


## ORIGINAL ARTICLE

# Argentinian clinical genomics in a leukodystrophies and genetic leukoencephalopathies cohort: Diagnostic yield in our first 9 years

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## Abstract

**Introduction and objectives:** Leukodystrophies and genetic leukoencephalopathies constitute a vast group of pathologies of the cerebral white matter. The large number of etiopathogenic genes and the frequent unspecificity on the clinical-radiological presentation generate remarkable difficulties in the diagnosis approach. Despite recent and significant developments, molecular diagnostic yield is still less than 50%. Our objective was to develop and explore the usefulness of a new diagnostic procedure using standardized molecular diagnostic tools, and next-generation sequencing techniques.

**Materials and methods:** A prospective, observational, analytical study was conducted in a cohort of 46 patients, evaluated between May 2008 and December 2016, with a suspected genetic leukoencephalopathy or leukodystrophy. A diagnostic procedure was set up using classical monogenic tools in patients with characteristic phenotypes, and next-generation techniques in nonspecific ones.

**Results:** Global diagnostic procedure yield was 57.9%, identifying the etiological pathogenesis in 22 of the 38 studied subjects. Analysis by subgroups, Sanger method, and next-generation sequencing showed a yield of 64%, and 46.1% respectively. The most common pathologies were adrenoleukodystrophy, cerebral autosomal-dominant arteriopathy with subcortical infarcts (CADASIL), and vanishing white matter disease.

**Conclusions:** Our results confirm the usefulness of the proposed diagnostic procedure expressed in a high diagnostic yield and suggest a more optimal cost-effectiveness in an etiological analysis phase.

**KEY WORDS**

diagnostic procedure, genetic leukoencephalopathies, leukodystrophies, next-generation sequencing

## 1 | INTRODUCTION

Genetic leukodystrophies and leukoencephalopathies constitute a vast group of neurological diseases that affect all age groups, with the involvement of cerebral white matter as a common denominator. Under the term leukodystrophies are those genetic disorders induced by primary damage of glial cells or myelin of the central nervous system, with or without involvement of the peripheral nervous system. On the other hand, genetic leukoencephalopathies include hereditary disorders of the white matter that cannot be included in the term leukodystrophies, such as those entities with primary involvement of the gray matter or systemic pathologies with secondary neurological involvement (example: mitochondrial diseases with hepatic, muscular, or cardiac involvement and additional commitment of central nervous white matter) (Vanderver et al., 2015). Between 1990 and 2010, many of the genes linked to these pathologies were identified. In the last few years, thanks to the incorporation of massive sequencing techniques, a noticeable expansion in the knowledge of the genetic causes of white matter diseases was made possible, thus allowing the identification of more than 100 leukodystrophies and genetic leukoencephalopathies.

Despite these significant advances, the molecular diagnostic yield is still less than 50% (Kevelam et al., 2016). The nonspecificity of the symptoms, the clinical superposition between one entity and another, the heterogeneous presentation, the limited existence of biochemical markers, and the little guidance in the findings in neuroimaging several of the disorders added to the high number of genes involved generate marked difficulties when making a diagnostic approach, even for medical specialists in the field. The need arises to find an ideally simple, economic, and reproducible procedure that allows us to reach the etiological diagnosis in a greater number of cases, which would have profound implications in the clinical follow-up, family counseling, and eventual therapeutic behavior, depending on the case. On the other hand, although there have been isolated reports of individual patients with leukodystrophies and genetic leukoencephalopathies, and small series of cases in the country and region (Amorosi et al., 2012; Arroyo, Grippo, Taratuto, Duffau, & Chamoles, 1991; de Kremer et al., 2002; Guarnaschelli & Sotelo, 2017; Rodríguez-Quiroga et al., 2015), there has not been a systematic analysis evaluating the

diagnostic performance of a comprehensive molecular diagnosis approach in the field of these disorders in Argentina.

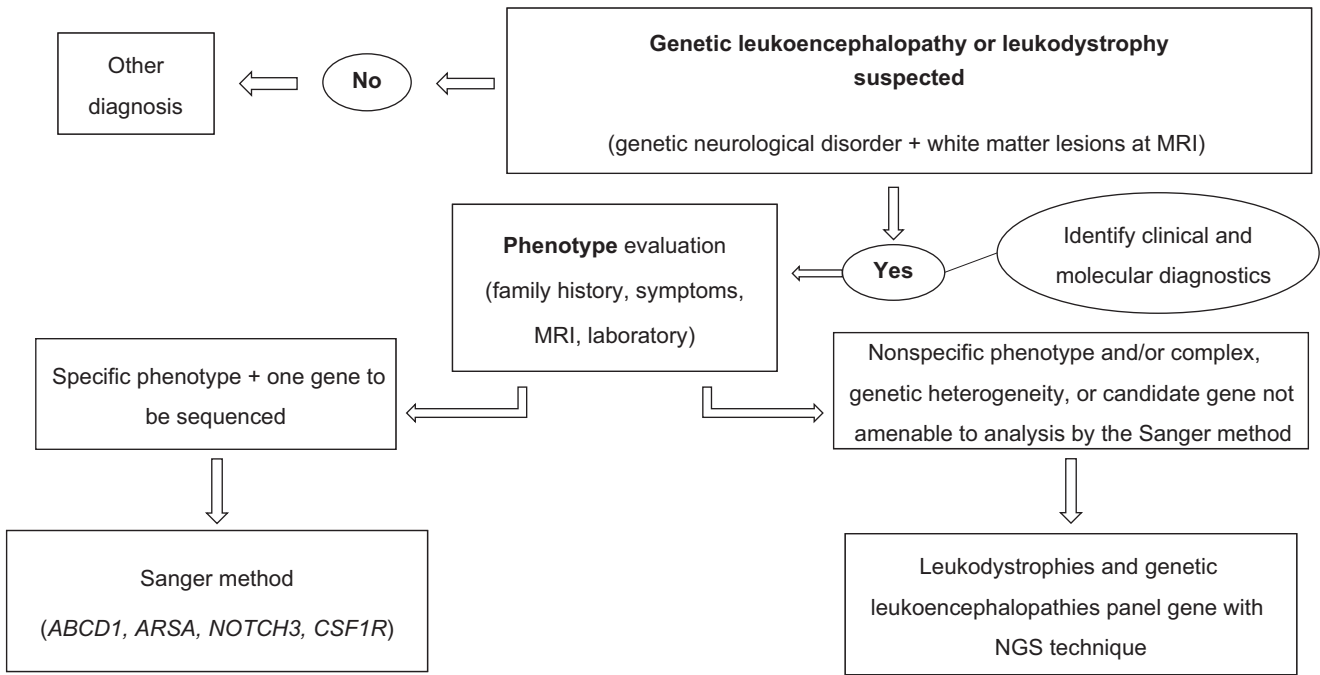
Therefore, the objectives of our work were (1) to characterize and stratify a population of adult and pediatric patients with leukodystrophies and genetic leukoencephalopathies, and (2) to develop and evaluate the diagnostic performance of a clinical-molecular procedure, which used classical and next-generation sequencing (NGS) techniques in a prospective cohort of patients with neurogenetic disorders.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients: Diagnostic procedure

A prospective, observational, and descriptive cohort study was conducted, including adult and pediatric patients enrolled at the Neurogenetic Clinic of the J.M. Ramos Mejía Hospital in Buenos Aires, from May 2008 to December 2016, with a clinical diagnosis of leukodystrophy or genetic leukoencephalopathy, based on the definition of both concepts by Vanderver et al. (2015). All the patients, prior to their participation in the study, provided informed consent through a form approved by the Ethics Committee of the institution, and in the case of minors, the consent was given by their parents. Demographic, family history, clinical, and paraclinical data were obtained.

For the selection of the molecular tests to be performed in each patient, the clinical characteristics, biochemical markers, and neuroimaging findings of each individual case were considered. In those who presented with a phenotype characteristic of a pathology linked to a single candidate gene and capable of being analyzed by the Sanger method, such as, but not limited to, X-linked adrenoleukodystrophy, metachromatic leukodystrophy, cerebral autosomal-dominant arteriopathy with subcortical infarcts (CADASIL), and hereditary diffuse leukoencephalopathy with spheroids, we proceeded to the sequencing of the etiopathogenic gene using the technique described for *ABCD1*, *ARSA*, *NOTCH3*, and *CSF1R*, respectively. In cases with non-specific phenotype and/or complex, genetic heterogeneity, or candidate gene not amenable to analysis by the Sanger method, a multigenic panel was used with the inclusion of more than 100 genes linked to leukodystrophies and genetic



**FIGURE 1** Diagnostic and methodological procedure to characterize patients to have a better diagnostic approach

leukoencephalopathies, with NGS techniques. Unfortunately, funding did not permit multigene panel testing in those subjects ( $n = 9$ ) for whom the Sanger method did not confirm an etiology (Figure 1; Supplementary Table 1).

## 2.2 | Molecular studies

### 2.2.1 | Genomic DNA purification

Two millimeters of whole blood were obtained from each patient by venipuncture. Total genomic DNA was purified using commercial systems, following the manufacturer's instructions. This was kept anonymized until further processing.

### 2.2.2 | Sequencing of individual genes by the Sanger technique

Primers were designed for the coding regions of the *ABCD1* gene (\*300371) using NCBI NM\_000033.3 on GRCh37 (hg19) as the reference sequence. The 10 exons, including the flanking regions of each exon, were amplified by “polymerase chain reaction” (PCR) and sequenced by the Sanger method, as described in Boehm, Cutting, Lachtermacher, Moser, & Chong (1999).

For the analysis of the *ARSA* gene (\*607574), the reference of GenBank NM\_000487.5 was followed, based on GRCh37 (hg19). The adjacent coding and intronic regions of the eight exons were amplified by PCR using oligonucleotides or primers designed for that purpose and subsequent sequencing by the Sanger method (conditions available as required).

For the analysis of the *NOTCH3* gene (\*600276), the NCBI NM\_000435.2 was used on the reference sequence GRCh37 (hg19) and the exons 3-4-5-6-11-12-18-19-20 were studied, while for the *CSF1R* gene analysis (\*221820), based on NCBI NM\_005211.3, the coding region and exon–intron junctions of exons 18, 19, and 20 were examined; in both cases, the same methodology to that already specified above was used (conditions available as required).

For the analysis of the *GFAP* gene (\*137780) and *EIF2B5* gene (\*603945), the NCBI NM\_000435.2 and NCBI NM\_003907.2 were used on the reference sequence GRCh37 (hg19), respectively, sequencing the coding region and exon–intron junctions following the Sanger method (conditions available as required).

### 2.2.3 | Multigene panel sequencing

The coding fraction of 4813 genes was enriched in solution following standardized procedures recommended by the manufacturer using the TruSight One Sequencing Panel commercial kit (Illumina, San Diego, CA). Later, in batches of three samples, sequencing runs were performed on Illumina MiSeq equipment, achieving average coverage greater than 70X, following standardized procedures recommended by the manufacturer. A total of three sequencing runs were performed for a total analysis of nine samples. Four other patients underwent similar procedures but in commercial genomic diagnostic laboratories (methods reported in individual study reports). As mentioned below, the clinical interpretation was based on the scrutiny of a subset of genes associated with white matter disorders (virtual panel analysis).

## 2.3 | Bioinformatic analysis

The product of the sequencing runs was aligned with the reference sequence of the human genome of the National Center for Biotechnology Information of the National Institutes of Health of the United States version 37/hg19 using the BWA tool (version 0.5.9, requirement) (Li & Durbin, 2009). Subsequently, single-nucleotide variants and small insertions/deletions were identified, using the tools Picard (version 1.59) and GATK (version 3.6, parameters used available upon request) (Van der Auwera et al., 2013). From a search of genes reported as causing leukodystrophies and genetic leukoencephalopathies in OMIM and HGMD, a virtual multigenic panel (Annex I) of the pathologies under study was constructed, which was useful for the analysis and clinical interpretation of the variant files obtained from sequencing runs, using procedures developed by our group and described in Córdoba et al. (2018).

## 3 | RESULTS

### 3.1 | Cohort

Of the 882 patients evaluated in the Neurogenetic Clinic of our hospital from May 2008 to December 2016, we included 46 subjects with diagnostic presumptions of leukodystrophy or genetic leukoencephalopathy.

The implementation of the diagnostic procedure presented in Figure 1, based on the analysis of clinical data, family history, and paraclinical studies, allowed us to systematize the study of our population, dividing them into two large groups:

1. a group of patients with a phenotype characteristic of a pathology linked to a single candidate gene, and capable of being analyzed by the Sanger method, in which individual sequencing was carried out using the technique described; such is the case of X-linked adrenoleukodystrophy, metachromatic leukodystrophy, CADASIL, vanishing white matter disease, Alexander's disease, and diffuse hereditary leukoencephalopathy with axonal spheroids and pigmented cells, and
2. a group of patients with nonspecific phenotype and/or complex, genetic heterogeneity, or candidate genes not amenable to analysis by the Sanger method, who were evaluated through extended gene sequencing with next-generation techniques.

The methodology used and the cohort studied by strata are represented in Figure 2 with the diagnostic yield obtained in each case. The demographic and clinical characteristics of the included patients are shown in Figure 3. The details of the clinical presentation, radiology, family history,

diagnostic presumption, and method of molecular study used in each patient, are presented in Table 1.

### 3.2 | Diagnostic yield of the comprehensive approach

From this study methodology and using the proposed diagnostic procedure, we arrived at the molecular diagnosis that confirmed the genetic etiology in 22 subjects of the 38 studied (57.9%). It was not possible to perform molecular studies in eight patients that were not available for blood testing. The details of the recognized mutations are summarized in Supplementary Table 2.

In the case of patients with suspected X-linked adrenoleukodystrophy, based on family history, very long-chain fatty acids, endocrine comorbidity with adrenal insufficiency, and suggestive radiological abnormalities, the *ABCD1* gene was sequenced by the Sanger method, confirming the molecular diagnosis in six of the seven patients evaluated (85.7%).

In three patients, there was a diagnostic suspicion of metachromatic leukodystrophy based on the biochemical profile, which shows a decrease in the plasmatic activity of the enzyme arylsulfatase A and an increase in the excretion of urinary sulfatides. The sequencing of the *ARSA* gene showed the presence of pathogenic variants in homozygosis in two of them (66.7%). In the patient in whom only one heterozygous variant was found, the occurrence of a structural variant in the other allele or the presence of a pathogenic variant in areas that are not sequenced by the Sanger method are possible.

The presumptive diagnosis of CADASIL was based on the occurrence of acute neurovascular events, migraine, or cognitive impairment in individuals under 50 years old, with or without family history compatible with autosomal-dominant inheritance and associated with neuroimaging with multifocal involvement of cerebral white matter. The diagnosis was confirmed by the finding of pathogenic variants in the *NOTCH3* gene in four of the 10 patients studied (40%).

In the cases of a progressive adult-onset neurological disorder, characterized by cognitive-behavioral impairment and asymmetric pyramidal motor deficit associated with magnetic resonance imaging (MRI) showing involvement of cerebral white matter with frontoparietal predominance, with or without an autosomal-dominant inheritance pattern, we suspected diffuse hereditary leukoencephalopathy with axonal spheroids and pigmented giant cells. The sequencing of the *CSF1R* gene revealed a pathogenic variant in heterozygosis in one of the two patients evaluated with these characteristics (50%).

In 13 patients, we proceeded to study a multigenic panel by massive sequencing in parallel, with next-generation techniques through the analysis of more than 100 genes linked with leukodystrophies and genetic leukoencephalopathies. All

**TABLE 1** Patients with clinical diagnosis of leukodystrophy or genetic leukoencephalopathy: Demographic, clinical presentation, brain MRI, family history/inherited pattern, suspected clinical diagnosis based on phenotype, and gene sequencing method used to achieve a molecular diagnostic

Patient number	Gender	Age of onset (years)	Clinical	Brain MRI	Family history inherited pattern	Clinical diagnosis	Gene sequencing method
1	M	10	Epilepsy; spastic quadriparesis, language disorder, evolutive, Addison syndrome, increased VLCFA	White matter images: hyperintense in T2 and hypointense in T1, bilateral parietal and occipital, Gd (+)	Six cousins affected X-linked	X-linked adrenoleukodystrophy	Sanger method: <i>ABCD1</i> gene
2	M	9	Epilepsy, spastic quadriparesis, language and cognitive disorder, evolutive, Addison syndrome increased VLCFA	White matter images, hyperintense in T2 and hypointense in T1, bilateral parietal and occipital	Grandfather and brother affected X-linked	X-linked adrenoleukodystrophy	Sanger method: <i>ABCD1</i> gene
3	M	7	Epilepsy, spastic quadriparesis, language and cognitive disorder, evolutive, Addison syndrome, increased VLCFA	White matter images, hyperintense in T2 and hypointense in T1, bilateral parietal and occipital, Gd (+)	Sister carrier, X-linked	X-linked adrenoleukodystrophy	Sanger method: <i>ABCD1</i> gene
4	M	6	Epilepsy, spastic quadriparesis, language and cognitive disorder, evolutive, Addison syndrome, increased VLCFA	White matter images, hyperintense in T2 and hypointense in T1, bilateral frontal, parietal and occipital, Gd (+)	Uncle affected, sister carrier, X-linked	X-linked adrenoleukodystrophy	Sanger method: <i>ABCD1</i> gene
5	M	18	Learning disorders, pyramidal syndrome, periferic neuropathy, evolutive, increased VLCFA	White matter images, hyperintense in T2 and hypointense in T1, bilateral parietal and occipital with involvement of brainstem and cerebellum	Brother with increased VLCFA	X-linked adrenoleukodystrophy	Sanger method: <i>ABCD1</i> gene
6	M	20	Peripheral neuropathy, pyramidal syndrome, language and cognitive disorder, evolutive, Addison syndrome, increased VLCFA	Right periventricular white matter image hyperintense in T2, tractography: decrease in the fibers of the pyramidal path	Sister carrier, X-linked	X-linked adrenoleukodystrophy	Sanger method: <i>ABCD1</i> gene

(Continues)

TABLE 1 (Continued)

Patient number	Gender	Age of onset (years)	Clinical	Brain MRI	Family history inherited pattern	Clinical diagnosis	Gene sequencing method
7	M	3	Epilepsy, without symptoms for 13 years, then ataxia, spastic quadriparesis, language disorder, evolutive, increased VLCFA	White matter images, hyperintense in T2 and hypointense in T1, bilateral parietal and occipital	Mother with peripheral neuropathy, X-linked	X-linked adrenoleukodystrophy	Sanger method: <i>ABCD1</i> gene
8	F	47	TIA, migraine with aura, mood disorders	Bihemispheric white matter images hyperintense in T2, with temporal pole involvement	Autosomal dominant	CADASIL	Sanger method: <i>NOTCH3</i> gene
9	M	34	TIA, migraine without aura	Bihemispheric white matter images hyperintense in T2, with temporal pole involvement	Sporadic	CADASIL	Sanger method: <i>NOTCH3</i> gene
10	F	32	Epilepsy, migraine with aura	Bihemispheric white matter images hyperintense in T2, with temporal pole involvement	Autosomal dominant	CADASIL	Sanger method: <i>NOTCH3</i> gene
11	M	27	TIA	Bihemispheric white matter images hyperintense in T2, with temporal pole involvement	Autosomal dominant	CADASIL	Sanger method: <i>NOTCH3</i> gene
12	F	30	Stroke, migraine without aura, epilepsy, cognitive disorder	Bihemispheric white matter images hyperintense in T2, with temporal and frontal pole involvement	Autosomal dominant	CADASIL	Sanger method: <i>NOTCH3</i> gene
13	F	21	Stroke	Bihemispheric white matter images hyperintense in T2, with supra and infratentorial involvement.	Sporadic	CADASIL	Sanger method: <i>NOTCH3</i> gene
14	F	40	Migraine, cognitive disorder	Bihemispheric white matter images hyperintense in T2, with temporal pole involvement	Sporadic	CADASIL	Sanger method: <i>NOTCH3</i> gene

(Continues)

TABLE 1 (Continued)

Patient number	Gender	Age of onset (years)	Clinical	Brain MRI	Family history inherited pattern	Clinical diagnosis	Gene sequencing method
15	M	44	Migraine, arterial hypertension	Bihemispheric white matter images hyperintense in T2, with temporal pole and external capsule involvement	Autosomal dominant	CADASIL	Sanger method: <i>NOTCH3</i> gene
16	M	46	Migraine, cognitive disorder	Bihemispheric white matter images hyperintense in T2, with temporal pole and external capsule involvement	Autosomal dominant	CADASIL	Sanger method: <i>NOTCH3</i> gene
17	M	43	Stroke, migraine with aura, arterial hypertension	Bihemispheric white matter images hyperintense in T2, with temporal pole and external capsule involvement	Sporadic	CADASIL	Sanger method: <i>NOTCH3</i> gene
18	M	3	Epilepsy, intellectual disability, low weight and short stature, macrocephaly	Bilateral periventricular white matter images, hyperintense in T2, with infratentorial and spinal cord involvement	Sporadic	Unspecific phenotype	Panel gene sequencing with NGS technique
19	M	1	Epilepsy, intellectual disability, macrocephaly	Bihemispheric white matter images, hyperintense in T2, with frontal lobe involvement	Sporadic	Alexander disease	Sanger method: <i>GFAP</i> gene
20	F	0	Epilepsy, intellectual disability, macrocephaly, spastic quadriparesis, ataxia	Bihemispheric white matter images, hyperintense in T2, with frontal lobe involvement	Sporadic	Alexander disease	Sanger method: <i>GFAP</i> gene
21	F	1	Epilepsy, intellectual disability, microcephaly, spastic quadriparesis, ataxia, strabismus and nystagmus, hypoaacusis, low weight and short stature, dysmorphism, hypogonadism, hypoparathyroidism	Diffuse cortical atrophy, hyperintensity of lenticular nucleus in T1, bilateral periventricular images hyperintense in T2 hypointense in T1, corpus callosum atrophy	Sporadic	Unspecific phenotype	Panel gene sequencing with NGS technique

(Continues)

TABLE 1 (Continued)

Patient number	Gender	Age of onset (years)	Clinical	Brain MRI	Family history inherited pattern	Clinical diagnosis	Gene sequencing method
22	M	6	Ataxia, cognitive and conductal disorder, visual involvement and nystagmus, hypoaacusis	Bilateral periventricular images hyperintense in T2, hypointense in T1, corpus callosum and brainstem atrophy	Sporadic	Unspecific phenotype	Panel gene sequencing with NGS technique
23	M	1	Epilepsy, intellectual disability, ataxia, spastic quadriparesis, hypodontia, dysmorphia	Bihemispheric white matter images hyperintense in T2, hypointense in T1	Sporadic	4 "H" leukodystrophy	Plan: panel gene sequencing with NGS technique (genetic heterogeneity) (this patient could not be studied)
24	M	10	Epilepsy, intellectual disability, ataxia, spastic quadriparesis evolutive, decreased in arylsulfatase A activity and increased in urine sulfatides	Diffuse cortical atrophy, bilateral periventricular images hyperintense in T2 hypointense in T1, corpus callosum atrophy	Autosomal recessive	Metachromatic leukodystrophy	Sanger method: ARSA gene
25	M	1	Intellectual disability, spastic quadriparesis evolutive, decreased in arylsulfatase A activity and increased in urine sulfatides	Bilateral periventricular images hyperintense in T2 hypointense in T1	Sporadic	Metachromatic leukodystrophy	Sanger method: ARSA gene
26	M	1	Epilepsy, intellectual disability, ataxia, spastic quadriparesis evolutive, decreased in arylsulfatase A activity and increased in urine sulfatides	Bilateral periventricular images hyperintense in T2 hypointense in T1 with infratentorial and corpus callosum involvement	Sporadic	Metachromatic leukodystrophy	Sanger method: ARSA gene
27	M	27	Evolutive, spastic quadriparesis, saccadic eyes movement disorder, cognitive impairment	Asymmetric bihemispheric white matter images, hyperintense in T2, with frontal lobe involvement	Autosomal dominant	Hereditary diffuse leukoencephalopathy with spheroids and pigmented cells	Sanger method: CSF/R gene
28	F	71	Ataxia, cognitive disorder, evolutive	Asymmetric bihemispheric white matter images, hyperintense in T2, with frontal lobe involvement	Sporadic	Hereditary diffuse leukoencephalopathy with spheroids and pigmented cells	Sanger method: CSF/R gene

(Continues)



TABLE 1 (Continued)

Patient number	Gender	Age of onset (years)	Clinical	Brain MRI	Family history inherited pattern	Clinical diagnosis	Gene sequencing method
29	F	1	Intellectual disability, epilepsy, macrocephaly, Ataxia	Bihemispheric hypomyelination with subcortical temporal cysts	Unknown	Megalencephalic leukodystrophy with subcortical cysts	Panel gene sequencing with NGS technique (this gene is not candidate to individual sequencing)
30	F	15	Epilepsy, left hemiparesis, pyramidal syndrome, ataxia, cognitive disorder, evolutive	Asymmetric bihemispheric white matter images, hyperintense in hyperintense in T2	Sporadic	Unspecific phenotype	Panel gene sequencing with NGS technique
31	F	3	Ataxia, status epilepticus and posterior progressive encephalopathy, spastic quadriparesis, microcephaly, Primary amenorrhea and absence of secondary sexual characteristics	Bihemispheric white matter images, hyperintense in T2 with tendency to cysts formations, diffuse atrophy	Sporadic	Unspecific phenotype	Panel gene sequencing with NGS technique
32	F	5	Brain injury then ataxia and posterior recovery, acute left hemiparesis, migraine, epilepsy, premature ovarian failure	Asymmetric bihemispheric white matter images, hyperintense in T2 with tendency to cysts formations, diffuse atrophy	Sporadic	Vanishing white matter disease	Sanger method: <i>EIF2B5</i> gene, exon 7
33	M	2	Brain injury then ataxia and posterior recovery, other episode of brain injury then left hemiparesis and posterior partial recovery, pyramidal syndrome	Bihemispheric white matter images, hyperintense in T2 with tendency to cysts formations, diffuse atrophy	Sporadic	Vanishing white matter disease	Panel gene sequencing with NGS technique (genetic heterogeneity)
34	M	4	Evolutive cognitive disorder	Bihemispheric white matter images, hyperintense in T2 with tendency to cysts formations	Sporadic	Vanishing white matter disease	Plan: panel gene sequencing with NGS technique (genetic heterogeneity) (this patient could not be studied)

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TABLE 1 (Continued)

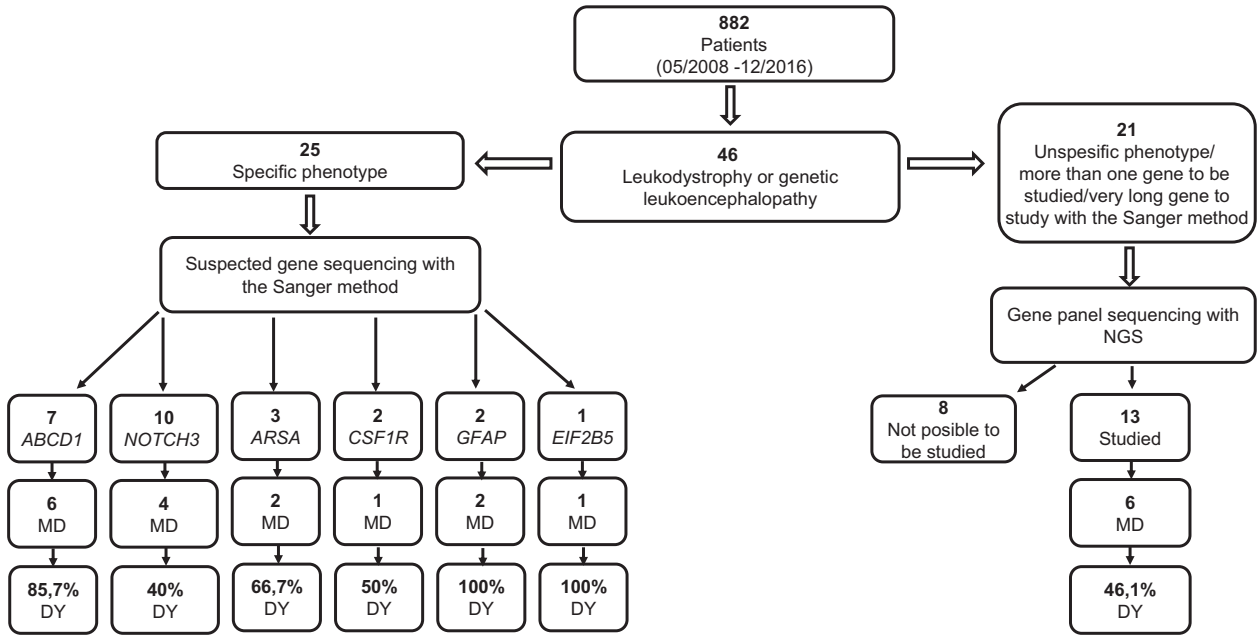
Patient number	Gender	Age of onset (years)	Clinical	Brain MRI	Family history inherited pattern	Clinical diagnosis	Gene sequencing method
35	M	1	Intellectual disability, spastic quadriparesis, visual disorders	Bihemispheric white matter images, hyperintense in T2 with tendency to cyst formation, diffuse atrophy	Sporadic	Vanishing white matter disease	Plan: panel gene sequencing with NGS technique (genetic heterogeneity) (this patient could not be studied)
36	M	3	Epilepsy, spastic quadriparesis, ataxia, cognitive disorder, hypoaacusis, evolutive	Bihemispheric white matter images, hyperintense in T2 with corpus callosum and infratentorial involvement	Sporadic	Unspecific phenotype	Plan: panel gene sequencing with NGS technique (this patient could not be studied)
37	M	33	Visual disorders, transient focal neurological disorders, migraine, hypoaacusis	Bihemispheric white matter images, hyperintense in T2 with tendency to cysts formations	Sporadic	Unspecific phenotype	Plan: panel gene sequencing with NGS technique (this patient could not be studied)
38	M	0	Epilepsy, intellectual disability, spastic quadriparesis, ataxia, cognitive disorder	Bihemispheric white matter images, hyperintense in T2 with supra and infratentorial involvement	Sporadic	Unspecific phenotype	Plan: panel gene sequencing with NGS technique (this patient could not be studied)
39	F	4	Epilepsy, spastic quadriparesis, language disorder, evolutive, systemic lupus erythematosus	Bihemispheric white matter images, hyperintense in T2 with basal ganglia calcification	Sporadic	Aicardi-Goutières	Plan: panel gene sequencing with NGS technique (this patient could not be studied)
40	M	40	Episodic vertigo, hypothyroidism	Bihemispheric white matter images, hyperintense in T2 with supra and infratentorial involvement	Sporadic	Unspecific phenotype	Panel gene sequencing with NGS technique

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TABLE 1 (Continued)

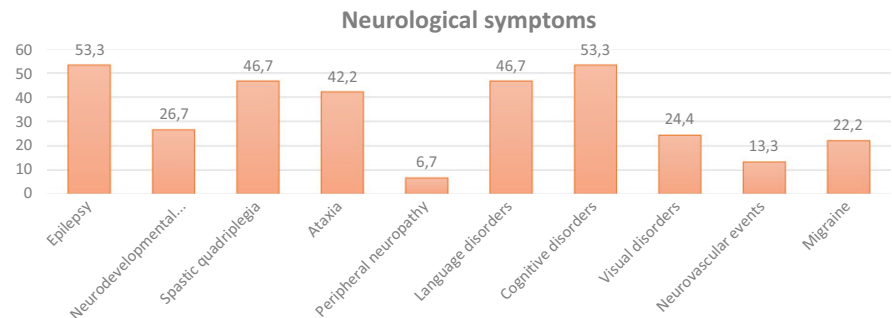
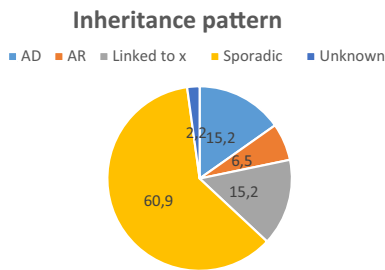
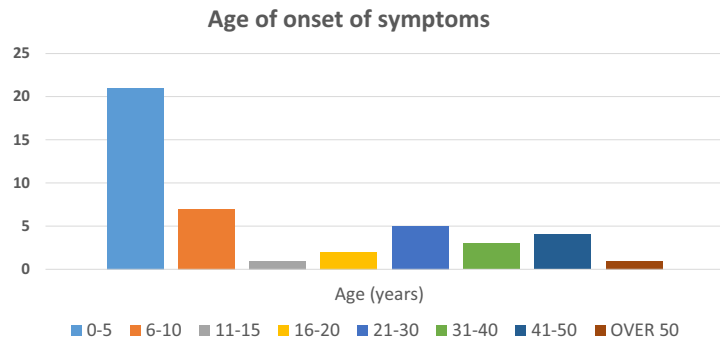
Patient number	Gender	Age of onset (years)	Clinical	Brain MRI	Family history inherited pattern	Clinical diagnosis	Gene sequencing method
41	M	1	Epilepsy, intellectual disability, spastic quadriparesis, cognitive disorder, microcephaly	Bilateral periventricular white matter images, hyperintense in T2	Autosomal recessive	Unspecific phenotype	Panel gene sequencing with NGS technique
42	F	28	Epilepsy, ataxia, cognitive disorder, evolutive, premature ovarian failure, hypothyroidism	Bihemispheric white matter images, hyperintense in T2 with supra and infratentorial involvement	Sporadic	Unspecific phenotype	Plan: panel gene sequencing with NGS technique (this patient could not be studied)
43	M	1	Epilepsy, intellectual disability, spastic quadriparesis, cognitive and visual disorders	Bihemispheric white matter images, hyperintense in T2 with tendency to cysts formations	Sporadic	Unspecific phenotype	Panel gene sequencing with NGS technique
44	M	10	Migraine, cognitive disorder, pyramidal syndrome	Bihemispheric white matter images, hyperintense in T2	Sporadic	Unspecific phenotype	Panel gene sequencing with NGS technique
45	M	2	Progressive spastic paraparesis, hollow foot	Supratentorial bihemispheric white matter images, hyperintense in T2	Sporadic	Unspecific phenotype	Panel gene sequencing with NGS technique
46	F	1	Intellectual disability, evolutive, ataxia, spastic quadriparesis	Bihemispheric white matter images, hyperintense in T2 with corpus callosum involvement	Autosomal recessive	Unspecific phenotype	Panel gene sequencing with NGS technique

Abbreviations: MRI, Magnetic resonance imaging; M, male; F, female; VLCFA, very long-chain fatty acids; TIA, transient ischemic attack; CADASIL, cerebral autosomal-dominant arteriopathy with subcortical infarcts; Gd (+), gadolinium positive.



**FIGURE 2** Cohort of patients studied following the proposed procedure and diagnostic yield by groups of pathologies. Gene, disease associations; *ABCD1*, X-linked adrenoleukodystrophy; *NOTCH3*, CADASIL; *ARSA*, metachromatic leukodystrophy; *CSF1R*, hereditary diffuse leukoencephalopathy with spheroids and pigmented cells; *GFAP*, Alexander disease; *EIF2B5*, vanishing white matter disease. Abbreviations: MD, Molecular diagnosis; DY, diagnostic yield

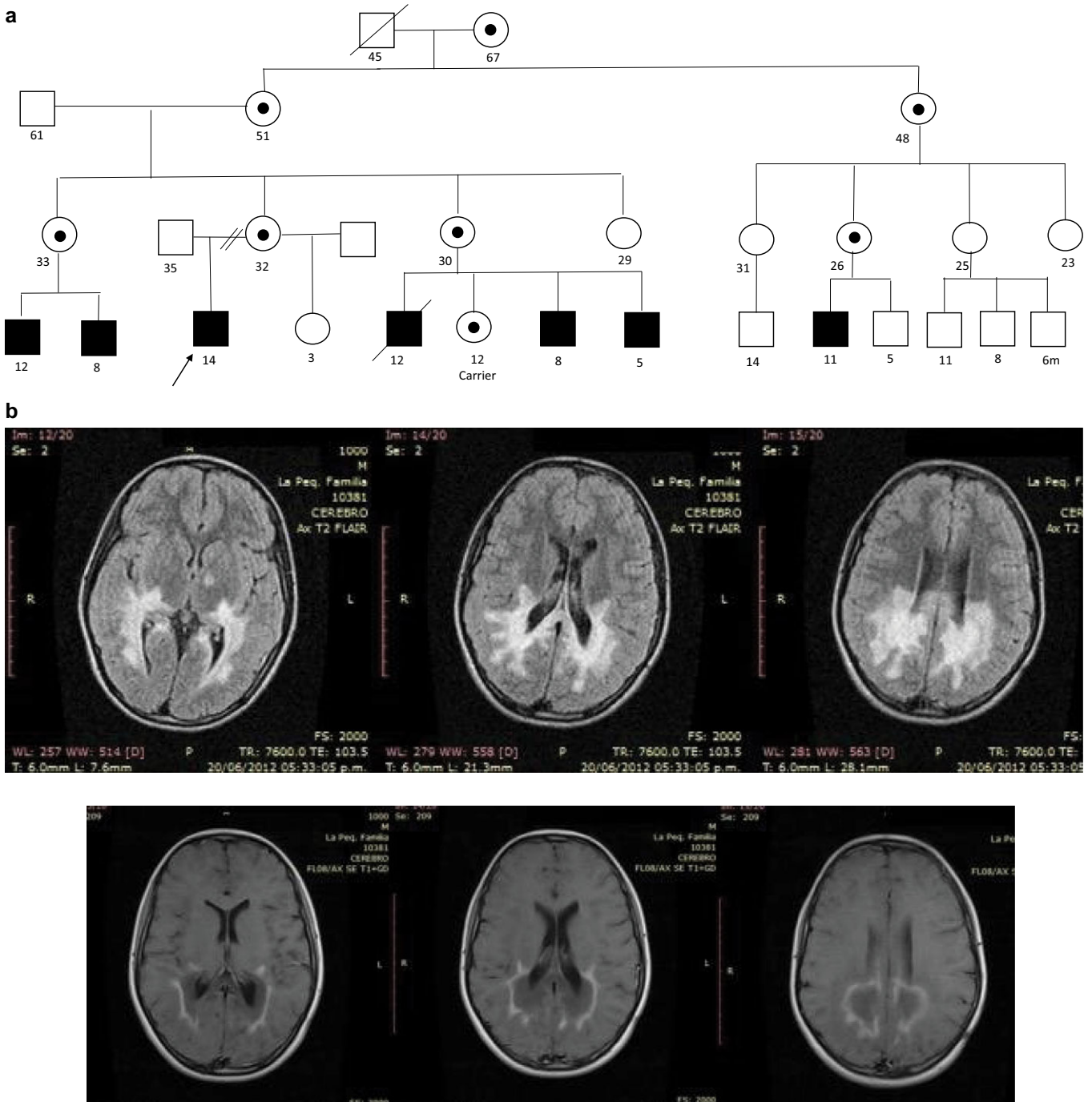
Patients	46
Male sex	31
Female sex	15



**FIGURE 3** Demographic and clinical characteristics of the included patients. Abbreviations: AD, Autosomal dominant; AR, autosomal recessive [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

of them presented variable clinical forms, without radiological signs or neurometabolic studies suggestive of any particular entity, or because of genetic heterogeneity or the

size of the candidate gene made the individual approach difficult. Molecular diagnosis was obtained in six of them (46.1%).



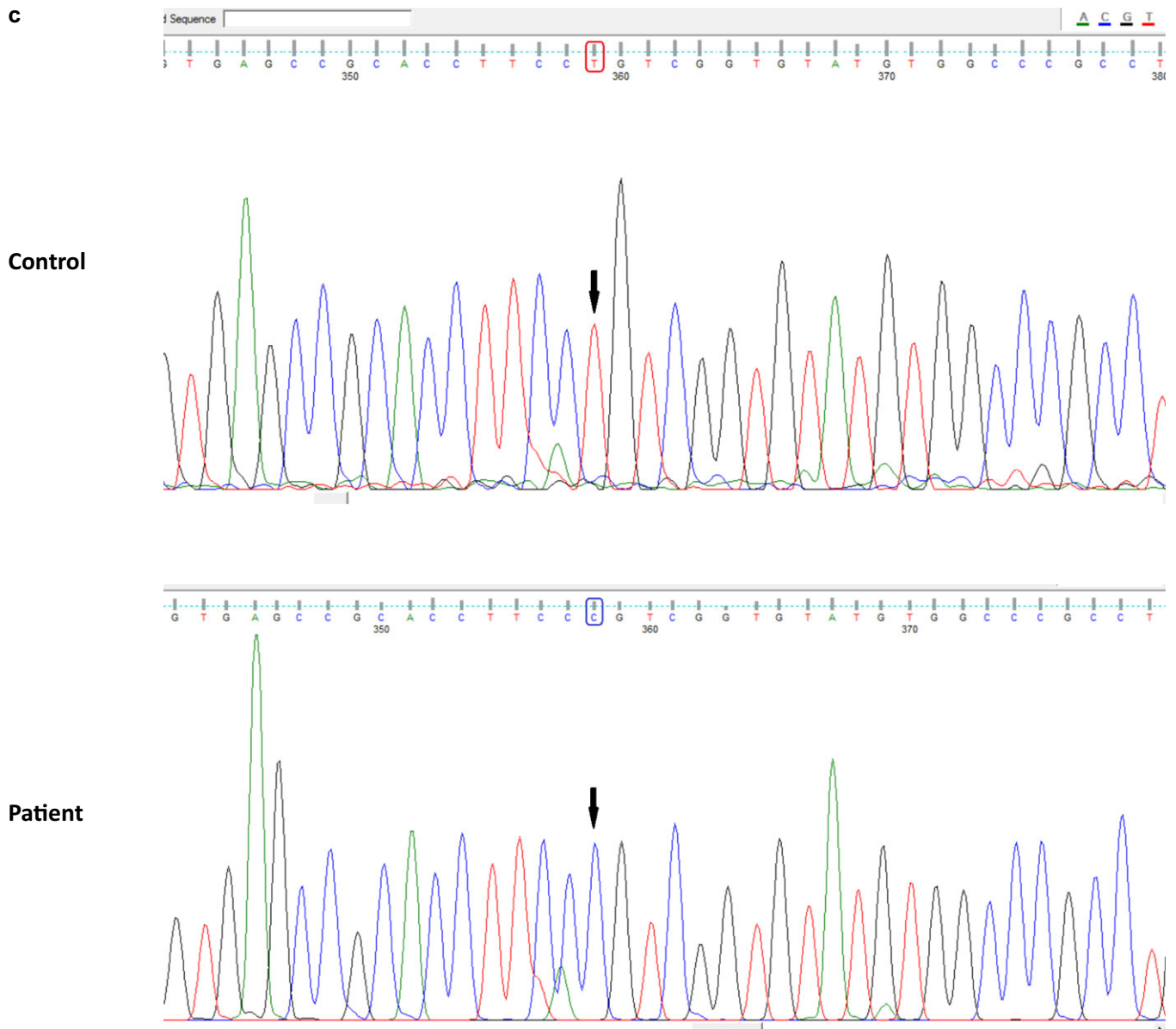
**FIGURE 4** (a) Family tree Patient No. 1. (b) Brain magnetic resonance imaging of patient No. 1: White matter images, hyperintense in T2 and hypointense in T1, bilateral periventricular and subcortical in parietal and occipital lobes. (c) Electropherogram of patient No. 1: Representation of c.320T>C (p.Leu107Pro) variant in exon 1 of ABCD1 gene. Those have a peripheral enhancement with gadolinium [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

### 3.3 | Illustrative cases

#### 3.3.1 | Case No. 1 (Patient No. 1)

A male patient, at 10 years old, presented with convulsions, progressive neurologic deterioration manifesting as pyramidal motor compromise, changes in skin color, visual symptoms, and a family history suggestive of X-linked

inheritance (Figure 4a). Neurological examination revealed severe phonological deglutition disorder, emission of incomprehensible sounds, bilateral visual acuity affection with homonymous hemianopsia and convergent strabismus, spastic quadriparesis with pyramidal signs, and cervical dystonic posture. MRI of the brain showed confluent images in posterior periventricular white matter, hyperintense in T2 and spin echo



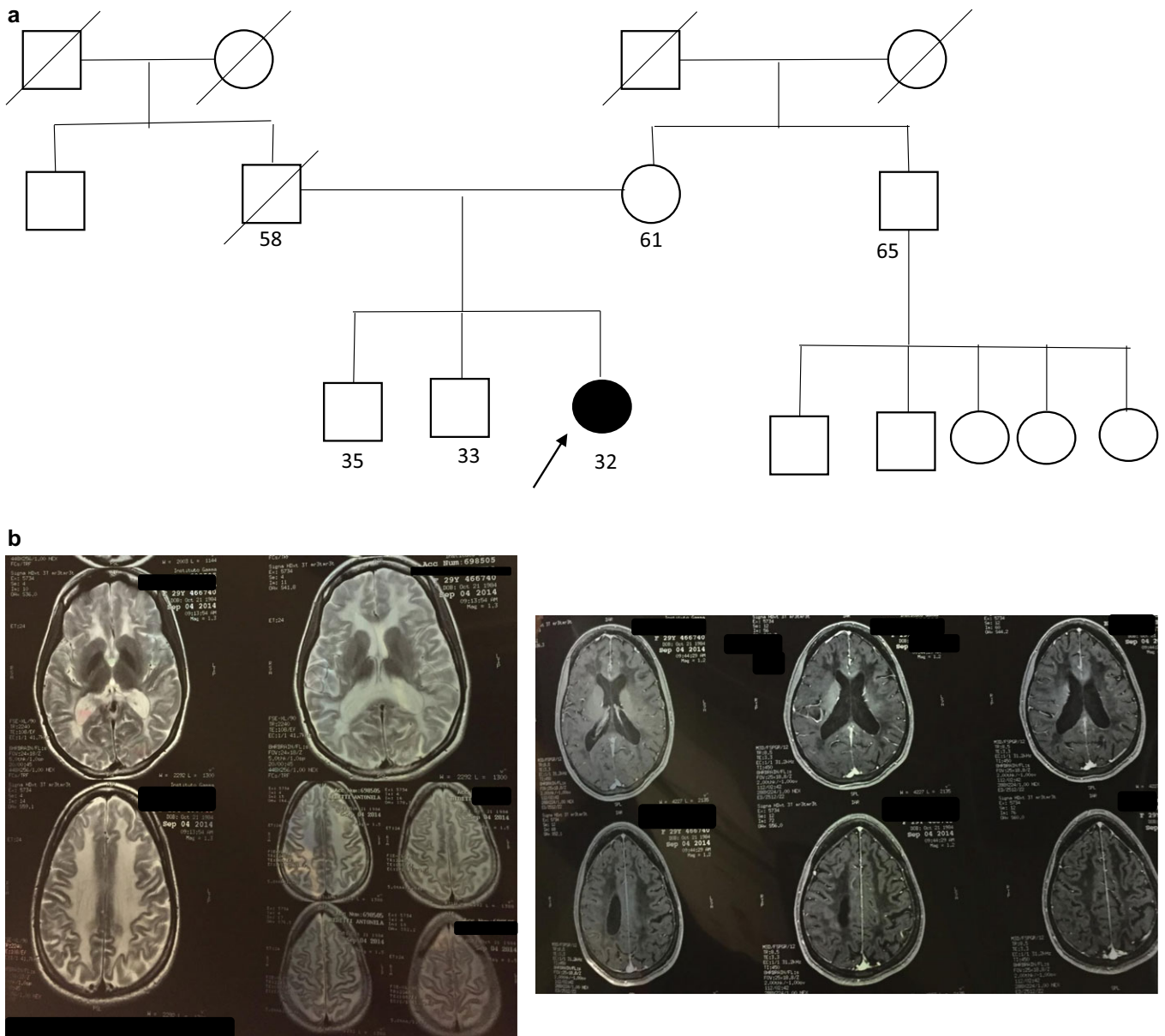
**FIGURE 4** Continued

sequences enhanced in T2, hypointense in T1, with peripheral enhancement after contrast administration (Figure 4b). Laboratory findings included increased adrenocorticotrophin and decreased cortisol compatible with adrenal insufficiency, and a neurometabolic study indicated an increase in very long-chain fatty acids. With presumptive diagnosis of X-linked adrenoleukodystrophy, the *ABCD1* gene was sequenced, and c.320T > C variant (p.Leu107Pro) was detected in exon 1, classifiable as pathogenic and previously reported in the literature (Figure 4c; Korenke et al., 1997; Krasemann, Meier, Korenke, Hunneman, & Hanefeld, 1996).

### 3.3.2 | Case No. 2 (Patient No. 30)

A 32-year-old female patient had presented at 15 years of age with progressive neurological symptoms, characterized by

focal seizures, ataxia, motor deficit in the left side of the body, and cognitive impairment, with no family history (Figure 5a). A metabolic laboratory without relevant findings, and brain MRI with confluent lesions in bilateral periventricular and subcortical white matter, hyperintense in T2 and hypointense in T1, with tractography showed an asymmetric bilateral pyramidal pathway compromise (Figure 5b). Given that the phenotype did not approach a defined clinical-radiological syndrome, a multigenic panel sequencing was resorted to using next-generation techniques. A pathogenic variant in homozygosity was identified in exon 3 of the *EIF2B3* gene (\*606273): NM\_001261418: c.260C > T (p.A87V), which has been previously reported in the literature as pathogenic (van der Knaap et al., 2002), concluding in the diagnosis of vanishing white matter disease.



**FIGURE 5** 5 Patient No. 1: Electropherogram representation of c.320T > C (p.Leu107Pro) variant in exon 1 of the *ABCD1* gene. (a) Family tree patient No. 30. (b) Brain magnetic resonance imaging of patient No. 30: bihemispheric white matter images, periventricular and subcortical, hyperintense in T2, hypointense in T1, associated with asymmetric ventriculomegaly [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

## 4 | DISCUSSION

In the present work, a pediatric and adult population of patients with genetic leukoencephalopathies and leukodystrophies was characterized, obtaining a genetic diagnosis in a significant number of individuals. This has been possible because of the development of a diagnostic procedure by means of which the molecular study path is addressed according to the presumption that arises from the phenotypic evaluation.

Although these entities are characterized by a marked clinical heterogeneity and involve a high number of candidate genes (Lynch et al., 2017), the identification of the causal

defect was achieved in 22 of the 38 patients studied. Our results confirm the usefulness of the proposed diagnostic procedure, expressed by the significant diagnostic yield (57.9%). Noteworthy, the yield for Sanger sequencing in the CADASIL phenotype was 40%, whereas the yield for the remaining phenotypes was higher at 80%.

In patients with an uncharacteristic and/or complex phenotype, the use of a directed capture of genes through the NGS technology reaffirmed the performance of these techniques for the diagnostic approach in these pathologies (Wang et al., 2016). This constituted a quick and effective instrument, allowing us to arrive at a definite etiology even when

the clinical manifestation is not eloquent (Gordon, Letsou, & Bonkowsky, 2014), reducing costs by shortening the long process of complementary methods of study that patients are often subjected to (Córdoba, González Morón, Rodríguez-Quiroga, & Kauffman, 2014).

This work covers the largest series of patients affected by genetic diseases of the cerebral white matter reported in Argentina, with the inclusion of clinical and molecular data. In relation to similar publications in other countries, we found similarities in clinical manifestations and in the genes studied, with individual differences in the frequency of each of the pathologies, probably because of the different epidemiological characteristics of the populations evaluated (Lynch et al., 2017; Stellitano, Winstone, van der Knaap, & Verity, 2016). Likewise, there was a greater proportion of adult patients because of the high number of people belonging to this age group being evaluated at our center, which is reflected in the diagnostic approach to pathologies characteristic of this group, such as CADASIL, and diffuse hereditary leukoencephalopathy with axonal spheroids and pigmented cells.

In other cohorts that studied populations of similar ages, the suspicion based on the clinical-radiological characterization was similar, with higher frequency of adult-onset vanishing white matter disease, and other pathologies such as leukodystrophy with ovarian failure related to the mutation in the *AARS2* gene, which we did not have in our series (Lynch et al., 2017). The sequence of the *NOTCH3* gene confirmed the diagnosis in only four of the patients studied with suspected CADASIL, unlike other reports where the diagnostic yield was higher (Chen et al., 2017). In our particular case, we recognize the laxity in the selection criteria, as we studied those patients who met at least one clinical and one radiological criterion described by Davous (1998) without being strict in the sum of 15 or more points on the diagnostic probability scale of CADASIL proposed by Pescini, Nannucci, & Pantoni (2013), there is a better performance in the molecular study of the *NOTCH3* gene.

On the other hand, with respect to the patients studied with suspicion of X-linked adrenoleukodystrophy, in a single patient no pathogenic variants were detected in the *ABCD1* gene, presuming that they may be found in noncoding regions of the gene or because of the presence of a deletion that escapes detection by the Sanger method. A similar case occurs in the patient with suspected metachromatic leukodystrophy in whom a confirmatory molecular diagnosis could not be reached because a single heterozygous pathogenic variant was detected. Therefore, it is believed that there may be another variant in noncoding regions or structural anomalies not identifiable by this method, requiring additional techniques such as MLPA assays not performed by our group. Finally, the case with suspicion of diffuse hereditary leukoencephalopathy with axonal spheroids and pigmented cells that we could

not confirm, corresponded to a patient with a nonconclusive clinical phenotype, with difficulty in the subsequent follow-up.

The illustrated cases are paradigmatic. The first patient with phenotypic characteristics allowed suspicion of a particular entity, leading to the sequencing of an individual gene by classical techniques, with a high probability of achieving a definitive etiological diagnosis through a single molecular study. The second case represents a sufficiently complex phenotype to require a broader approach, feasibly to be analyzed through a next-generation technique as a directed panel of candidate genes, which in a single step allowed obtaining a molecular diagnosis, confirming a defined pathological entity, reducing time and costs (Richards, Korgenski, Taft, Vanderver, & Bonkowsky, 2015). The configuration of a diagnostic procedure allows the treating physician to have a better approach to this complex group of pathologies, optimizing the diagnostic resources.

The access to an etiological diagnosis derives in medical actions that originate from it, through the early detection or monitoring of the different comorbidities (Parikh et al., 2015). The possibility of providing adequate genetic counseling through the guidance on risk of recurrence is equally important, and, most of all, the obtaining of a greater proportion of molecular diagnoses expands the knowledge of the clinical spectrum of each genetic leukoencephalopathy, configuring a registry of the individualized causal defect for future therapeutic opportunities in current development. Moreover, comprehensive diagnostic approaches, such as the one used by our group, reduces the time that these patients must wait before getting a diagnosis thereby ending odysseys of many years, impacting in disorders such as ALD-X on their medical management, and optimizing the genetic counseling for these families. However, we must keep working on new methods that allow a rapid diagnosis of the genetic conditions of the cerebral white matter to reduce the individual and family impact.

The vast amount of knowledge that has emerged in recent years about the genes linked to leukodystrophies and genetic leukoencephalopathies will provide genomics an increasingly essential role as a tool for diagnostic use, which will lead to the growing need to develop therapeutic strategies to avoid the significant morbidity and mortality of these pathologies.

Despite the notorious advances achieved with the increasing use of massive sequencing techniques, a high percentage of affected individuals remain without genetic diagnosis. It is necessary to have larger groups of patients through collaborative work between the different groups of studies, to have a deeper and more extensive knowledge of these conditions. Research in the molecular diagnosis of genetic leukoencephalopathies and leukodystrophies continues to be a great challenge in this new era where knowledge and promising therapeutic advances evolve daily.



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## AUTHOR CONTRIBUTIONS

M.K. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The study concept and design were performed by L.C., P.V., and M.K. Acquisition of data was done by L.C., A.M., H.A., N.S., M.C., and N.M. Analysis and interpretation of data was done by L.C., N.M., S.R.-Q., D.G.-M., J.R., and P.V. Manuscript preparation was performed by all authors.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

## INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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