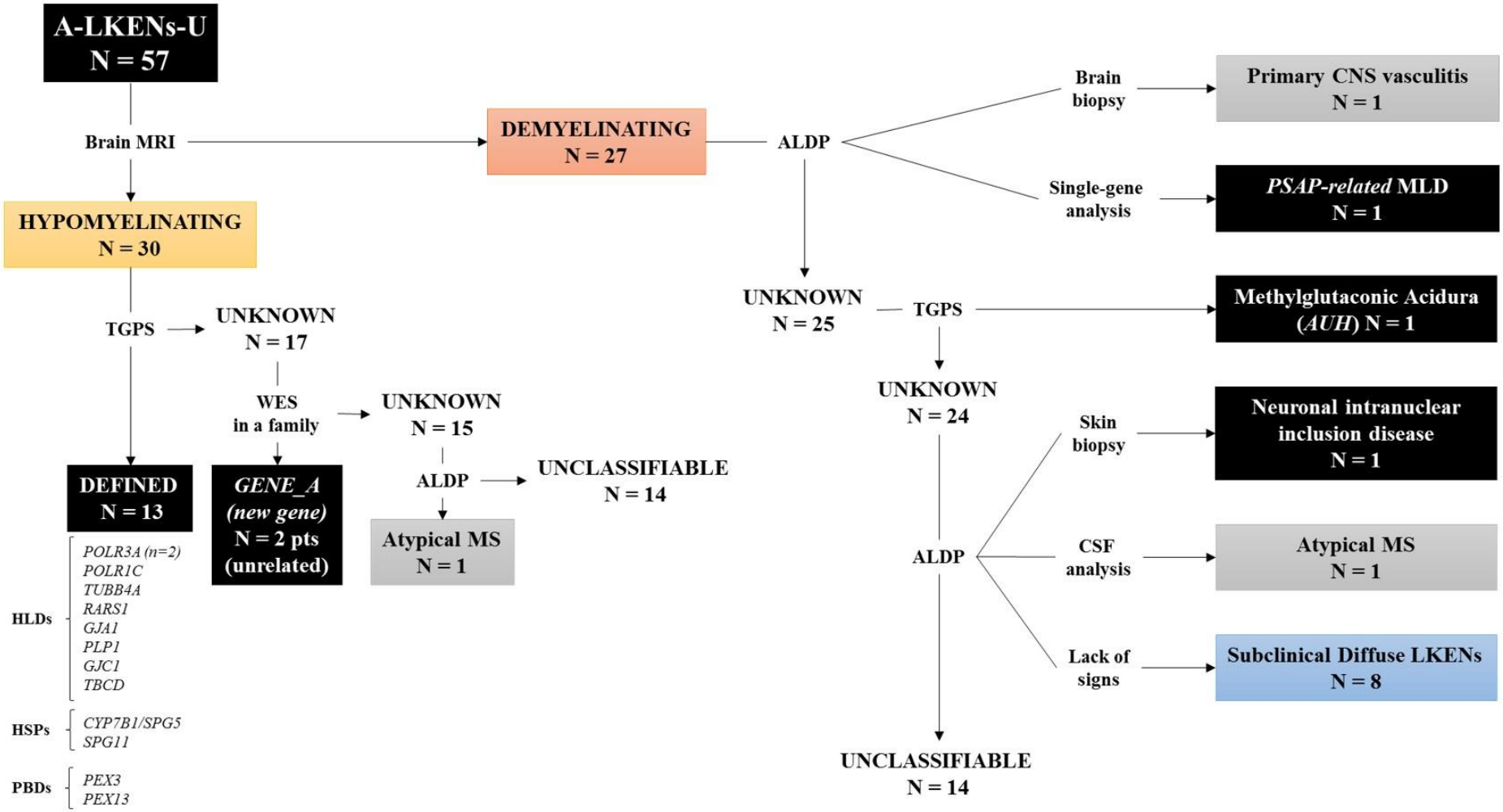

Figure 2. Flow diagram showing the results of the study. ***Abbreviations***: **A-LKENs-U**, adulthood leukoencephalopathies of unknown cause; **ALDP**, Adult Leukodystrophy Diagnostic Protocol; **CNS**, central nervous system; **CSF**, cerebrospinal fluid; **HLDs**, hypomyelinating leukodystrophy; **HSPs**, hereditary spastic paraplegias; **LKENs**, leukoencephalopathies; **PBDs**, peroxisomal biogenesis disorders; pts, patients; **MS**, multiple sclerosis; **TGPS**, targeted gene panel sequencing; **WES**, whole-exome sequencing.

HYPOMYELINATING LEUKOENCEPHALOPATHIES (HypoLKENs)

Thirteen patients (index cases, patients 1-13) out of the 30 HypoLKEN individuals were diagnosed by using our custom TGPS. In two other index patients (patients 14a and 15) and in the affected sister of the proband (patient 14B), the diagnosis was made thanks to the identification of a novel disease-causing gene in the two siblings by using WES. Finally, in one patient (patient 16) out of the residual 15 undiagnosed individuals, we suspected an atypical variant of MS based on further review of the clinical and paraclinical features of the disease.

The crucial clinical and genetic findings of the fifteen diagnosed patients (and of the sister of the patient 14a) are summarized in **Table 2**. Briefly, from a clinical perspective, key manifestations are slowly progressive walking difficulties associated with lower limb spasticity and/or cerebellar ataxia, combined with mild cognitive impairment. The cognitive impairment is usually the result of overlooked learning disabilities followed by slowly progressive cognitive decline. Details of the patients 1-11 have been previously reported (Di Bella et al. 2021), while details of the remaining patients (patients 12, 13, 14A, 14B and 15) are reported below.

CYP7B1 (SPG5, OMIM # 270800)

Patient 12 was a 40-year-old man born to consanguineous parents. He received medical attention because of a 7-year history of urinary urgency and a 3-year history of walking difficulties associated with leg stiffness and unsteadiness. The disease course was very slowly progressive. At our assessment, he had very mild spastic gait and could still run. He had poor scholar performances, but the Sartori's brief intelligence test estimated a normal premorbid IQ (107.6) (Sartori et al. 1997), and Montreal Cognitive Assessment (MoCA) revealed no cognitive impairment (26/30; normal value (n.v.) $\geq$ 26/30). CSF analysis showed the

Table 2. Key clinical features of the adult patients with hypomyelinating leukoencephalopathies and pathogenic or possibly pathogenic variants[†]

Patient ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14A	P14B	P15
Gene	*POLR3A*	*POLR3A*	*POLR1C*	*RARS1*	*TUBB4A*	*GJA1*	*PLP1*	*GJC2*	*PEX3*	*PEX13*	*TBCD*	*SPG5/CYP7B1*	*SPG11*	*GENE_A*		*GENE_A*
Sex	M	M	F	M	M	F	F	F	F	F	F	M	M	M	F	F
Psychomotor development	N	N	N	Transitory U and PN	N	N	Severe PMR (IW at 5 yrs)	Delayed (IW at 2½ yrs)	N	N	N	N	N	N	N	N
Education (years)	11	8	10	10	8	13	None	13	8	13	13	8	8	13	14	17
School performances[‡]	Poor	Poor	Poor	Poor	Poor	N	N/A	N	Poor	N	N	Poor	Poor	Poor	N	N
Age at first reported symptoms (years)[§]	39	38	~20	~45	33	~20	< 1	< 1	~15	~15	~20	~35	47	19	12	32
Initial reported symptoms	D, U	WD	TCS	WD	WD	WD	Severe PMR	WD	WD	WD, HL	WD	WD	WD	Visual loss	UL tremor	UL tremor
Estimated age of disease onset (years)[¶]	~16	~13	~15	~15	~13	~20	< 1	N/A	~10	~15	~20	~35	~12	~15	12	32
Age at first examination	39	45	38	50	39	36	21	39	57	40	45	40	52	19	15	34
Key clinical findings	CA, CI	SpG, tremor, CI	SpG, CA, CI	SpG, CI	SpG, CI	SpG	Severe CI	SpG, Dy, CI	SpG, CI	SpG, NB	SpG, CA, T, CI	SpG	SpG, CI	OA, SpG, CI	CI, tremor	SpG, tremor
Walking aid	None	Unilateral	None	None	None	None	None	None	Wheelchair-bound	Wheelchair-bound	Wheelchair-bound	None	None	None	None	None
Course / years from first reported symptoms	Slow P / 5	Slow P / 8	Slow P / 20	Slow P / 10	Slow P / 10	Slow P / 30	Stable / 25	Slow P / 43	Slow P / 45	Slow P / 30	Slow P / 25	Slow P / 8	Slow P / 10	Slow P / 9	Slow P / 8	Slow P / 15

[†]Patients 1-12 have pathogenic (class 5) or likely pathogenic (class 4) variants; Patient 13 has two class 3 variants with potential pathogenic relevance; Patients 14A, 14B and 15 have mutations in a novel gene.

[‡]Patients with poor performances experienced difficulty at school, but none had special educational support.

[§]Based on the patient's and caregiver's reports of the symptoms which led to medical attention.

[¶]Estimated in retrospect on the basis of: i) age at which the patient experienced overt difficulty at school; ii) scores on intelligence tests in adulthood (when performed).

Abbreviations: CA, cerebellar ataxia; CI, cognitive impairment; D, depression; Dy, dystonia; HL, hearing loss, HT, head tremor; IW, Independent Walking; LL, lower limb; N, normal; N/A, not applicable; NB, neurogenic bladder; P, progression; PMR, psychomotor retardation; PN, pendular nystagmus; SpG, Spastic Gait; TCS, tonic-clonic seizure; U, unsteadiness; UL, upper limb; WD, walking difficulties; yr(s), year(s).

presence of oligoclonal bands (type 3 pattern) and very mild pleocytosis. Brain MRI findings are shown in **Figure 3A**. In this patient, the already reported (Arnoldi et al. 2012) homozygous pathogenic variant c.845A>T (p.His285Leu) in the *CYP7B1* gene was identified. Consistently, biochemical analysis showed significantly elevated level of 27OH-cholesterol (519 ng/mL; n.v., 70-200).

***SPG11* (SPG11, OMIM # 604360)**

Patient 13 was a 52-year-old man who developed a slowly progressive gait disturbance due to leg stiffness since the age of 45. He had a history of learning disability, but he had a normal life (with a job, wife and one daughter). At our assessment, he had moderate, autonomous spastic gait. Mini Mental State Examination (MMSE) score was 27/30, and formal neuropsychological tests revealed difficulties in attentional and executive abilities. Sartori's brief intelligence test estimated a pathological premorbid IQ (87.2) (Sartori et al. 1997). Brain MRI findings are shown in **Figure 3B**.

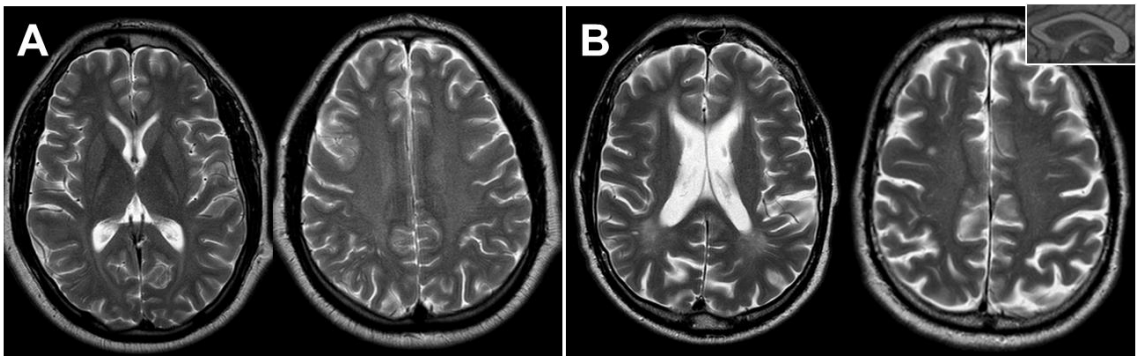

Figure 3. MRI features of SPG5 and SPG11. A) *CYP7B1* (SPG5) in T2 axial images. There is a mild, symmetric and diffuse hyperintensity of the supratentorial WM, with sparing of the subcortical regions. **B) *SPG11* in T2 axial images**. There is a mild hyperintensity of the WM especially evident in the frontal and parietal periventricular regions, as well as the typical, more marked cone-shaped hyperintensity at the tip of the frontal horns (so-called ears of the lynx sign) (Pascual et al. 2019). Mild thinning of the anterior part of the corpus callosum is present (inset).

Neurophysiological studies showed signs of mild sensory-motor neuropathy in lower limbs. Gene panel analysis revealed the presence of two compound heterozygous variants in the *SPG11* gene: the variant c.1891+1G>T previously reported in the literature

(Giannoccaro et al. 2014) and predicted to abolish the donor site of exon 9, along with the novel missense variant c.497T>C (p.Leu166Pro) on the other allele.

GENE_A (novel hypomyelinating leukodystrophy)

We identified two heterozygous, possibly pathogenic variants in a new gene (henceforward termed *GENE_A*) involved in gene expression and translational processes by using whole exome sequencing (WES) in a family (# H2042) consisting of two healthy non-consanguineous parents, two affected siblings (one male, patient 14A, and one female, patient 14B), and one healthy brother (**Figure 4**).

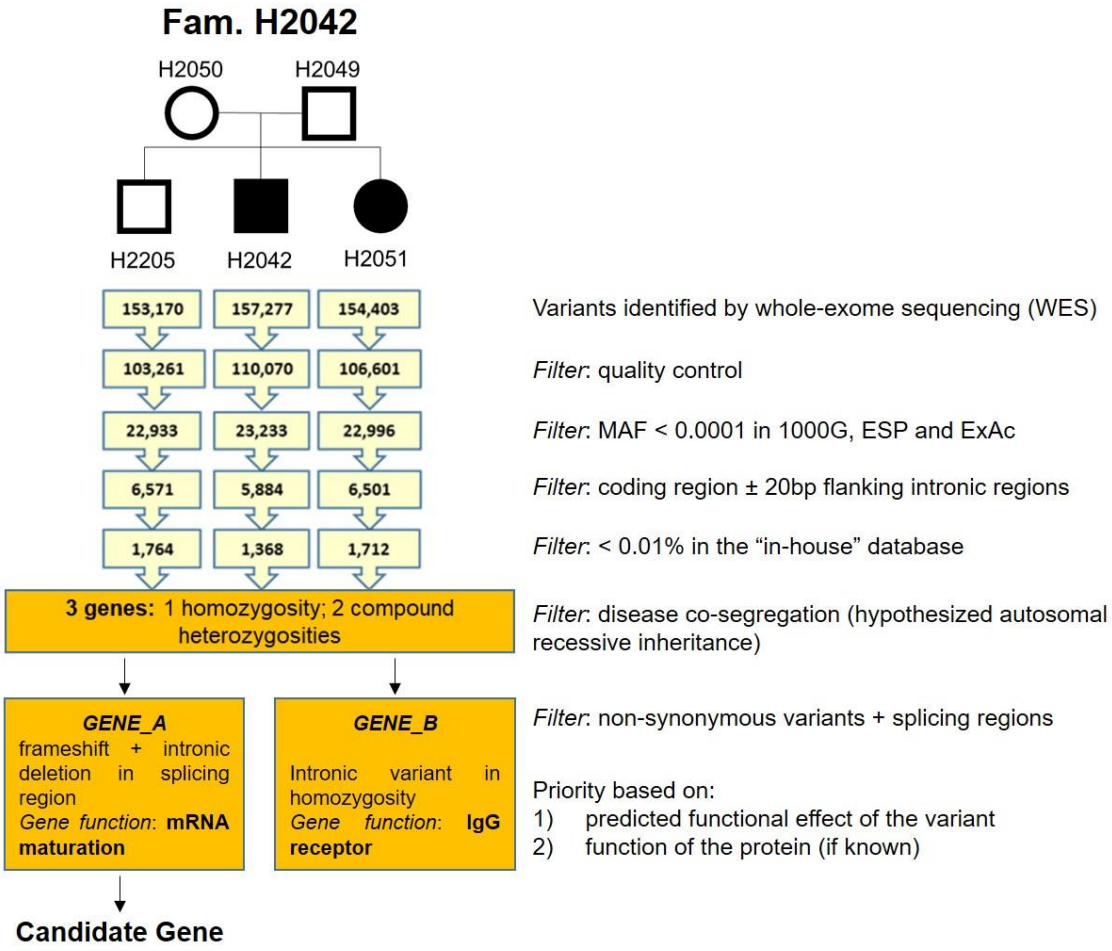

Figure 4. **Whole-exome sequencing (WES) filtering diagram**. Flowchart for WES filtering strategies according to quality, rareness, disease co-segregation, deleteriousness, and biological filters. More than 150,000 variants were identified in each patient, clearly demonstrating that filtering is crucial. Patient H2042 and H2051 are indicated in the main text as patient 14A and 14B, respectively. Abbreviations: 1000G, 1000 Genome Project; ESP, Exome Sequencing Project; ExAC, Exome Aggregation Consortium; MAF, minor allele frequency. *Courtesy of Stefania Magri, Daniela Di Bella and Franco Taroni*.

Of note, the same variants were identified in an unrelated female presenting with similar manifestations (patient 15), other genes involved in gene expression and translational processes (e.g., *POLR3A*, *POLR3B* and *POLR3K*) are responsible for HLDs (**Appendix Table S1**), and functional studies support the pathogenic role of those variants. Indeed, the GENE_A protein expression in fibroblasts from the three affected subjects was significantly reduced (**Figure 5**), and the results from zebrafish experiments are as follows (zebrafish (*Danio rerio*) is an *in vivo* model useful to assess the role of genes in myelin formation, given that the pathways involved in CNS development are preserved among vertebrates) (**Figure 6**). We found a defective response in a touch-response motility assay in 2.5- and 4-days post-fertilization embryos in a *gene_A* knockdown zebrafish model generated by the injection of an antisense oligonucleotide called morpholino (*gene_A*-MO) (**Figure 6, B and E**; results 4-days post-fertilization are not shown). Further: i) at molecular level, by using real-time quantitative PCR and *in situ* hybridization, the *gene_A* knock-down was found to reduce the expression of two markers, the oligodendrocyte transcription factor 2 (Olig2) and myelin basic protein (Mbp), which are commonly used for the screening of defects in myelin formation (**Figure 6, F and G**); ii) the injection of the zebrafish mRNA of *geneA* (not shown) and

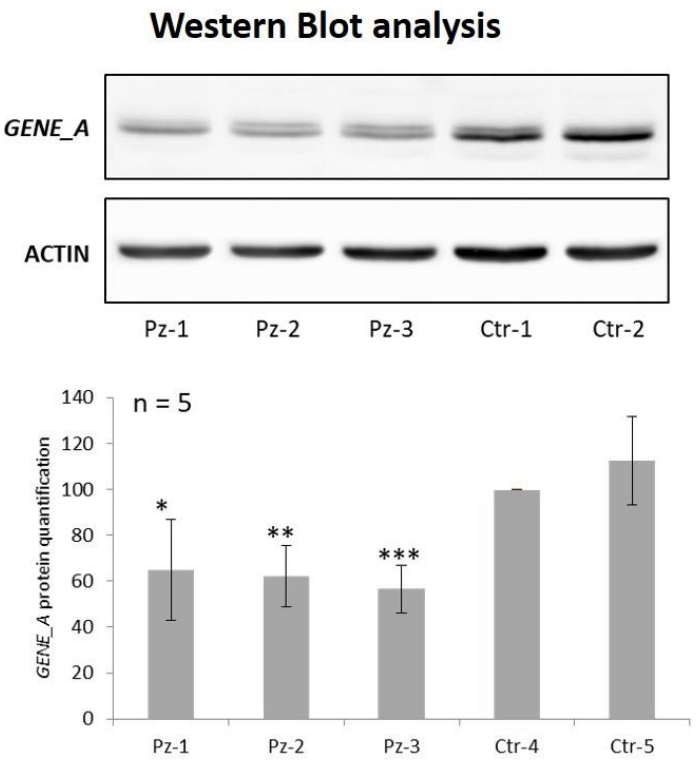

Figure 5. Western blot analysis of the GENE_A protein expression in the patients' fibroblasts. In all the three patients (Pz-1, Pz-2 and Pz-3), there is a significant reduction of the GENE_A protein expression in the fibroblasts, when compared to two healthy controls (Ctr-4 and Ctr-5). *Courtesy of Stefania Magri, Daniela Di Bella and Franco Taroni*.

of the human mRNA of *GENE_A* (**Figure 6C**) into *gene_A*-MO embryos was able to rescue the motor defect (**Figure 6E**) and to recover the olig2 and mbp expressions (**Figure 6, F and G**), confirming that the observed phenotype is specifically caused by *gene_A* down-regulation. In contrast, the injection of the human mRNA of *GENE_A* carrying the mutation identified in the HLD patients (**Figure 6D**) did not rescue the phenotype of *gene_A*-MO injected embryos (**Figure 6, F and G**), confirming the pathogenicity of the mutations.

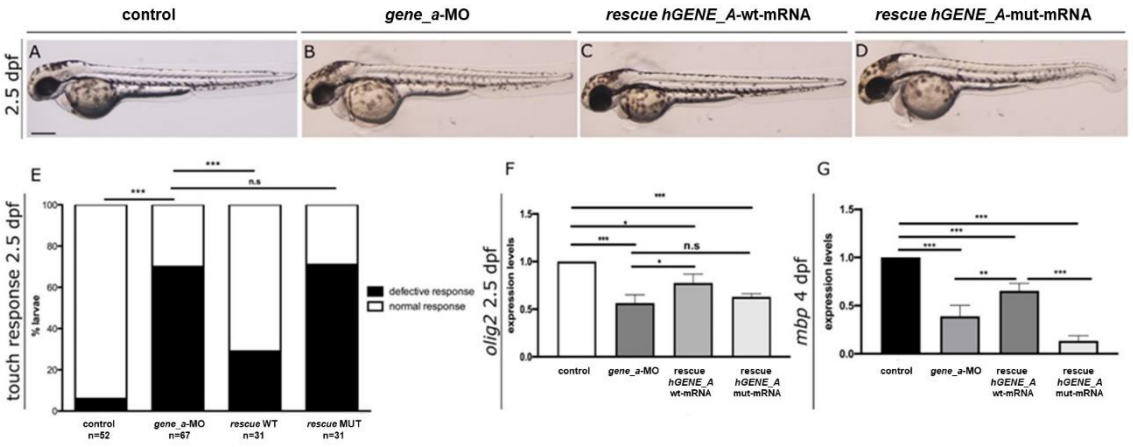

Figure 6. Results of the functional studies in a zebrafish model of *Gene_a* deficiency 2.5 days post-fertilization. See the main text for details (manuscript in preparation). Olig2 is a basic helix-loop-helix transcription factor essential for oligodendrocyte development; Mbp is a membrane protein expressed by oligodendrocytes in the central nervous system and required for maintenance of the compact multi-lamellar membrane structure of mature myelin. Abbreviations: dpf, days post-fertilization; *hGENE_A*, human *GENE_A*; MO, Morpholino antisense oligonucleotide; MUT (or mut), mutant; WT (or wt), wild-type. *Courtesy of Anna Silvia Pistocchi*.

Clinically, all the three patients had a late-onset phenotype (**Table 2**, patients 14A-B and 15). Mild action tremor in the upper limbs was the most consistent feature at onset, and it was the symptom leading the patient to the medical assessment in two cases (patient 14B and patient 15). Visual loss was the initial complaint in the third case, i.e., the proband (patient 14A), and progressively developed in the others (patients 14B and 15). Intellectual disability was present in one case (patient 14B), only a very mild cognitive impairment was observed in her brother (patient 14A), while a severe cognitive decline developed in the last case during the follow-up (patient 15). Gait difficulties associated

with lower limb spasticity or cerebellar ataxia were absent or minimal at presentation, but they became evident or even severe over about a decade in two patients (patient 14A and 15). Brain MRI showed the typical discrepancy between the T2/FLAIR-weighted and T1-weigthed images observed in hypomyelinating leukodystrophies, i.e., an evident, but relatively mild, diffuse T2-hyperintensity combined with (almost-)normal T1 signal (**Figure 7**). Further, there were the involvement of subcortical U fibers and internal capsules, a varying degree of brain and corpus callosum atrophy (more severe in the oldest patient), and a proton MR spectroscopic pattern showing (near-)normal profiles of choline (Cho), creatine (Cr), N-acetyl-aspartate (NAA), and lactate (Lac), as classically seen in hypomyelinating leukodystrophies (Bizzi et al. 2008; Di Bella et al. 2021).

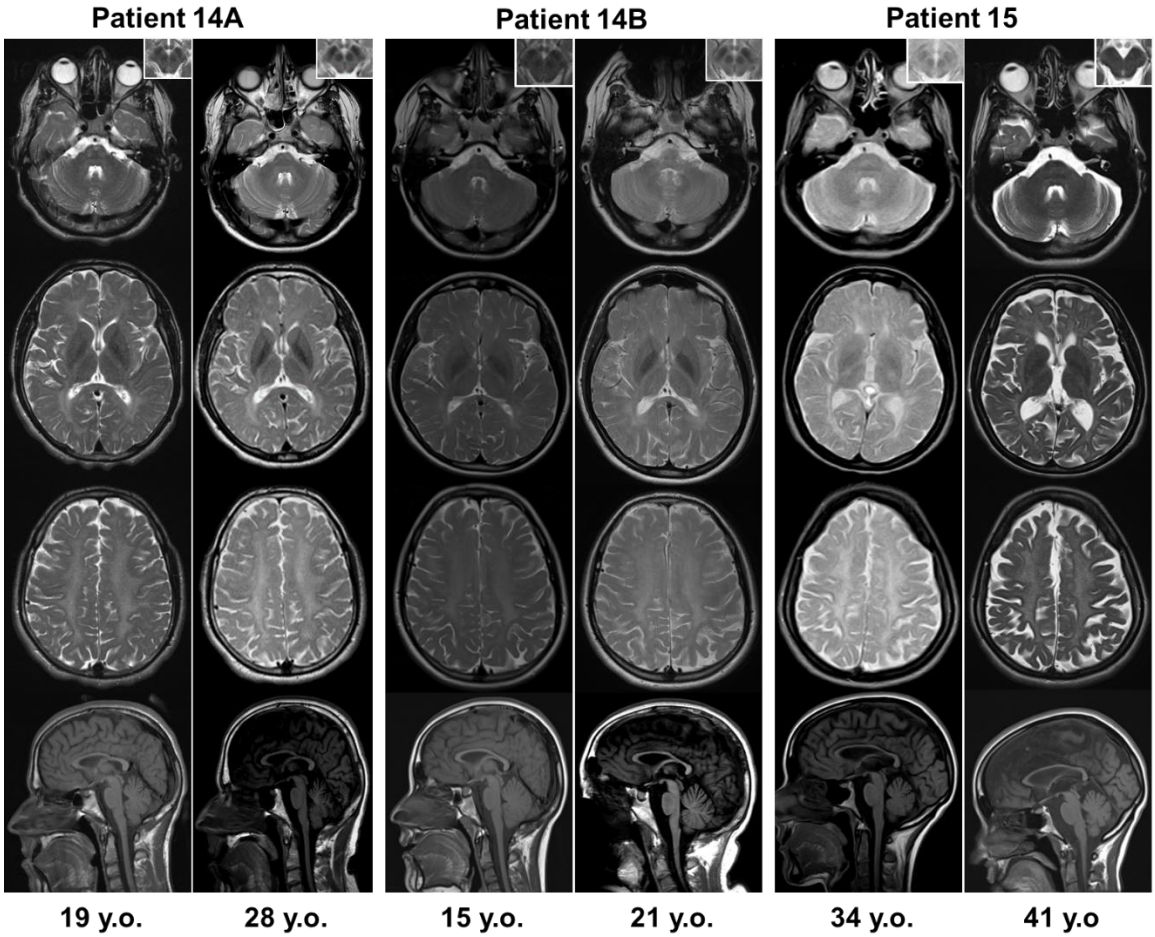

Figure 7. Brain MR imaging of the three patients with mutations in a novel gene associated with late-onset HLD. Transversal T2-weighed images showing a diffuse, mild T2-hyperintensity (involving also internal capsules and subcortical U-fibers) combined with thinning of corpus callosum (sagittal T1-weighted images). T1 signal was normal (not shown) in all patients. No overt change can be appreciated in the repeat brain MRI more than five years apart. Abbreviations: y.o., year-old.

Finally, neurophysiological studies demonstrated the lack of peripheral nerve involvement, and marked bilateral prolongation of the central conduction times without definite clinical correlates early in the disease course, as typically observed in diseases with central hypomyelination (Orthmann-Murphy et al. 2009; Abrams et al. 2014; Di Bella et al. 2021).

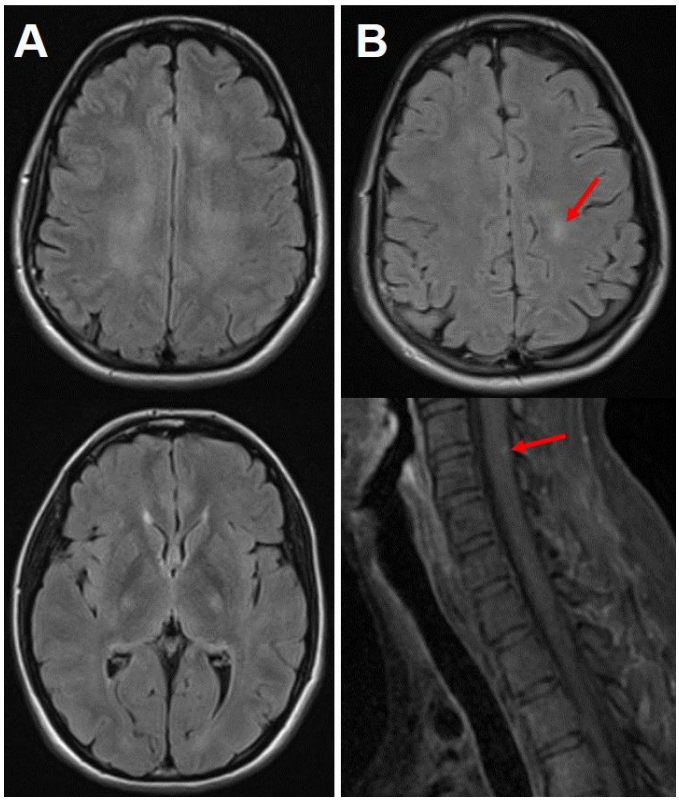

Figure 8. Atypical multiple sclerosis mimcking leuko-dystrophy. Brain MR images of patient 16. A) Diffuse, mild FLAIR hyperintensity, more marked at the tip of the frontal horns. B) Focal, more marked FLAIR hyperintensity in the centrum semiovale (upper subfigure, transversal section, red arrow), and minimal contrast enhancement in the posterior part of the cervical spinal cord (lower subfigure, sagittal section, red arrow).

Multiple sclerosis mimicking leukodystrophy

Patient 16 was a 31-year-old female complaining of subacute onset of asymmetric lower and upper limb dysesthesias combined with a transient electric shock-like sensation travelling down to the spine at neck flexion (Lhermitte's sign). Based on the brain MRI finding, showing a mild, diffuse, bilateral and symmetrical WM abnormality on T2/FLAIR-weighted images, a diagnosis of hypomyelinating LKEN was suspected **(Figure 8A)**. At our evaluation, there were mild reduction of vibration sensitivity in the distal lower limbs (left > right), exaggerated deep tendon reflexes, including left knee and ankle clonus, and minimal gait ataxia. Spinal cord MRI revealed a loss of grey-white matter discrimination, and proton MR spectroscopy showed an

increased choline peak. Motor, visual, auditory and somatosensory evoked potentials were diffusely altered with prolongation of central conduction times, and CSF analysis showed the presence of oligoclonal bands. High dose bolus methylprednisolone (500mg/day × 5 days) markedly improved her symptoms and signs. The patient was followed-up over the next three years during which she underwent high dose bolus methylprednisolone (500mg/day × 5 days) other two times one year apart because of the recurrence (or mild increase) of her dysesthesias. At last evaluation, she still complained of (very) mild hand and foot sensory disturbances, associated with mild-to-moderate loss of vibration sense at both feet (left > right). Deep tendon reflexes were (near-)normal, and she was living a normal life. Brain MRI showed the presence of focal, more T2/FLAIR hyperintense areas (**Figure 8B**, upper part), while cervical spinal cord MRI demonstrated a minimal area of enhancement in posterior columns (**Figure 8B,** lower part). Gene panel analysis was negative, and a diagnosis of atypical multiple sclerosis mimicking leukodystrophy was made (X. Ayrignac, Carra-Dallière, and Labauge 2018).

DEMYELINATING LEUKODYSTROPHIES

Two out of the 28 patients with DemLKENs were diagnosed based on the review of their clinical and laboratory data (Adult Leukodystrophy Diagnostic Protocol, ALDP). Specifically, patient 17 suffered from an atypical variant of primary CNS vasculitis, and her diagnosis was confirmed by brain biopsy; patient 18 was the first case of adult-onset MLD caused by pathogenic variants in the *PSAP* (prosaposin) gene (Metachromatic Leukodystrophy due to Saposin B deficiency, OMIM #249900) and her diagnosis was confirmed by *PSAP* gene analysis. Both patients have been described in detail elsewhere (Caputi et al. 2019; Fenu et al. 2019). Key clinical manifestations were as follows: in the individual with primary CNS vasculitis (female, 45-year-old), subacute onset of headache with episodes of vomiting and slow heart rate, suggesting intracranial hypertension

(Caputi et al. 2019); in the individual with *PSAP*-related MLD (female, 29-year-old), behavioural and cognitive decline with gradual onset and slowly progressive course over a period of ~7 years, combined with a diffuse leukoencephalopathy predominantly involving the frontal lobes and sparing the peri-rolandic regions (Fenu et al. 2019).

Only one patient (patient 19) was diagnosed by using our custom TGPS, which allowed the identification of a homozygous pathogenic variant in the *AUH* (AU-specific RNA-binding protein) gene. However, by reviewing again the clinical and laboratory data of the remaining 25 undiagnosed individuals, we could make two further diagnoses: one (patient 20) was an atypical cavitary multiple sclerosis (X. Ayrignac, Carra-Dallière, and Labauge 2018), the other (patient 21) was a neuronal intranuclear inclusion disease probably related to *NOTCH2NLC* gene (OMIM # 618025) (Sone et al. 2016). Finally, eight out of the remaining 22 undiagnosed individuals (~35%) were considered as having a new entity we termed "subclinical diffuse leukoencephalopathy" (SDL) (Salsano et al. 2019). Details of the patients 19-21 and of the individuals with SDL are reported below.

3-Methylglutaconic Aciduria, type 1 (*AUH*, OMIM #250950)

Patient 19 was a 61-year-old man, born to consanguineous parents. His psychomotor development was normal, he had eight year of schooling, and worked as a craftsman. He had a seven-year history of very slowly progressive walking difficulties associated with unsteadiness and weakness. Neurological examination showed mild cerebellar ataxia,

pyramidal signs and mild cognitive impairment (MoCA score was 23/30) with partial lack of insight. Brain MRI findings are shown in **Figure 9**.

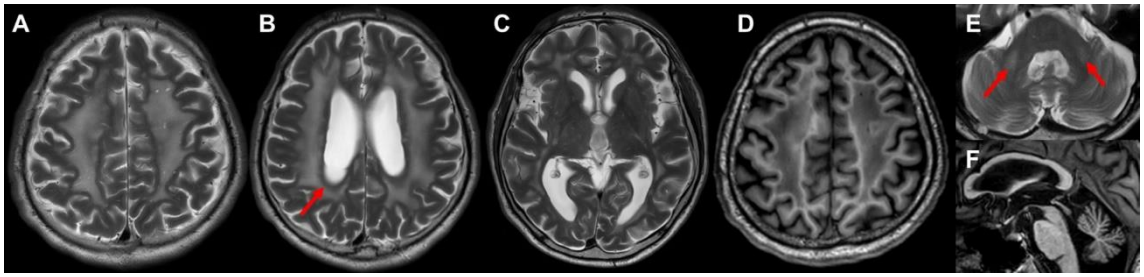

Figure 9. Brain MRI findings in a case of methylglutaconic aciduria (*AUH*-related leukoencephalopathy). The MRI pattern is characterized by prominent, diffuse T2 hyperintensity (A) combined with prominent T1 hypointensity (D), as seen by definition in demyelinating leukoencephalopathies. The typical sparing of the periventricular regions (B, red arrow) and the bilateral involvement of the middle cerebellar peduncles (E, red arrows) are also shown.

Gene panel analysis revealed the presence of a novel homozygous pathogenic variant in *AUH* gene (c.996_1004delGCCCCCTCG, p.Arg332_Arg335delinsSer). Accordingly, urinary analysis showed that 3-methylglutaconic acid (3-MGA) was markedly increased (102 μg/mg creatinine, n.v. < 15).

Cavitary multiple sclerosis

Patient 20 was a 48-year-old female with gradual onset of asymmetric lower and upper limb postural/action tremor and blurred vision five years earlier. At our evaluation, there were minimal stance ataxia, pendular nystagmus (most evident in the right eye), asymmetric postural/action tremor (~4 Hz; most evident in the left upper limb and in the right lower limb), and mild cognitive impairment. Brain MRI findings was suggestive of

an inherited leukoencephalopathy, and more specifically of a leukodystrophy with vanishing white matter (LVWM) **(Figure 10)**.

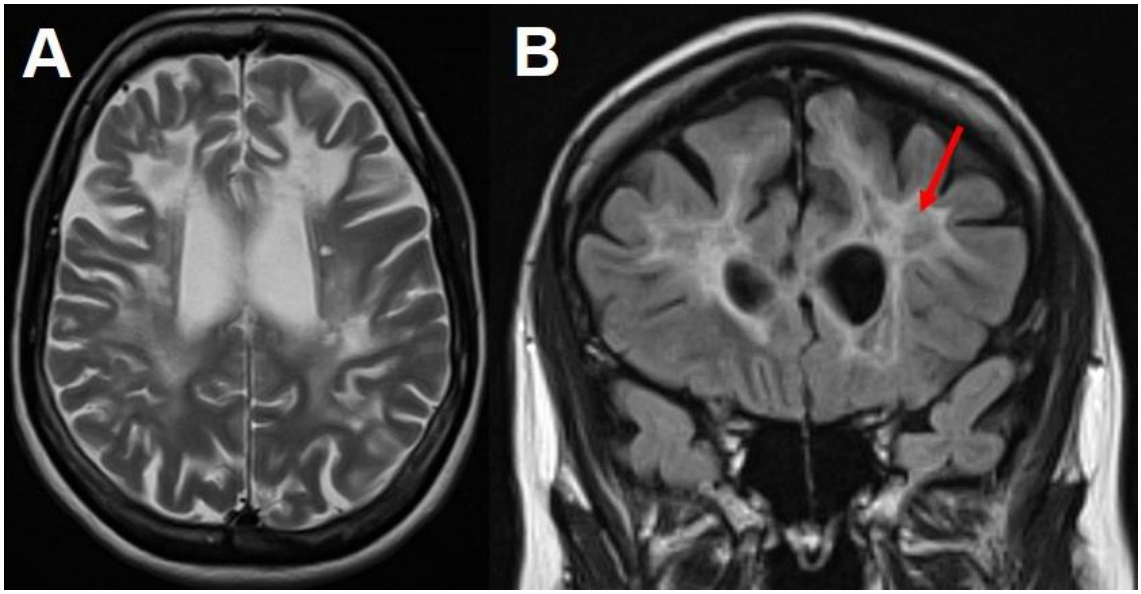

Figure 10. Brain MRI findings in a case of cavitary multiple sclerosis. The MRI pattern is characterized by diffuse, prominent T2 hyperintensity (A) with areas of FLAIR hypointensities (B, arrow red) suggesting white matter rarefaction (as seen in the leukodystrophy with vanishing white matter caused by mutations in *EIF2B1-B5* genes).

The disease course over a period of more than 5 years was (near-)stationary, repeat CSF analysis showed the presence of oligoclonal bands, gene panel analysis (including the *EIF2B1-5* genes responsible of LVWM) was negative, and a diagnosis of cavitary multiple sclerosis was made (X. Ayrignac, Carra-Dallière, and Labauge 2018).

Neuronal Intranuclear Inclusion Disease (*NOTCH2NLC*, OMIM # 603472)

Patient 21 was a 61-year-old female with recurrent headache from her young adulthood. At age 49, she underwent her first brain MRI because she started to complain of ‘pain in the nose’. The brain MRI unexpectedly showed diffuse WM abnormalities (**Figure**

11C1).

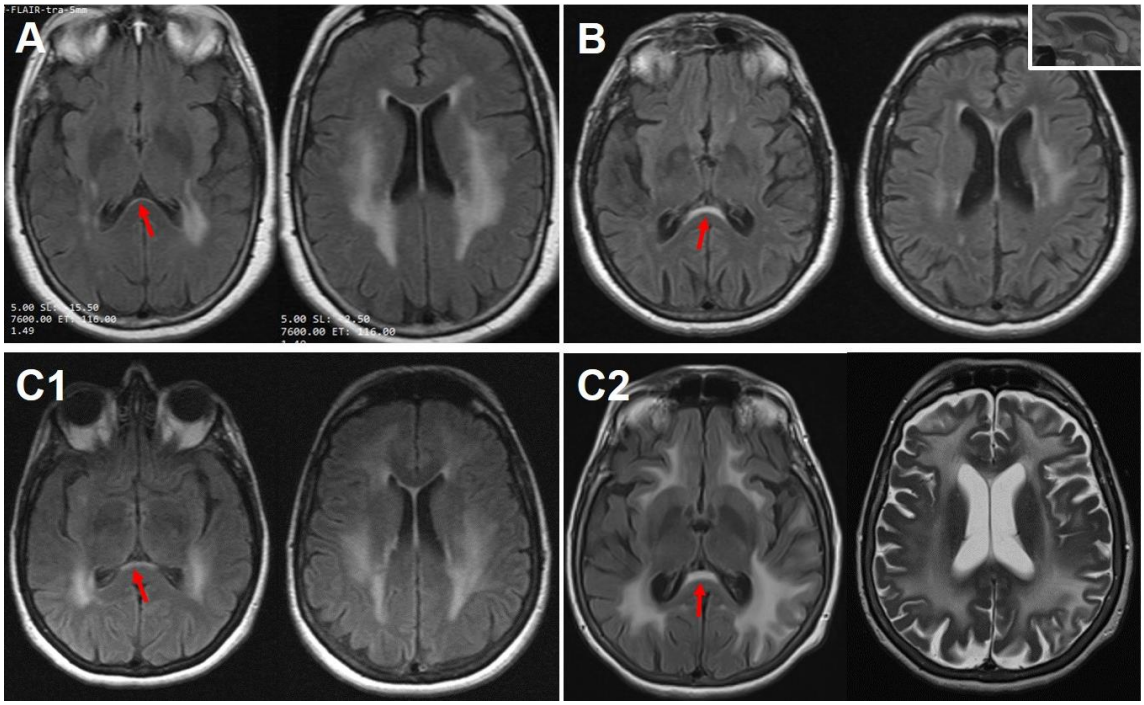

Figure 11. Brain MRI findings in a case of neuronal intranuclear inclusion disease (NIID). The first brain MRI of the proband (patient 21) is shown in C1 (FLAIR), while the last one, 13 years later, is shown in C2 (FLAIR on the left, and T2 on the right), where the marked increase of the extension of the white matter changes can be appreciated. Brain MRI of the proband's mother, performed at age 76, is shown in A (FLAIR), whereas the MRI of the proband's brother is shown in B (FLAIR). Of note, in the proband's brother, the white matter changes were much less extensive, despite his severe clinical manifestations. The varying involvement of the splenium is indicated by red arrows.

Less extensive WM abnormalities without overt clinical manifestations were found in her mother (**Figure 11A**), who was investigated because the patient's brother had had recurrent confusional episodes followed by progressive cognitive decline, and associated with much less extensive WM abnormalities (**Figure 11B**). At age 50, the patient had a transient amnesia attack, and at age 56, she started to complain of mild, inconstant action tremor in the left upper limb. Muscle biopsy combined with mitochondrial respiratory chain complex activities, and CSF and gene panel analyses were unremarkable. From age 58 on, she had recurrent encephalitic-like episodes (n = 5) characterized by severe headache, fever and disturbance of consciousness. Cognitive decline with prominent language disturbance was left from the last episode, when she was admitted to our Institution. Brain MRI findings are shown in **Figure 11C2**. EMG with nerve conduction studies revealed a subclinical mixed (demyelinating-axonal) peripheral neuropathy, as

reported in her brother. The review of a brain MRI performed in another hospital during an encephalitic-like episode showed a mildly high intensity signal in the corticomedullary junction on diffusion-weighted imaging (DWI) (**Figure 12, left**). Hence, we performed a skin biopsy, which showed eosinophilic, p62-positive intranuclear inclusions (**Figure 12, right**), and a diagnosis of neuronal intranuclear inclusion disease (NIID) was made (Sone et al. 2016). *NOTCH2NLC* analysis is still ongoing (Sone et al. 2019).

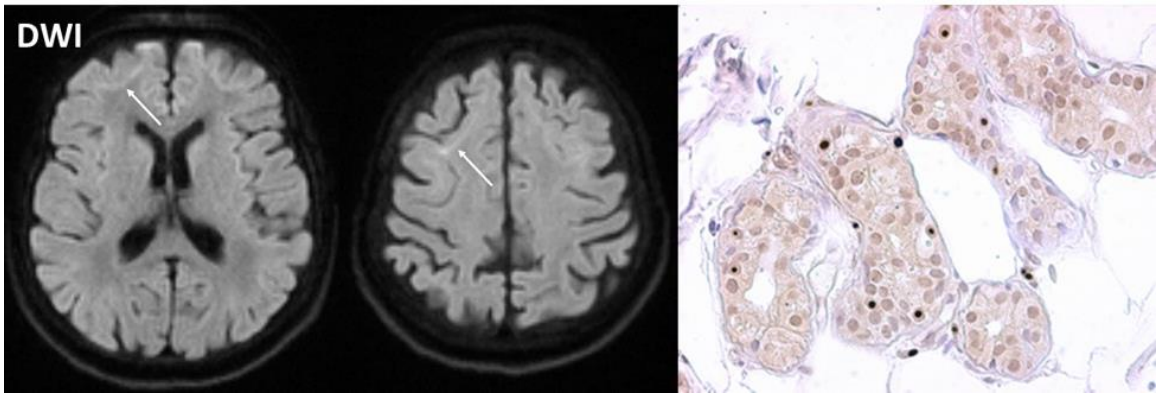

Figure 12. Diffusion-weighted imaging and skin biopsy findings in a case of neuronal intranuclear inclusion disease (NIID). Brain MRI shows a mildly high intensity signal in the corticomedullary junction on DWI (*courtesy of Marco Moscatelli*), while p62-positive intranuclear inclusions are present in the skin (magnification: 400×; *courtesy of Gianluca Marucci*).

Subclinical Diffuse Leukoencephalopathies (SDLs)

The clinical features of the patients with subclinical diffuse leukoencephalopathy (SDL) are summarized in **Table 3** (patients 22-29).

Table 3. Clinical and paraclinical features of the eight individuals with subclinical diffuse leukoencephalopathies.

Patient	22	23	24	25	26	27	28	29
Age at first MRI / Sex	39/F	52/M	72/F	50/F	60/F	57/F	46/M	59/F
Follow-up (yrs)	11	4	2	5	5	10	22	4
Symptoms at first MRI	Vertigo	Unilateral SHL & Tinnitus	Unilateral SHL & Tinnitus	Transient sensory disturbances	Recurrent phantom smells	Dizziness	Head-positional vertigo and vomiting	Recurrent dizziness
Vascular risk factors	Preeclampsia	NO	NO	Treated PFO	NO	NO	NO	NO
Key negative findings	CSF, MuB	BAEPs[1], MuB	BAEPs[1]	-	EEG	CSF	BAEPs	BAEPs
Other findings	Blood-related parents	-	Blood-related parents	Joint pain (hands and feet)	Spontaneous symptom remission	Unilateral SHL	-	-

[1]Performed a long time after the first MRI

Abbreviations: **BAEPs**, brainstem auditory-evoked potentials; **BPPV**, benign paroxysmal positional vertigo; **MuB**, Muscle Biopsy; **PFO**, patent foramen ovale; **SHL**, sudden hearing loss

Briefly, we identified eight sporadic cases (mean age 54 years (range 39-72); 6 females), who had undergone MRI because of transient symptoms such as dizziness, vertigo, sensory disturbances, hearing loss and tinnitus, and were referred to our Institute because of a possible genetic LKEN. Key brain MRI findings are showed in **Figure 13**. No neurological or extra-neurological sign was observed after a mean follow-up of 8 years (range 2-22), and neuroimaging findings remained stationary. There was no evidence of brain injury at birth or vascular risk factor. Neuropsychological assessment, neurophysiological testing, and CSF analysis (when performed) were unremarkable, as well as our comprehensive custom TGPS.

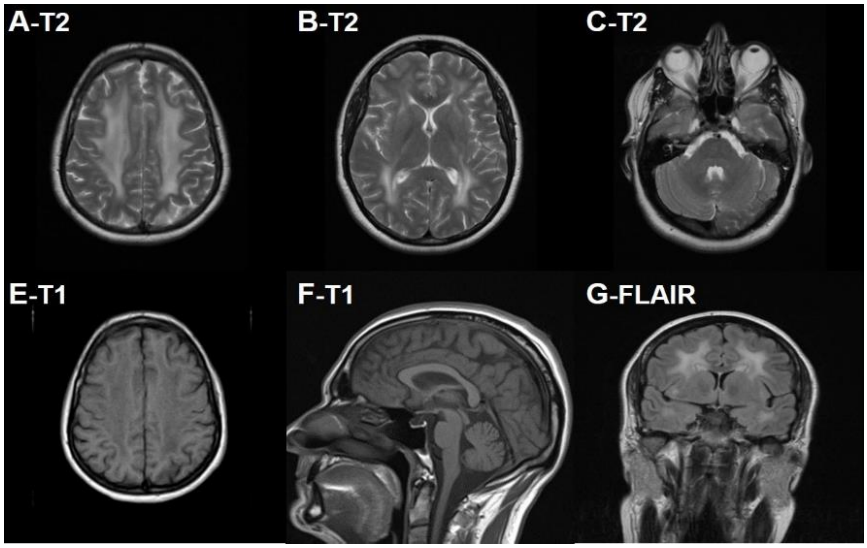

Figure 13. MRI findings in subclinical diffuse leukoencephalopathies (SDLs). The MRI pattern is characterized by diffuse, prominent T2/FLAIR hyperintensity (A, B, G) combined with prominent T1 hypointensity (E). There is no thinning of corpus callosum (F), and there are no pontine T2 hyperintensities (C). No microhemorrhage was present on T2-star sequences. External capsules, thalami and infratentorial regions can be occasionally observed.

DISCUSSION

Little is known about leukoencephalopathies of unknown cause in adults, and only a few cohorts of patients have been previously analyzed (Xavier Ayrignac et al. 2015; Lynch et al. 2017; Kunii et al. 2018). In this study, we systematically examined a cohort of adult patients with leukoencephalopathy of unknown cause. The major findings of this study are as follows.

First, an MRI pattern suggestive of hypomyelination is common among adult patients with undiagnosed leukoencephalopathy (henceforward termed "hypomyelinating leukoencephalopathies", HypoLKENs), is almost always associated with monogenic diseases. *Second*, in adults, HypoLKENs are a group of (quite) clinically homogeneous degenerative diseases caused by pathogenic variants in genes associated with classical, *early-onset* hypomyelinating leukodystrophies (HLDs), unexpected known genes or even new disease-causing genes. *Third*, after a systematic review of the clinical and laboratory data, in adult patients with undiagnosed leukoencephalopathies showing an MRI pattern suggestive of demyelination (henceforward termed "demyelinating leukoencephalopathies", DemLKENs), a definite diagnosis is only occasionally made by using a targeted test for genetic causes of leukoencephalopathy. *Forth*, a (likely small) subset of sporadic adult patients with a diffuse leukoencephalopathy may have an atypical form of acquired diseases resembling a leukodystrophy. *Fifth*, a subgroup of adult individuals can have a diffuse leukoencephalopathy with an MRI pattern suggestive of demyelination which remains asymptomatic over a long-time frame.

Leukoencephalopathies with an MRI pattern suggestive of hypomyelination (HypoLKENs) are common in adults and genetic in nature

Unlike previously reported case series (Xavier Ayrignac et al. 2015; Lynch et al. 2017; Kunii et al. 2018), we provide evidence that an MRI pattern suggestive of hypomyelination, i.e., mild T2-hyperintensity in combination with normal or almost normal T1 signal (Schiffmann and van der Knaap 2009) characterizes a large number (~50%, n = 30) of adult patients with leukoencephalopathy. Half of these patients (n = 15) were diagnosed by using a next generation sequencing (NGS) approach, which is probably the most cost-effective way for the diagnosis of these diseases. In fact, they are considered by definition as genetic in nature, and are difficult to differentiate one from another, particularly in the late-onset cases (Macaron et al. 2019; Di Bella et al. 2021). Indeed, clinical manifestations (usually extra-neurological, such as dental anomalies or hand malformations) and/or characteristic brain MRI findings unequivocally guiding clinicians to the proper diagnosis (e.g., optic radiation sparing in *POLR3A*-related HLDs or typical brainstem tract involvement at the level of the pons in *GJA1*-related oculodentodigital dysplasia) are infrequent (Di Bella et al. 2021; Harting et al. 2019; Wolf et al. 2014), discouraging a single-gene analysis approach. In the remaining half of patients (all sporadic), no disease-causing gene was found, but a diagnosis of an atypical form of multiple sclerosis was made in one patient, suggesting that acquired diseases can (very) rarely be misdiagnosed as HLDs (X. Ayrignac, Carra-Dallière, and Labauge 2018).

Genetic and clinical features of the diagnosed HypoLKENs in adults

Thirteen out of 15 patients were diagnosed by using a custom targeted gene panel sequencing (TGPS) for leukoencephalopathies. In the remaining two patients, one familial, the other sporadic, we identified pathogenic variants in a novel disease-causing gene by using WES in the familial case, and by using single gene analysis in the sporadic

one (selected on the basis of her clinical and paraclinical findings). Only in two subjects (patients 6 and 7, classified as "early-onset" patients), one with a *PLP1*-related disease (HLD1/Pelizaeus-Merzbacher disease), the other with a *GJC2*-related disease (HLD2/Pelizaeus-Merzbacher-like disease 1), the onset was unequivocally in the first year(s) of life, as classically seen in HLDs (in both cases the pathogenic variants in *PLP1* and *GJC2* were missed on targeted single-gene analysis because of technical issues (Di Bella et al. 2021)). They represent examples of patients with an inherited disease having onset in childhood, and persisting into adulthood, because of the relatively benign evolution or improvement of palliative cares. In the remaining 13, the onset was during the second decade or later. In seven cases, the disease-causing genes (i.e., *POLR3A*, *POLR1C*, *TUBB4A*, *RARS1*, *GJA1* and *TBCD*) have been previously associated with HLDs. In contrast, two patients had two forms of hereditary spastic paraplegias (i.e., SPG5 and SPG11) which are characterized by WM abnormalities that might resemble central hypomyelination (see also below). Two further patients had two distinct peroxisomal biogenesis disorders (PBDs) related to *PEX3* and *PEX13*, which are diseases habitually excluded from the differential diagnosis of leukoencephalopathies in adults. The last two subjects (patients 14A and 15) present a new disease (manuscript in preparation), providing evidence that there may be leukodystrophies with hypomyelinating features (HLDs) caused by still-unidentified genes, and presenting later in life.

From the clinical perspective, all these late-onset patients presented walking difficulties caused by spasticity or, rarely, cerebellar ataxia, combined with mild cognitive impairment. In the two patients with the novel disease-causing gene (termed *GENE_A* throughout the manuscript), a crucial manifestation was vision loss from optic atrophy. Visual loss from optic atrophy has been associated with hypomyelinating leukodystrophies, such as PMD/HLD1 (*PLP1*), HLD2 (*GJC2*), HLD6 (*TUBB4A*), HLD7

(*POLR3A*), HLD12 (*VPS11*) and HLD14 (*UFM1*), but it is rarely a prominent manifestation, especially when the disease onset is late in life. Therefore, we hypothesize that this finding might be a specific sign of this novel disease.

As to the neuroimaging features, two main subgroups can be further identified, based on extension and degree of brain T2 hyperintensity. The first subgroup is characterized by widespread and relatively marked T2 WM hyperintensity, also involving U-fibres and internal capsules, and includes patients with leukodystrophies caused by genes classically associated with hypomyelination (e.g., *PLP1*). The second subgroup is characterized by milder WM T2 hyperintensity, which can be diffuse, as in HLD2 (*GJC2*) (**Figure 1A**), or localized in the periventricular regions as in the two patients with SPG5 and SPG11 (**Figure 3A** and **Figure 3B**). Less extensive, incomplete and patchy WM abnormalities have been reported in classical hypomyelinating disorders (Schiffmann and van der Knaap 2009; Sagnelli et al. 2016), but they may also suggest different underlying pathophysiological bases, as in SPG5 and SPG11. In fact, in these diseases (which are not considered as HLDs), there can be a pattern of diffuse, mild T2 hyperintensity and almost normal T1 signal, which may lead to an *in vivo* diagnosis of hypomyelination in the clinical practice (Parikh et al. 2015; Dreha-Kulaczewski et al. 2006; Riverol et al. 2009; Mignarri et al. 2015; Pascual et al. 2019). Interestingly, the same occurs for SPG35 (*FA2H*) not classified as HLD (Pensato et al. 2014), but characterized by myelin formation defect (Edvardson et al. 2008). Little is known about the mechanism underlying SPG5 and SPG11 WM abnormalities (Aghakhanyan et al. 2014; Roos et al. 2014), although myelin involvement is more likely secondary to axonal damage. Whatever the mechanism underlying WM changes is, our findings corroborate the clinical overlap between hypomyelinating leukodystrophies (HLDs) and hereditary spastic paraplegias (HSPs), and suggest that all the genes causing hereditary spastic paraplegias with WM

changes should be considered in patients with hypomyelinating imaging findings, and HLD genes should be considered in patients with progressive spastic paraplegias.

Leukoencephalopathies of unknown cause with an MRI pattern suggestive of demyelination (DemLKENs) are rarely diagnosed by targeted gene panel sequencing (TGPS)

Unlike adults with undiagnosed HypoLKENs, adults with DemLKENs were diagnosed only occasionally by TGPS, if a diagnostic work-up combining clinical manifestations, MRI-based recognition pattern and other laboratory data is systematically applied in clinical practice. Of note, many LKENs supposed to be genetic remain without diagnosis also if WES is used (Lynch et al. 2017). In our work, the diagnosis was made as a result of the TGPS in one patient only (patient 19), who had methylglutaconic aciduria type 1 (MGCA1), i.e., an extremely rare autosomal recessive disorder of the leucine catabolism caused by mutations in the *AUH* gene. The diagnosis was also missed because urinary organic acid analysis was not performed as screening test because: i) organic acidurias are typically characterized by onset early in life, (overlooked) psychomotor delay/learning disabilities, and basal ganglia alterations, subcortical predominance of the WM abnormalities (2L-hydroxyglutaric aciduria) and/or frontotemporal hypoplasia and subependymal nodules (glutaric acidaemia type I) on brain MRI (Wajner 2019); ii) the disease was not considered in the differential diagnosis (no more than a couple of MGCA1 cases has been described in adults (Eriguchi et al. 2006; Wortmann et al. 2010)). Actually, our patient presented the crucial clinical and neuroimaging features of the late-onset form of MGCA1, i.e., a progressive spastic-ataxic syndrome with cognitive decline and diffuse leukoencephalopathy, also involving the middle cerebellar peduncles (Eriguchi et al. 2006), and sparing the subcortical and periventricular WM regions (Steenweg et al. 2009; Wortmann et al. 2010) (**Figure 9**). Unlike MGCA1, the other

definitive diagnosis of a genetic leukoencephalopathy was made thanks to the revision of the clinical and paraclinical data. In fact, in a young adult (patient 18), the combination of: i) progressive behavioral and cognitive decline, ii) demyelinating peripheral neuropathy with characteristic MLD inclusions at sural nerve biopsy, and iii) leukoencephalopathy sparing the periventricular regions and predominantly involving the frontal lobes was strongly suggestive of metachromatic leukodystrophy (MLD) (Fenu et al. 2019; Benzoni et al. 2021). Hence, given the lack of mutations in the *ARSA* gene, the *PSAP* gene was subsequently analyzed by direct sanger sequencing because of our very focused question, and the first case of *PSAP*-related MLD of adult onset was identified (Fenu et al. 2019). In a third case (patient 21), the diagnosis of a genetic leukoencephalopathy was suspected on the basis of the family history, but no mutation was found through our TGPS approach. Again, it was the revision of the clinical and paraclinical data, mostly the combination of recurrent encephalitic-like episodes and a stripe of high intensity signal in the corticomedullary junction of the frontal lobe on diffusion-weighted images (DWI) (Ye et al. 2021), which led us to hypothesize that our patient could have a neuronal intranuclear inclusion disease (NIID). This hypothesis was confirmed by the presence of eosinophilic, p62-positive intranuclear inclusions identified on a skin biopsy, and considered as hallmark of the disease. NIID is an autosomal dominant, slowly progressive neurodegenerative disorder characterized by a wide range of clinical manifestations, so far reported only in individuals of East Asian descent (Yau et al. 2020), and recently related to *NOTCH2NLC* gene (Sone et al. 2019). In our case, the gene analysis of *NOTCH2NLC* is still ongoing, because the mutation consists of a heterozygous trinucleotide repeat expansion (GGC) in the 5-prime untranslated region, and a specific protocol to detect tandem repeats is required (Sone et al. 2019). This case clearly shows two limitations of NGS, when no disease-causing mutation is identified: i) when a TGPS approach is used, the possibility that a gene not included in the panel is

responsible for the disease (e.g., because it is discovered as a cause of leukoencephalopathy after the panel is designed); ii) when a WES (or even a whole genome sequencing, WGS) approach is used, the possibility that the new, still-unidentified gene has mutations which cannot be easily identified, e.g., because they may require specific protocols.

Leukoencephalopathies of unknown cause can be caused by atypical variants of acquired diseases mimicking a leukodystrophy

In clinical practice, a targeted gene panel sequencing (TGPS) could be performed with the aim to rule out genetic diseases of known cause in difficult-to-classify patients. When no disease-causing mutation is identified, it should be always considered the hypothesis that the cause of the leukoencephalopathy is acquired, particularly in sporadic adult cases. Our work provides evidence that acquired leukoencephalopathies may look like leukodystrophies, although rarely. Indeed, one patient (patient 17) had a leukodystrophy-like CNS vasculitis. In this case, the diagnosis was suspected because of her clinical manifestations (i.e., intracranial hypertension syndrome), and confirmed by brain biopsy, without the support of any genetic testing. Brain biopsy should be systematically considered as a diagnostic option in adult subjects with diffuse leukoencephalopathy of unknown cause, despite its invasive nature (Caputi et al. 2019; Markovic and Basic 2020). By contrast, in two other patients, the diagnosis of an atypical multiple sclerosis mimicking a genetic leukoencephalopathy was considered and/or corroborated on the basis of clinical manifestations such as asymmetry, positive sensory symptoms, and response to steroids, presence of oligoclonal bands and lack of mutations on the TGPS. None of these features can be considered as definitive sign of acquired/inflammatory disease. For instance, though exceptionally, response to steroids has been reported in genetic leukoencephalopathies (Wolf et al. 2015), and the presence of an intratechal

immunoglobulin synthesis should not automatically lead to exclusion of non-inflammatory neurological diseases (Pannewitz-Makaj et al. 2020). In patient 20, TGPS allowed leukodystrophy with vanishing white matter to be ruled out, and in patient 16, who presented an MRI pattern suggestive of hypomyelination, TGPS allowed HLDs and other genetic diseases with hypomyelinating-like abnormalities to be formally excluded. Nevertheless, we cannot exclude the presence of pathogenic variants in genes not included in the panel or the possibility that certain pathogenic variants such as deep intronic variants are missed by this test.

Subclinical diffuse leukoencephalopathies: proposal of a new entity.

There is a subgroup of adult patients with a diffuse leukoencephalopathy with demyelinating features who remained asymptomatic during their long-lasting follow-up (years/decades). Their diffuse white matter changes were discovered incidentally, when they underwent brain imaging because of a variety of transient symptoms difficult to correlate with their leukoencephalopathy. We showed that their white matter changes are not associated with known genes responsible for leukoencephalopathies. The patients are about 35% of the undiagnosed subjects with demyelinating features (eight out of 22), are frequently middle-aged females, and it is impossible to predict when they develop clinical manifestations, if ever. The condition could be referred to as “subclinical diffuse leukoencephalopathy”, may lead to extensive, unsuccessful investigations, and may cause anxiety to the patients by mimicking severe leukodystrophies. The pathogenesis of this condition remains unknown, but it may reflect a brain water content increase due to blood-brain barrier permeability changes possibly related to benign micro-vessel dysfunction, as seen in mitochondrial neurogastrointestinal encephalopathy syndrome (MNGIE), *TYMP*-related (Gramegna et al. 2018).

In the future, it will be worth to verify whether advanced neuroimaging techniques, including quantitative MRI and PET imaging using specific myelin radioligands, might allow an *in vivo* characterization of the pathological substrate of these subclinical diffuse leukoencephalopathies, as well as a clusterization of the patients with still-undiagnosed leukoencephalopathies in addition to the MRI-based recognition pattern. Indeed, we believe that NGS approaches are unlikely to identify new disease-causing genes in sporadic cases, unless they are grouped in homogenous clusters.

Conclusions

In summary, a hypomyelinating pattern characterizes a large number (~50%) of undefined leukoencephalopathies in adults, which can be usually due to genes causing severe early onset hypomyelinating leukodystrophies, hereditary spastic paraplegias, and even peroxisomal biogenesis disorders or novel disease-causing genes. Among the undefined leukoencephalopathies with demyelinating features, only few (<20%) are definitively diagnosed by the review of the clinical and laboratory data and/or thanks to a targeted-gene panel sequencing approach. The possibilities of acquired diseases (e.g., rare variants) or genetic diseases caused by unconventional, difficult-to-detect mutations should be always considered, since in these cases, targeted gene panel sequencing and even whole-exome or whole-genome sequencing may be useless (or of limited utility). Finally, we found that, in adulthood, a subgroup of undiagnosed leukoencephalopathies with demyelinating features is characterized by lack of neurological signs even after a follow-up of many years. Despite their etiopathogenesis is unknown, they may represent a new benign syndrome termed “subclinical diffuse leukoencephalopathy” which deserves further investigations.

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APPENDIX

Table S1. List of leukoencephalopathies (LKENs) with hypomyelinating (Hypo) or demyelinating (Dem) features.

HypoLKENs	OMIM	Inheritance pattern / Gene
Pelizeus-Merzbacher disease (leukodystrophy, hypomyelinating, 1: HLD1)	312080	XLR / PLP1
HLD2 (Pelizaeus-Merzbacher-Like Disease, 1: PMLD1)	608804	AR / GJC2
HLD3	260600	AR / AIMP1
HLD4	612233	AR / HSPD1
HLD5 (Hypomyelination and Congenital Cataract: HCC)	610532	AR / FAM126A
HLD6 (Hypomyelination with atrophy of basal ganglia and cerebellum: HABC)	612438	AD / TUBB4A
HLD7 (Hypomyelination, hypogonadotropic hypogonadism and hypodontia; 4H syndrome)	607694	AR / POLR3A
HLD8	614381	AR / POLR3B
HLD9	616140	AR / RARS1
HLD10	616420	AR / PYCR2
HLD11 (4H leukodystrophy 3)	616494	AR / POLR1C
HLD12	616683	AR / VPS11
HLD13	616881	AR / HIKESHI
HLD14	617899	AR / UFM1
HLD15	617951	AR / EPRS
HLD16	617964	AD / TMEM106B
HLD17	618006	AR / AIMP2
HLD18	618404	AR / DEGS1
HLD19 (Leukodystrophy, hypomyelinating, 19, transient infantile)	618688	AD / TMEM63A
HLD20	619071	AR / CNP
HLD21	619310	AR / POLR3K
HLD22	619328	AD / CLDN11
HLD23 (Leukodystrophy, hypomyelinating, 23, with ataxia, deafness, liver dysfunction, and dilated cardiomyopathy	619688	AR / RNF220
Peripheral demyelinating neuropathy, central dysmyelination, Waardenburg syndrome, and Hirschsprung disease (PCWH)	609136	AD / SOX10
Oculodentodigital dysplasia (ODDD)	257850	AR / GJA1
DemLKENs	**OMIM**	**Inheritance pattern / Gene**
Metachromatic Leukodystrophy	250100	AR / ARSA
Metachromatic Leukodystrophy due to saposin B deficiency	249900	AR / PSAP
Krabbe Disease	245200	AR / GALC
Adrenoleukodystrophy	300100	XLR / ABCD1
Alexander disease	203450	AD / GFAP
Leukoencephalopathy with vanishing white matter	603896	AR / EIF2B1-5
Leukoencephalopathy, diffuse hereditary, with spheroids 1	221820	AD / CSF1R
Leukoencephalopathy, progressive, with ovarian failure	615889	AR / AARS2
Nasu-Hakola disease	221770	AR / TYROBP
	618193	AR / TREM2
Leukoencephalopathy with ataxia	615651	AR / CLCN2
Polyglucosan body disease, adult form	263570	AR / GBE1
Leukodystrophy, Demyelinating, Adult-Onset, Autosomal Dominant (ADLD)	169500	AD / LMNB1
Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL)	611105	AR / DARS2
L-2-hydroxyglutaric aciduria	236792	AR / L2HGDH
Cerebrotendinous xanthomatosis (CTX)	213700	AR / CYP27A1
Fragile X tremor/ataxia syndrome (FXTAS)	300623	XLD / FMR1
Neuronal intranuclear inclusion disease (NIID)	603472	AD / NOTCH2NLC
Leuko-vasculopathies (CADASIL, CARASIL, CARASAL, Fabry, LCC, CAA, subcortical arteriosclerotic encephalopathy, WM lesions of the elderly, vasculitis, Susac syndrome)	Acquired or genetic (AD, AR or XL)	
Multiple sclerosis and related disorders	Acquired	
Infectious disorders (HIV, brucellosis, subacute sclerosing panencephalitis, congenital and perinatal CMV infection, Whipple disease, PML)		
Toxic Encephalopathies		
Posterior Reversible Encephalopathy Syndrome (PRES)		
Gliomatosis and lymphomatosis cerebri		
Histiocytosis		
Post-Hypoxic-Ischemic damage		

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; CAA, cerebral amyloid angiopathy; CADASIL, cerebral AD arteriopathy with subcortical infarcts and leukoencephalopathy; CARASAL, cathepsin A-related arteriopathy with strokes and leukoencephalopathy; CARASIL, cerebral AR arteriopathy with subcortical infarcts and leukoencephalopathy; CMV, cytomegalovirus; LCC, leukoencephalopathy with calcifications and cysts; PML, progressive multifocal leukoencephalopathy; WM, white matter; XL, X-linked; XLD, X-linked Dominant; XLR, X-linked Recessive.

Table S2. List of the 142 genes associated with genetic leukoencephalopathies and included in our custom targeted gene panel sequencing (Di Bella et al. 2021)**.**

GENE	DISEASE	GENE NAME	PHENOTYPE
AARS2	LKENP (AR)	ALANYL-tRNA SYNTHETASE 2	#615889 LEUKOENCEPHALOPATHY, PROGRESSIVE, WITH OVARIAN FAILURE
ABCD1	ALD (XL)	ATP-BINDING CASSETTE, SUBFAMILY D, MEMBER 1;	#300100 ADRENOLEUKODYSTROPHY; ALD
ACOX1	ACOADEF (AR)	ACYL-CoA OXIDASE 1, PALMITOYL	#264470. PEROXISOMAL ACYL-CoA OXIDASE DEFICIENCY
ADAR	AGS6 (AR)	ADENOSINE DEAMINASE, RNA-SPECIFIC	#615010. AICARDI-GOUTIERES SYNDROME 6; AGS6
AIMP1	HLD3 (AR)	ARS-INTERACTING MULTIFUNCTIONAL PROTEIN 1	#260600. LEUKODYSTROPHY, HYPOMYELINATING, 3; HLD3
ALDH3A2	SLS (AR)	ALDEHYDE DEHYDROGENASE, FAMILY 3, SUBFAMILY A, MEMBER 2	#270200. SJOGREN-LARSSON SYNDROME; SLS
ARSA	MLD (AR)	ARYLSULFATASE A	#250100. METACHROMATIC LEUKODYSTROPHY; MULTIPLE SULFATASE DEFICIENCY
ASPA	CANAVAN (AR)	ASPARTOACYLASE	#271900. CANAVAN DISEASE
ATP7A	MENKES (AR)	ATPase, Cu(2+)-TRANSPORTING, ALPHA POLYPEPTIDE; ATP7A	#309400. MENKES DISEASE
ATP7B	WILSON (AR)	ATPase, Cu(2+)-TRANSPORTING, BETA POLYPEPTIDE; ATP7B	#277900. WILSON DISEASE
AUH	MGCA1 (AR)	AU-SPECIFIC RNA-BINDING PROTEIN	#250950 3-METHYLGLUTACONIC ACIDURIA, TYPE I
BCAP31	DDCH (XLR)	B-CELL RECEPTOR-ASSOCIATED PROTEIN 31	#300475. DEAFNESS, DYSTONIA, AND CEREBRAL HYPOMYELINATION; DDCH
BOLA3	MMDS2 (AR)	BolA, E. COLI, HOMOLOG OF, 3; BOLA3	#614299. MULTIPLE MITOCHONDRIAL DYSFUNCTIONS SYNDROME 2; MMDS2
CLCN2	LKPAT (AR)	CHLORIDE CHANNEL 2	#615651. LEUKOENCEPHALOPATHY WITH ATAXIA; LKPAT
COL4A1	POREN1 (AD)	COLLAGEN, TYPE IV, ALPHA-1	#175780. PORENCEPHALY 1; POREN1
COL4A2	POREN2 (AD)	COLLAGEN, TYPE IV, ALPHA-2; COL4A2	#614483. PORENCEPHALY 2; POREN2
CSF1R	HDLS (AD)	COLONY-STIMULATING FACTOR 1 RECEPTOR	#221820. LEUKOENCEPHALOPATHY, DIFFUSE HEREDITARY, WITH SPHEROIDS; HDLS
CTC1	CRMCC1 (AR)	CONSERVED TELOMERE MAINTENANCE COMPONENT 1	#612199 CEREBRORETINAL MICROANGIOPATHY WITH CALCIFICATIONS AND CYSTS
CYP27A1	CTX (AR)	CYTOCHROME P450, SUBFAMILY XXVIIA, POLYPEPTIDE 1	#213700. CEREBROTENDINOUS XANTHOMATOSIS; CTX
CYP2U1	SPG56 (AR)	CYTOCHROME P450, FAMILY 2, SUBFAMILY U, POLYPEPTIDE 1	#615030 SPASTIC PARAPLEGIA 56, AUTOSOMAL RECESSIVE; SPG56
CYP7B1	SPG5 (AR)	CYTOCHROME P450, FAMILY 7, SUBFAMILY B, POLYPEPTIDE 1; CYP7B1	#270800. SPASTIC PARAPLEGIA 5A, AUTOSOMAL RECESSIVE; SPG5A
D2HGDH	D2HGA1 (AR)	D-2-HYDROXYGLUTARATE DEHYDROGENASE; D2HGDH	#600721. D-2-HYDROXYGLUTARIC ACIDURIA 1
DARS	HBSL (AR)	ASPARTYL-tRNA SYNTHETASE	#615281. HYPOMYELINATION WITH BRAINSTEM AND SPINAL CORD INVOLVEMENT AND LEG SPASTICITY; HBSL
DARS2	LBSL (AR)	ASPARTYL-tRNA SYNTHETASE 2	#611105 LEUKOENCEPHALOPATHY WITH BRAINSTEM AND SPINAL CORD INVOLVEMENT AND LACTATE ELEVATION; LBSL
DCAF17	Woodhouse-Sakati (AR)	DDB1- AND CUL4-ASSOCIATED FACTOR 17; DCAF17	#241080.WOODHOUSE-SAKATI SYNDROME
DNM1L	EMPF1 (AD, AR)	DYNAMIN 1-LIKE; DNM1L	#614388 ENCEPHALOPATHY, LETHAL, DUE TO DEFECTIVE MITOCHONDRIAL AND PEROXISOMAL FISSION; EMPF
EARS2	COXPD12 (AR)	GLUTAMYL-tRNA SYNTHETASE 2; EARS2	#614924. COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY 12; COXPD12
EIF2B1	VWM (AR)	EUKARYOTIC TRANSLATION INITIATION FACTOR 2B, SUBUNIT 1	#603896. LEUKOENCEPHALOPATHY WITH VANISHING WHITE MATTER; VWM
EIF2B2	VWM (AR)	EUKARYOTIC TRANSLATION INITIATION FACTOR 2B, SUBUNIT 2	#603896. LEUKOENCEPHALOPATHY WITH VANISHING WHITE MATTER; VWM
EIF2B3	VWM (AR)	EUKARYOTIC TRANSLATION INITIATION FACTOR 2B, SUBUNIT 3	#603896. LEUKOENCEPHALOPATHY WITH VANISHING WHITE MATTER; VWM
EIF2B4	VWM (AR)	EUKARYOTIC TRANSLATION INITIATION FACTOR 2B, SUBUNIT 4	#603896. LEUKOENCEPHALOPATHY WITH VANISHING WHITE MATTER; VWM
EIF2B5	VWM (AR)	EUKARYOTIC TRANSLATION INITIATION FACTOR 2B, SUBUNIT 5	#603896. LEUKOENCEPHALOPATHY WITH VANISHING WHITE MATTER; VWM
ERCC2	XPD (AR)	EXCISION-REPAIR, COMPLEMENTING DEFECTIVE, IN CHINESE HAMSTER, 2	#278730. XERODERMA PIGMENTOSUM, COMPLEMENTATION GROUP D; XPD
ERCC3	XPB (AR)	EXCISION-REPAIR, COMPLEMENTING DEFECTIVE, IN CHINESE HAMSTER, 3	#610651. XERODERMA PIGMENTOSUM, COMPLEMENTATION GROUP B; XPB
ERCC5	XPG (AR)	EXCISION-REPAIR, COMPLEMENTING DEFECTIVE, IN CHINESE HAMSTER, 5; ERCC5	#278780. XERODERMA PIGMENTOSUM, COMPLEMENTATION GROUP G; XPG
ERCC6	CSB (AR)	EXCISION-REPAIR CROSS-COMPLEMENTING, GROUP 6	#133540. COCKAYNE SYNDROME B; CSB
ERCC8	CSA (AR)	EXCISION-REPAIR CROSS-COMPLEMENTING, GROUP 8	#216400. COCKAYNE SYNDROME A; CSA
FA2H	SPG35 (AR)	FATTY ACID 2-HYDROXYLASE; FA2H	#612319. SPASTIC PARAPLEGIA 35, AUTOSOMAL RECESSIVE; SPG35
FAM126A	HLD5 (AR)	FAMILY WITH SEQUENCE SIMILARITY 126, MEMBER A	#610532. LEUKODYSTROPHY, HYPOMYELINATING, 5; HLD5
FUCA1	FUCOSISOSI (AR)	FUCOSIDASE, ALPHA-L, 1	#230000. FUCOSIDOSIS; Lysosomal storage disease
GALC	KRABBE (AR)	GALACTOSYLCERAMIDASE	#245200 KRABBE DISEASE: GLOBOID CELL LEUKODYSTROPHY

GAN	GAN1 (AR)	GAN GENE; GAN	#256850. GIANT AXONAL NEUROPATHY 1, AUTOSOMAL RECESSIVE; GAN1
GBE1	GSD4 (AR), APBN (AR)	GLYCOGEN BRANCHING ENZYME; GBE1	#263570 POLYGLUCOSAN BODY NEUROPATHY, ADULT FORM; APBN
GCDH	GA1 (AR)	GLUTARYL-CoA DEHYDROGENASE; GCDH	#231670 GLUTARIC ACIDEMIA I
GFAP	ALEXANDER (AD)	GLIAL FIBRILLARY ACIDIC PROTEIN; GFAP	#203450. ALEXANDER DISEASE
GJA1	ODDD (AD, AR)	GAP JUNCTION PROTEIN, ALPHA-1; GJA1	#257850 - OCULODENTODIGITAL DYSPLASIA, AUTOSOMAL RECESSIVE
GJB1	CMTX1 (XLD)	GAP JUNCTION PROTEIN, BETA-1; GJB1	#302800 CHARCOT-MARIE-TOOTH DISEASE, X-LINKED DOMINANT, 1; CMTX1
GJC2	HLD2 (AR)	GAP JUNCTION PROTEIN, GAMMA-2; GJC2	#608804. LEUKODYSTROPHY, HYPOMYELINATING, 2; HLD2
GLA	FABRY (XL)	GALACTOSIDASE, ALPHA; GLA	#301500. FABRY DISEASE
GLB1	GM1 (AR)	GALACTOSIDASE, BETA-1	#230500. GM1-GANGLIOSIDOSIS, TYPE I; MPS IV TYPE B; Malattia di Morquio tipo B; GM1-gangliosidosis, type I, II, III
GLRX5	SIDBA3 (AR)	GLUTAREDOXIN 5; GLRX5	#205950. ANEMIA, SIDEROBLASTIC, PYRIDOXINE-REFRACTORY, AUTOSOMAL RECESSIVE
GTF2H5	TTDP (AR)	GENERAL TRANSCRIPTION FACTOR IIH, POLYPEPTIDE 5; GTF2H5	#601675. TRICHOTHIODYSTROPHY, PHOTOSENSITIVE; TTDP
HEPACAM	MLC2A (AD, AR)	HEPATOCYTE CELL ADHESION MOLECULE; HEPACAM	#613925. MEGALENCEPHALIC LEUKOENCEPHALOPATHY WITH SUBCORTICAL CYSTS 2A; MLC2A
HEXA	TAY-SACHS (AR)	HEXOSAMINIDASE A; HEXA	#272800. TAY-SACHS DISEASE; TSD
HMGCL	HMGCLD (AR)	3-HYDROXY-3-METHYLGLUTARYL-CoA LYASE; HMGCL	#246450. 3-HYDROXY-3-METHYLGLUTARYL-CoA LYASE DEFICIENCY; HMGCLD
HSD17B4	D-BIFUNCTIONALDEF (AR)	17-BETA-HYDROXYSTEROID DEHYDROGENASE IV; HSD17B4	#261515. D-BIFUNCTIONAL PROTEIN DEFICIENCY
HSPD1	HLD4 (AD, AR)	HEAT-SHOCK 60-KD PROTEIN 1; HSPD1	#612233. LEUKODYSTROPHY, HYPOMYELINATING, 4; HLD4
HTRA1	CARASIL (AR)	HTRA SERINE PEPTIDASE 1; HTRA1	#600142. CEREBRAL AUTOSOMAL RECESSIVE ARTERIOPATHY WITH SUBCORTICAL INFARCTS AND LEUKOENCEPHALOPATHY; CARASIL
IBA57	MMDS3 (AR), SPG74 (AR)	IBA57, S. CEREVISIAE, HOMOLOG OF; IBA57	#615330. MULTIPLE MITOCHONDRIAL DYSFUNCTIONS SYNDROME 3; MMDS3
IFIH1	AGS7 (AD)	INTERFERON-INDUCED HELICASE C DOMAIN-CONTAINING PROTEIN 1; IFIH1	#615846. AICARDI-GOUTIERES SYNDROME 7; AGS7
ISCA2	MMDS4 (AR)	IRON-SULFUR CLUSTER ASSEMBLY 2, S. CEREVISIAE, HOMOLOG OF; ISCA2	#616370. MULTIPLE MITOCHONDRIAL DYSFUNCTIONS SYNDROME 4; MMDS4
KIF5A	SPG10 (AD), NEIMY (AD), ALS25 (AD)	KINESIN FAMILY MEMBER 5A; KIF5A	#604187 SPASTIC PARAPLEGIA 10, AUTOSOMAL DOMINANT; SPG10
L1CAM	MASA (XL), SPG1 (XL)	L1 CELL ADHESION MOLECULE; L1CAM	#303350 MASA SYNDROME
L2HGDH	L2HGA (AR)	L-2-HYDROXYGLUTARATE DEHYDROGENASE; L2HGDH	#236792. L-2-HYDROXYGLUTARIC ACIDURIA
LAMA2	MDC1A (AR)	LAMININ, ALPHA-2; LAMA2	#607855. MUSCULAR DYSTROPHY, CONGENITAL MEROSIN-DEFICIENT, 1A; MDC1A
LAMB1	LIS5 (AR)	LAMININ, BETA-1; LAMB1	#615191. LISSENCEPHALY 5; LIS5
LIAS	PDHLD (AR)	LIPOIC ACID SYNTHASE; LIAS	#614462. PYRUVATE DEHYDROGENASE LIPOIC ACID SYNTHETASE DEFICIENCY; PDHLD
LIPT1	LIPT1D (AR)	LIPOYLTRANSFERASE 1; LIPT1	#616299. LIPOYLTRANSFERASE 1 DEFICIENCY; LIPT1D
LMNB1	ADLD (AD)	LAMIN B1; LMNB1	#169500. LEUKODYSTROPHY, DEMYELINATING, ADULT-ONSET, AUTOSOMAL DOMINANT; ADLD
LYRM7	MC3DN8 (AR)	LYR MOTIF-CONTAINING PROTEIN 7; LYRM7	#615838. MITOCHONDRIAL COMPLEX III DEFICIENCY, NUCLEAR TYPE 8; MC3DN8
MAG	SPG75 (AR)	MYELIN-ASSOCIATED GLYCOPROTEIN; MAG	#616680 SPASTIC PARAPLEGIA 75, AUTOSOMAL RECESSIVE; SPG75
MCOLN1	ML4 (AR)	MUCOLIPIN 1; MCOLN1	#252650. MUCOLIPIDOSIS IV
MLC1	ML4 (AR)	MLC1 GENE; MLC1	#604004. MEGALENCEPHALIC LEUKOENCEPHALOPATHY WITH SUBCORTICAL CYSTS 1; MLC1
MOCS1	MOCODA (AR)	MOLYBDENUM COFACTOR SYNTHESIS GENE 1; MOCS1	#252150. MOLYBDENUM COFACTOR DEFICIENCY, COMPLEMENTATION GROUP A; MOCODA
MPLKIP	TTDN1 (AR)	CHROMOSOME 7 OPEN READING FRAME 11; C7ORF11	#234050. TRICHOTHIODYSTROPHY, NONPHOTOSENSITIVE 1; TTDN1
MTHFR	MTHFR (AR)	10-METHYLENETETRAHYDROFOLATE REDUCTASE; MTHFR	#236250 HOMOCYSTINURIA DUE TO DEFICIENCY OF N(5,10)-METHYLENETETRAHYDROFOLATE REDUCTASE ACTIVITY
MYT1	OAVS (AD)	MYELIN TRANSCRIPTION FACTOR 1	OCULO-AURICULO-VERTEBRAL SPECTRUM
NFU1	MMDS1 (AR)	NFU1, S. CEREVISIAE, HOMOLOG OF; NFU1	#605711. MULTIPLE MITOCHONDRIAL DYSFUNCTIONS SYNDROME 1; MMDS1
NOTCH3	CADASIL (AD)	NOTCH, DROSOPHILA, HOMOLOG OF, 3; NOTCH3	#125310. CEREBRAL ARTERIOPATHY, AUTOSOMAL DOMINANT, WITH SUBCORTICAL INFARCTS AND LEUKOENCEPHALOPATHY; CADASIL
NPC1	NPC1 (AR)	#257220. NPC1 GENE; NPC1	#257220 NIEMANN-PICK DISEASE, TYPE C1; NPC1
NPC2	NPC2 (AR)	NPC2 GENE; NPC2	#607625 NIEMANN-PICK DISEASE, TYPE C2; NPC2
NUBPL	NUBPL (AR)	NUCLEOTIDE-BINDING PROTEIN-LIKE PROTEIN; NUBPL	#252010. MITOCHONDRIAL COMPLEX I DEFICIENCY
OCLN	BLCPMG (AR)	OCCLUDIN; OCLN	BAND-LIKE CALCIFICATION WITH SIMPLIFIED GYRATION AND POLYMICROGYRIA; BLCPMG
OCRL	OCRL (XLR)	OCRL GENE; OCRL	LOWE OCULOCEREBRORENAL SYNDROME; OCRL

PEX1	PBD1A (AR)	PEROXISOME BIOGENESIS FACTOR 1; PEX1	#214100. PEROXISOME BIOGENESIS DISORDER 1A (ZELLWEGER); PBD1A
PEX10	PBD6A (AR)	PEROXISOME BIOGENESIS FACTOR 10; PEX10	#614870. PEROXISOME BIOGENESIS DISORDER 6A (ZELLWEGER); PBD6A
PEX11B	PEX14B (AR)	PEROXISOME BIOGENESIS FACTOR 11B; PEX11B	#614920. PEROXISOME BIOGENESIS DISORDER 14B; PEX14B
PEX12	PBD3A (AR)	PEROXISOME BIOGENESIS FACTOR 12; PEX12	#614859. PEROXISOME BIOGENESIS DISORDER 3A (ZELLWEGER); PBD3A
PEX13	PBD11A (AR)	PEROXISOME BIOGENESIS FACTOR 13; PEX13	#614883. PEROXISOME BIOGENESIS DISORDER 11A (ZELLWEGER); PBD11A
PEX14	PBD13A (AR)	PEROXISOME BIOGENESIS FACTOR 14; PEX14	#614887. PEROXISOME BIOGENESIS DISORDER 13A (ZELLWEGER); PBD13A
PEX16	PBD8B (AR)	PEROXISOME BIOGENESIS FACTOR 16; PEX16	#614877. PEROXISOME BIOGENESIS DISORDER 8B; PBD8B
PEX19	PBD12A (AR)	PEROXISOME BIOGENESIS FACTOR 19; PEX19	#614886. PEROXISOME BIOGENESIS DISORDER 12A (ZELLWEGER); PBD12A
PEX2	PBD5B (AR)	PEROXISOME BIOGENESIS FACTOR 2; PEX2	#614867. PEROXISOME BIOGENESIS DISORDER 5B; PBD5B
PEX26	PBD7A (AR)	PEROXISOME BIOGENESIS FACTOR 26; PEX26	#614872. PEROXISOME BIOGENESIS DISORDER 7A (ZELLWEGER); PBD7A
PEX3	PBD10A (AR)	PEROXISOME BIOGENESIS FACTOR 3; PEX3	#614882. PEROXISOME BIOGENESIS DISORDER 10A (ZELLWEGER); PBD10A
PEX5	PBD2B (AR)	PEROXISOME BIOGENESIS FACTOR 5; PEX5	#202370. PEROXISOME BIOGENESIS DISORDER 2B; PBD2B
PEX6	PBD4B (AR)	PEROXISOME BIOGENESIS FACTOR 6; PEX6	#614863. PEROXISOME BIOGENESIS DISORDER 4B; PBD4B
PEX7	PBD9B (AR)	PEROXISOME BIOGENESIS FACTOR 7; PEX7	#614879. PEROXISOME BIOGENESIS DISORDER 9B; PBD9B
PHGDH	PHGDHD (AR)	PHOSPHOGLYCERATE DEHYDROGENASE; PHGDH	#601815. PHOSPHOGLYCERATE DEHYDROGENASE DEFICIENCY
PHYH	REFSUM (AR)	PHYTANOYL-CoA HYDROXYLASE; PHYH	#266500. REFSUM DISEASE, CLASSIC
PLP1	PMD (XL)	PROTEOLIPID PROTEIN 1; PLP1	#312080. PELIZAEUS-MERZBACHER DISEASE; PMD
PNPLA6	SPG39 (AR), BNHS (AR)	PATATIN-LIKE PHOSPHOLIPASE DOMAIN-CONTAINING PROTEIN 6; PNPLA6	#612020 SPASTIC PARAPLEGIA 39, AUTOSOMAL RECESSIVE; SPG39
POLG	PEOA1 (AD), PEOB1 (AR), MTDPS4A (AR)	POLYMERASE, DNA, GAMMA; POLG	#203700 MITOCHONDRIAL DNA DEPLETION SYNDROME 4A (ALPERS TYPE); MTDPS4A
POLR1C	HLD11 (AR)	POLYMERASE I, RNA, SUBUNIT C; POLR1C	#616494 LEUKODYSTROPHY, HYPOMYELINATING, 11; HLD11
POLR3A	HLD7 (AR)	POLYMERASE III, RNA, SUBUNIT A; POLR3A	#607694. LEUKODYSTROPHY, HYPOMYELINATING, 7, WITH OR WITHOUT OLIGODONTIA AND/OR HYPOGONADOTROPIC HYPOGONADISM; HLD7
POLR3B	HLD8 (AR)	POLYMERASE III, RNA, SUBUNIT B; POLR3B	#614381. LEUKODYSTROPHY, HYPOMYELINATING, 8, WITH OR WITHOUT OLIGODONTIA AND/OR HYPOGONADOTROPIC HYPOGONADISM; HLD8
PPT1	CLN1 (AR)	PALMITOYL-PROTEIN THIOESTERASE 1; PPT1	#256730. CEROID LIPOFUSCINOSIS, NEURONAL, 1; CLN1
PRF1	FHL2 (AR)	PERFORIN 1; PRF1	#603553 FAM HEMOPHAGOC LYMPHOHISTIOCYTOSIS 2
PSAP	PSAP (AR)	PROSAPOSIN; PSAP	#249900. METACHROMATIC LEUKODYSTROPHY DUE TO SAPOSIN B DEFICIENCY
PSAT1	PSATD (AR)	PHOSPHOSERINE AMINOTRANSFERASE 1; PSAT1	#610992. PHOSPHOSERINE AMINOTRANSFERASE DEFICIENCY; PSATD
PYCR2	HLD10 (AR)	PYRROLINE-5-CARBOXYLATE REDUCTASE 2; PYCR2	#616420 LEUKODYSTROPHY, HYPOMYELINATING, 10; HLD10
RARS	HLD9 (AR)	ARGINYL-tRNA SYNTHETASE; RARS	#616140. LEUKODYSTROPHY, HYPOMYELINATING, 9; HLD9
RMND1	COXPD11 (AR)	REQUIRED FOR MEIOTIC NUCLEAR DIVISION 1, S. CEREVISIAE, HOMOLOG OF; RMND1	#614922 COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY 11; COXPD11
RNASEH2A	AGS4 (AR)	RIBONUCLEASE H2, SUBUNIT A; RNASEH2A	#610333. AICARDI-GOUTIERES SYNDROME 4; AGS4
RNASEH2B	AGS2 (AR)	RIBONUCLEASE H2, SUBUNIT B; RNASEH2B	#610181. AICARDI-GOUTIERES SYNDROME 2; AGS2
RNASEH2C	AGS3 (AR)	RIBONUCLEASE H2, SUBUNIT C; RNASEH2C	#610329. AICARDI-GOUTIERES SYNDROME 3; AGS3
RNASET2	RNASET2 (AR)	RIBONUCLEASE T2; RNASET2	#612951. LEUKOENCEPHALOPATHY, CYSTIC, WITHOUT MEGALENCEPHALY
RNF216	GDHS (AR)	RING FINGER PROTEIN 216; RNF216	#212840. GORDON HOLMES SYNDROME; GDHS
RPIA	RPIA (AR)	RIBOSE 5-PHOSPHATE ISOMERASE A; RPIA	#608611 RIBOSE 5-PHOSPHATE ISOMERASE DEFICIENCY
SAMHD1	AGS5 (AR)	SAM DOMAIN- AND HD DOMAIN-CONTAINING PROTEIN 1; SAMHD1	#612952. AICARDI-GOUTIERES SYNDROME 5; AGS5
SCP2	LKDMN (AR)	STEROL CARRIER PROTEIN 2; SCP2	#613724. LEUKOENCEPHALOPATHY WITH DYSTONIA AND MOTOR NEUROPATHY
SLC16A2	AHDS (XL)	SOLUTE CARRIER FAMILY 16 (MONOCARBOXYLIC ACID TRANSPORTER), MEMBER 2; SLC16A2	#300523. ALLAN-HERNDON-DUDLEY SYNDROME; AHDS
SLC17A5	SALLA (AR)	SOLUTE CARRIER FAMILY 17 (SODIUM PHOSPHATE COTRANSPORTER), MEMBER 5; SLC17A5	#604369. SALLA DISEASE; SD
SLC19A3	THMD2 (AR)	SOLUTE CARRIER FAMILY 19 (THIAMINE TRANSPORTER), MEMBER 3; SLC19A3	#607483. THIAMINE METABOLISM DYSFUNCTION SYNDROME 2 (BIOTIN- OR THIAMINE-RESPONSIVE TYPE); THMD2
SLC25A1	D2L2ADN (AR)	SOLUTE CARRIER FAMILY 25 (MITOCHONDRIAL CARRIER, CITRATE TRANSPORTER), MEMBER 1; SLC25A1	COMBINED D-2- AND L-2-HYDROXYGLUTARIC ACIDURIA; D2L2AD
SLC25A12	EIEE39 (AR)	SOLUTE CARRIER FAMILY 25 (MITOCHONDRIAL CARRIER, ARALAR), MEMBER 12; SLC25A12	#612949. HYPOMYELINATION, GLOBAL CEREBRAL
SLC33A1	CCHLND (AR), SPG42 (AD)	SOLUTE CARRIER FAMILY 33 (ACETYL-CoA TRANSPORTER), MEMBER 1; SLC33A1	#614482. CONGENITAL CATARACTS, HEARING LOSS, AND NEURODEGENERATION; CCHLND

SOX10	PCWH (AD)	SRY-BOX 10; SOX10	#609136. PERIPHERAL DEMYELINATING NEUROPATHY, CENTRAL DYSMYELINATION, WAARDENBURG SYNDROME, AND HIRSCHSPRUNG DISEASE; PCWH
SPG11	SPG11 (AR)	SPG11 GENE; SPG11	#604360. SPASTIC PARAPLEGIA 11, AUTOSOMAL RECESSIVE; SPG11
SPG20	SPG20 (AR)	SPG20 GENE; SPG20	#275900 SPASTIC PARAPLEGIA 20, AUTOSOMAL RECESSIVE; SPG20
SPG21	SPG21 (AR)	ACIDIC CLUSTER PROTEIN, 33-KD; ACP33	#248900 MAST SYNDROME
STX11	FHL4 (AR)	SYNTAXIN 11; STX11	#603552 FAM HEMOPHAGOCYTIC LYMPHOHISTIOCY 4
STXBP2	FHL5 (AR)	SYNTAXIN-BINDING PROTEIN 2; STXBP2	#613101 FAM HEMOPHAGOCYTIC LYMPHOHISTIOCY 5
SUMF1	MSD (AR)	SULFATASE-MODIFYING FACTOR 1; SUMF1	#272200. MULTIPLE SULFATASE DEFICIENCY; MSD
SUOX	ISOD (AR)	SULFITE OXIDASE; SUOX	#272300. SULFOCYSTEINURIA
TECPR2	SPG49 (AR)	TECTONIN BETA-PROPELLER REPEAT-CONTAINING PROTEIN 2; TECPR2	#615031 SPASTIC PARAPLEGIA 49, AUTOSOMAL RECESSIVE; SPG49
TREM2	PLOSL (AR)	TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS 2; TREM2	#221770 POLYCYSTIC LIPOMEMBRANOUS OSTEODYSPLASIA WITH SCLEROSING LEUKOENCEPHALOPATHY; PLOSL
TREX1	AGS1 (AD, AR)	3-PRIME REPAIR EXONUCLEASE 1; TREX1	#192315. VASCULOPATHY, RETINAL, WITH CEREBRAL LEUKODYSTROPHY; RVCL
TUBB4A	HLD6 (AD)	TUBULIN, BETA-4A; TUBB4A	#612438. LEUKODYSTROPHY, HYPOMYELINATING, 6; HLD6
TYROBP	PLOSL (AR)	TYRO PROTEIN TYROSINE KINASE-BINDING PROTEIN; TYROBP	#221770. POLYCYSTIC LIPOMEMBRANOUS OSTEODYSPLASIA WITH SCLEROSING LEUKOENCEPHALOPATHY; PLOSL
UNC13D	FHL3 (AR)	UNC13, C. ELEGANS, HOMOLOG OF, D; UNC13D	#608898 FAM HEMOPHAGOCYTIC LYMPHOHISTIOCY 3
ZFYVE26	SPG15 (AR)	ZINC FINGER FYVE DOMAIN-CONTAINING PROTEIN 26; ZFYVE26	#270700 SPASTIC PARAPLEGIA 15, AUTOSOMAL RECESSIVE; SPG15