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ORIGINAL ARTICLE

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Metachromatic leukodystrophy: A single-center longitudinal study of 45 patients

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Abstract

In this study, we characterize the natural course of metachromatic leukodystrophy (MLD), explore intra/inter group differences, and identify biomarkers to monitor disease progression. This is a longitudinal observational study. Genotype and characteristics at disease onset were recorded. Time-to-event analyses were performed to assess time to major disease-related milestones in different subgroups. Longitudinal trajectories of nerve conduction velocities (NCV), brain MRI score, and brainstem auditory evoked responses (BAERs) were described. We recruited 22 late-infantile, 14 early-juvenile, 5 late-juvenile, and 4 adult MLD patients. Thirty-four were prospectively evaluated (median FU time 43 months). In late-infantile patients, the attainment of independent walking was associated with a later age at dysphagia. In early-juvenile, the presence of isolated cognitive impairment at onset was not a favorable prognostic factor. Late-infantile and early-juvenile subjects showed similar rapid loss of ambulation and onset of seizures, but late-infantile displayed earlier loss of trunk control, dysphagia, and death. We found significant differences in all major disease-related milestones (except death) between early-juvenile and late-juvenile patients. Late-juvenile and adult patients both presented with a predominant cognitive impairment, mild/no peripheral neuropathy, lower brain MRI score at plateau compared to LI/EJ, and later cerebellar involvement. NCV and BAER were consistently severely abnormal in late-infantile but not in older subjects, in whom both NCV and BAER were

Francesca Fumagalli and Alberto A. Zambon contributed equally to this study.

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variably affected, with no deterioration over time in some cases. This study clarifies intra/inter group differences between MLD subtypes and provides additional indications regarding reliable clinical and instrumental tools to monitor disease progression and to serve as areference to evaluate the efficacy of future therapeutic interventions inthe different MLD variants.

KEYWORDS
longitudinal study, metachromatic leukodystrophy, MLD, natural history

1 | INTRODUCTION

Metachromatic leukodystrophy (MLD-OMIM 250100) is an autosomal recessive lysosomal storage disorder primarily caused by mutations in the *ARSA* gene, which encodes for the arylsulfatase A (ARSA) enzyme.[1,2] The lack of enzymatic activity leads to the accumulation of toxic substrates in tissues such as gallbladder, pancreas, kidneys, and, most importantly, myelin-forming cells; this causes progressive dysfunction of the central (CNS) and peripheral nervous system (PNS).[1,3-6]

Pathogenic variants in the *ARSA* gene (MIM# 607574) are classified as severe (0) or mild (R) based on residual enzymatic activity. Genotype-phenotype correlation has been variably reported.[7-10] Three main clinical phenotypes have been described: the late-infantile (LI, onset 0-2.5 years) form (40-60% of cases),[10] usually associated with two 0 alleles, and characterized by early motor impairment, rapid psychomotor regression, and premature death[11]; the juvenile (J, onset 2.6-16 years) variant (20-40% of cases, with a higher prevalence in specific geographic areas[12]), characterized by a combination of motor and cognitive deficits at onset and a more heterogenous progression than LI[13]; the adult (AD, onset >16 years) form (18-20% of cases), frequently presenting with cognitive decline, behavioral and psychiatric disturbances, and with variable outcomes.[14] The J and AD variants are either heterozygous or homozygous for R alleles.[12] Juvenile patients are commonly subdivided into early-juvenile (EJ) and late-juvenile (LJ). Although usually 6 years of age is the division between EJ and LJ, such threshold appears arbitrary and a consensus on this is lacking.[8,15]

In the past few years, advances in translational research have brought hope to patients affected by MLD, with different therapeutic strategies showing promising results in slowing disease progression.[16-22] However, the evaluation of efficacy of such treatments is sometimes hindered by the complexity and variability of the disorder.[13,23,24]

Here we present the results of a natural history study conducted over 17 years on a large group of MLD patients. We provide an extensive overview of the natural course of the disease and explore the reliability and applicability of different biomarkers in characterizing and distinguishing the evolution of the different variants of the disease.

2 | METHODS

2.1 | Study design and participants

This is a longitudinal, retrospective, and prospective observational study conducted on 45 MLD patients from 43 unrelated families (patients J04-J11 and J08-J14 are siblings). Patients were assessed at the San Raffaele Scientific Institute in Milan, Italy, between 2000 and 2017. All participants were enrolled in a natural history study approved by our institutional review board, after obtaining informed consent from parents/guardians. Since 2010, patients eligible for lentiviral-based Gene therapy (clinical trials EudraCT numbers: 2009-017349-77 and 2017-001730-26) were not considered for the study.[16] Clinical history and information regarding early milestones of motor development and disease onset were collected from medical reports and family interviews. *ARSA* gene sequencing, ARSA enzymatic activity and urinary sulfatides were investigated in patients not yet characterized elsewhere to confirm the diagnosis of MLD. Pathogenic variants were classified according to criteria described in detail in section 1.1 of Data S1, Supporting Information.[25-29] The diagnosis of MLD was defined by the presence of two out of three of the following criteria: (a) defective ARSA activity on peripheral blood mononuclear cells or fibroblasts, (b) presence of biallelic mutations in the *ARSA* gene, (c) increase of urine sulfatides in a 24-h sample. Patients were grouped according to age of onset. Two subjects with onset at 75 (Pt J13) and 81 (Pt J14) months were

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included in the EJ cohort because both patients presented a phenotype at onset more consistent with EJ (eg, coexistence of cognitive-including loss of sphincter controls-, and motor symptoms)[14]; in addition, in the sibling of Pt J14 (Pt J08), first symptoms were recognized at 5 years of age. Therefore, the EJ group analyzed in this study includes patients with an onset before the seventh birthday.[14] All LJ subjects manifested their first symptoms after the seventh birthday. Patients were prospectively assessed with regular clinical and instrumental evaluations (every 6-12 months when possible), as detailed in sections 1.1 and 1.2 of Data S1).

2.2 | Outcome measures

For the description of disease progression, we focused on survival, gross motor, and bulbar function. Clinical and instrumental endpoints selected for time-to-event analysis were the following: death, GMFC-MLD > 1 (*loss of independent ambulation*), GMFC-MLD > 4 (*loss of trunk control*), dysphagia, percutaneous gastrostomy (PEG) insertion, epilepsy (onset of first documented seizure), brainstem auditory evoked response (BAER) > 0 (*Abnormal exam*), BAER > 2 (*disappearance of both peripheral [Wave I] and central [Wave III and V] components*), first signs of cerebellar white matter alteration, cerebellar atrophy and severe cerebellar atrophy at brain magnetic resonance imaging (MRI).

Additional outcome measures were the longitudinal trajectory of peroneus nerve conduction velocity and Brain MRI score.

2.3 | Statistical analysis

Time-to-event curves were estimated with the Kaplan-Meier method (considering interval-censored data for all events except death). The log-rank test was used to assess the difference in overall survival (OS) between groups, while the permutation-based log-rank test was employed for interval-censored data. Cox-proportional hazard model was used to assess the impact of time-varying covariate on OS.

Nonlinear mixed-effects (NLME) model was used to estimate the longitudinal trend of the total MRI score and to compare the trend between MLD groups. Detailed statistical methods are described in section 1.3 of Data S1. For all tests, the significance level was set at 0.05. All statistical analyses were performed using R 3.5.0 (http://www.R-project.org/).

3 | RESULTS

3.1 | Cohort description

We recruited 45 patients (21 males; 46%). LI, J, and AD patients represented 49% (n = 22), 42% (14 EJ and 5 LJ) and 9% (n = 4) of our cohort, respectively. Fourteen LI, 16 J and all AD performed more than one evaluation at our hospital. The median time from onset to the first assessment at our center was 24 months (range 5-159 months). The median length of prospective FU was 43 months (max 18 years), with a median of four visits per patient (range 1-23). Three patients (2 LI and 1 J) dropped out from the study after receiving other experimental treatments. Nine additional patients (4 LI and 5 J) were lost at follow-up (FU). These patients did not present with peculiar features compared to subjects who remained in the study.

Four patients were offspring of consanguineous marriages. Forty-one patients were White-Caucasians (Caucasian European heritage), while four were White-Arabs (North African heritage). Demographics, age of onset, and genotype are presented in Table 1. Genotype, symptoms at onset, neurodevelopment, prospective cognitive assessment, and extra neurological involvement are detailed in section 2 in Data S1.

3.2 | Clinical progression

Time-to-event analysis for the major disease-related events (namely, death, loss of ambulation, loss of trunk control, dysphagia/PEG insertion, and epilepsy) according to MLD subtype and genotype are shown in Figures 1A-F and S1, respectively. Relevant comparisons of event-free survivals between groups are displayed in Tables 2 and S1. Comparisons were performed not only with respect to subtypes and genotypes, but also grouping patients according to specific clinical features (LI subjects who had attained independent ambulation vs those who did not; gross-motor symptoms vs isolated cognitive symptoms at onset for EJ).

3.2.1 | Survival

Overall survival from disease onset was statistically different between LI and EJ patients ($P = 0.013$), but not between EJ and LJ subjects ($P = 0.248$) (Figure 1A and Table S1). Notably, the insertion of PEG was not associated with prolonged survival in late-infantile patients (HR [95% CI] = 0.78 [0.33;1.83]; *P*-value 0.572).

3.2.2 | Gross motor function

Twenty-one LI subjects (95%) had a GMFC-MLD score > 1 (*inability to walk independently*) at 36 months

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TABLE 1 Demographics, genotype, and onset

Pt code, sex	Type	Age of onset (months)	Allele 1[b]	Allele 2[b]	Mutation effect	Reference[c]
LI01, F	LI	<15	c.161T>C; p.(Leu54Pro)	c.449C>T; p.(Pro150Leu)	0/Unc	9,26
LI02, F	LI	<15	c.1228_1229del; p.(Thr410Hisfs*16)	c.1228_1229del; p.(Thr410Hisfs*16)	0/0	9,26
LI03, F	LI	<15	c.465+1G>A	c.465+1G>A	0/0	9
LI04, M	LI	<15	c.763G>A; p.(Glu255Lys)	c.465+1G>A	0/0	9
LI05, M	LI	<15	c.827C>T; p.(Thr276Met)	c.827C>T; p.(Thr276Met)	0/0	26,29
LI06, F	LI	<15	c.1114C>T; p.(Arg372Trp)	c.1114C>T; p.(Arg372Trp)	0/0	9
LI07, M	LI	<15	c.1222_1230del; p.(Ser408_Thr410del)	c.917C>T; p.(Thr306Met)	0/0	9,26
LI08, M	LI	<15	c.465+1G>A	c.640G>C; p.(Ala214Pro)	0/0	9,26
LI09, F	LI	<15	c.465+1G>A	c.465+1G>A	0/0	9
LI10, M	LI	<15	c.883G>A; p.(Gly295Ser)	c.938G>A; p.(Arg313Gln)	0/0	9
LI11, M	LI	<15	c.350G>T; p.(Gly117Val)	paternal uniparental disomy	0/0	25
LI12, F	LI	15	c.465+1G>A	c.465+1G>A	0/0	9
LI13, M	LI	15	c.449C>G; p.(Pro150Arg)	c.449C>G; p.(Pro150Arg)	0/0	25
LI14, M	LI	16	c.465+1G>A	c.510C>A; p.(Cys170*)	0/0	9,26
LI15, F	LI	15-18	c.465+1G>A	c.855-1G>A	0/0	5
LI16, F	LI	15-18	c. 418dupC	c.1210+1G>A	0/0	
LI17, M	LI	19	c.465+1G>A	c.980-1G>A	0/0	25
LI18, F	LI	24	c.465+1G>A	c.465+1G>A	0/0	9
LI19, F	LI	24	c.827C>T; p.(Thr276Met)	c.1222A>G; p.(Ser408Gly)	0/0	9
LI20, F	LI	24	c.465+1G>A	c.855-1G>A	0/0	9
LI21, F	LI	24-27	c.736C>T; p.(Arg246Cys)	c.737G>A; p.(Arg246His)	0/0	25,29
LI22, M	LI	26	c.1108-2A>G	c.1108-2A>G	Unc/Unc	25
J01, M	EJ	30	c.465+1G>A	c.608A>G; p.(Tyr203Cys)	0/R	9
J02, M	EJ	30	c.931G>A; p.(Gly311Ser)	c.931G>A; p.(Gly311Ser)	R/R	25
J03, M	EJ	32	c.925G>A; p.(Glu309Lys)	c.418C>G; p.(His140Asp)	0/R	9,26
J04, F[a]	EJ	45	c.465+1G>A	c.256C>T; p.(Arg86Trp)	0/R	9
J05, M	EJ	47	c.465+1G>A	c.256C>T; p.(Arg86Trp)	0/R	9
J06, F	EJ	50	c.251C>T; p.(Pro84Leu)	c.256C>T; p.(Arg86Trp)	Unc/R	9
J07, F	EJ	60	c.465+1G>A	c.608A>G; p.(Tyr203Cys)	0/R	9
J08, F[a]	EJ	60	c.608A>G; p.(Tyr203Cys)	c.608A>G; p.(Tyr203Cys)	R/R	9,26
J09, F	EJ	61	**c.200C>T; p.(Pro67Leu)**	c.1283C>T; p.(Pro428Leu)	Unc/R	
J10, M	EJ	62	c.465+1G>A	c.1283C>T; p.(Pro428Leu)	0/R	25
J11, F[a]	EJ	66	c.465+1G>A	c.256C>T; p.(Arg86Trp)	0/R	9
J12, F	EJ	66	c.465+1G>A	c.1283C>T; p.(Pro428Leu)	0/R	25
J13, F	EJ	75	c.465+1G>A	c.1283C>T; p.(Pro428Leu)	0/R	
J14, M[a]	EJ	81	c.608A>G; p.(Tyr203Cys)	c.608A>G; p.(Tyr203Cys)	R/R	9,26
J15, F	LJ	10 y	c.465+1G>A	c.1471T>G; p.(Cys491Gly)	0/R	25
J16, M	LJ	11 y	c.256C>T; p.(Arg86Trp)	c.256C>T; p.(Arg86Trp)	R/R	9,26
J17, M	LJ	11 y	c.868C>T; p.(Arg290Cys)	c.938G>C; p.(Arg313Pro)	R/Unc	
J18, M	LJ	12 y	c.465+1G>A	c.542T>G; p.(Ile181Ser)	0/R	25
J19, F	LJ	14 y	c.1108-3C>G	c.542T>G; p.(Ile181Ser)	0/R	25

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TABLE 1 (Continued)

Pt code, sex	Type	Age of onset (months)	Allele 1[b]	Allele 2[b]	Mutation effect	Reference[c]
AD01, F	AD	24 y	c.1174C>T; p.(Arg392Trp)	c.604C>T; p.(Arg202Cys)	0/Unc	25
AD02, M	AD	31 y	c.256C>T; p.(Arg86Trp)	c.256C>T; p.(Arg86Trp)	R/R	9,26
AD03, F	AD	33 y	c.256C>T; p.(Arg86Trp)	c.931G>A; p.(Gly311Ser)	R/R	25,26
AD04, M	AD	35 y	c.465+1G>A	c.1471T>G; p.(Cys491Gly)	0/R	25

Note: Age of onset is defined as <15 months either when the patient never acquired independent walking or when first symptoms appeared before 15 months of age. According to the WHO normal ranges of motor milestones achievement, 15 months correspond to the age at which the 95% of healthy children reach independent walking. J09 new mutation is highlighted in (bold).

Abbreviations: AD, adults; EJ, early-juvenile; LI, late-infantile; LJ, late-juvenile; 0, null mutation; R, residual activity; Unc, pathogenic variant of uncertain severity; y, years.

[a]Indicate two siblings pairs

[b]Variants are named according to reference sequence NM_000487.5 (NP_000478.3) and HGVS nomenclature (www.hgvs.org/mutnomen).

[c]Pts previously described elsewhere. In addition to the classical 0 mutation c.465+1G>A, a mutation was classified as 0 (severe) if (1) displaying a null in vitro ARSA activity or (2) causing the disruption of big portions of the reading frame, (3) described in homozygosis or heterozygosis with another known 0 mutation in patients with LI form (28), or (4) described in heterozygosis with a R mutation in patients with LI form (28). In addition to the classical R mutation c.1283C>T, a mutation was classified as R (mild) if (1) retaining some residual enzymatic activity (29-31) or (2) described in homozygosis or in heterozygosis with a 0 mutation in a patient with a late onset form of MLD (either juvenile or adult onset).

of age (Figure 1B). The increase of the GMFC-MLD score from level 1 to level 2 was recorded in 12/14 EJ patients and occurred after a median time of 0.75 years (range 0.09-3.92), and in 1/5 LJ patient after 1.02 year (median age at the last follow-up 22.8 years, range 17.6-36.6).

No significant difference was observed in the time from onset to loss of independent ambulation between LI and EJ subjects ($P = 0.055$, comparison performed only considering ambulant LI at onset; Table 2), while there was a significant difference in the time to loss of trunk control (estimated median time 1.12 vs 2.70 years from onset; $P = 0.001$) (Figure 1C). In late-infantile patients, we observed only descriptively a later age at loss of trunk control in those patients who had achieved independent walking (2.62 vs 2.12 years from birth; $P = 0.1791$).

In EJ patients, we observed a similar time to loss of ambulation in those who presented with gross-motor symptoms at onset versus those with isolated cognitive abnormalities (median time in years 0.57 vs 0.58; $P = 0.1279$). Loss of trunk control occurred earlier in the latter group (Table 2).

Time from disease onset to GMFC-MLD > 1 (Figure 1B) and to GMFC-MLD > 4 (Figure 1C) differed significantly between EJ and LJ subjects (P-value <0.001 and 0.003, respectively). All but one AD patients, who lost ambulation at 43.9 years and trunk control at 46.3 years, were still walking at last follow-up (44.2 years of age, range 43.5-44.5).

The GMFM scale was also prospectively tested in all LI and 16 J subjects (Figure S3). As shown by GMFM score two EJ siblings (J08 and J14, genotype R/R) displayed slightly less marked motor involvement compared to other patients in the same group.

3.2.3 | Bulbar function

In the LI group, in those patients who had achieved the milestone of independent walking versus those who did not, we observed a significant difference in the age at onset of dysphagia (median time 2.42 vs 1.92 years, $P = 0.048$). LI subjects overall experienced dysphagia earlier than EJ subjects (median time from onset 0.88 vs 1.58 years; $P = 0.011$) and within the EJ group no differences were observed (Table 2).

Time from disease onset to PEG was not significantly different between LI and EJ (median 3.75 vs 9.83 years; $P = 0.084$), which might be due to the small sample size. Only one LJ and none of the adult subjects displayed bulbar dysfunction during follow-up. None of LJ and AD subjects required a PEG.

3.2.4 | Seizures

Seizures were observed in all MLD variants. The estimated median time from onset to the first seizure was 2.17 years for LI subjects, and 1.75 years for EJ subjects ($P = 0.943$). Only one LJ and two AD experienced seizures during follow-up. Neither the achievement of independent walking in LI nor the quality of symptoms at onset (namely, only cognitive vs also motor) in EJ were associated with a different time at first seizure.

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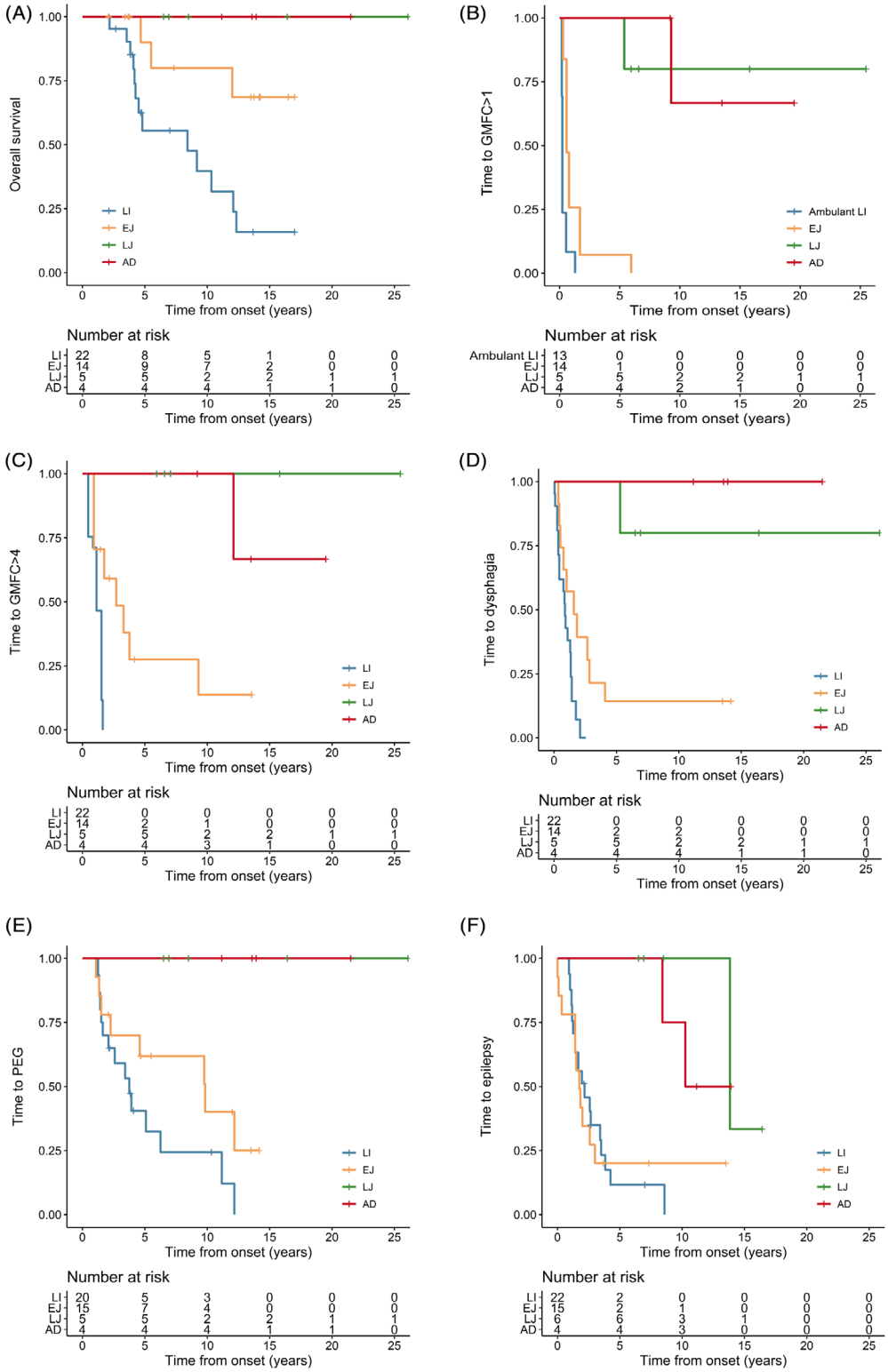


FIGURE 1 Time to event analysis for major disease-related events. A, Kaplan-Meier estimate of overall survival from disease onset. (B) Kaplan-Meier estimates for time interval from onset to entry into GMFC-MLD level >1 (meaning inability to walk independently), (C) from onset to entry into level >4 (meaning loss of trunk control), (D) from onset to dysphagia, (E) from onset to percutaneous gastrostomy insertion, (F) from onset to first documented seizures. Only LI patients who had achieved independent ambulation were considered in panel B. AD, adults; EJ, early-juvenile; GMFC, gross motor function classification; LI, late-infantile; LJ, late-juvenile; OS, overall survival; PEG, percutaneous gastrostomy

3.3 | Longitudinal instrumental assessment

3.3.1 | Nerve conduction studies

Longitudinal recordings of individual patients are displayed in Figure 2.

In all LI patients, nerve conduction studies (NCS) showed a severe sensory-motor demyelinating neuropathy with nerve conduction velocities (NCV) < 20 m/s after the second birthday (Figure 2B).

A greater variability was observed within the EJ group (Figure 2A,C). Out of 12 patients, 4 showed severe demyelinating neuropathy (NCVs<30 m/s), other 4 patients (J05,

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TABLE 2 Time-to-event analyses of major disease-related milestones

	Median time from onset in years (n) [*P*-value]					
Comparison	**OS**	**Time to GMFC>1**	**Time to GMFC>4**	**Time to dysphagia**	**Time to PEG**	**Time to epilepsy**
LI Ambulant vs LI non-Ambulant	9.17 (n = 13) vs 8.42 (n = 9) [$P = 0.9424$]		1.52 (n = 13) vs 1.12 (n = 9) [$P = 0.7855$]	0.88 (n = 13) vs 0.92 (n = 9) [$P = 0.7584$]	5.08 (n = 11) vs 3.75 (n = 9) [$P = 0.7882$]	1.96 (n = 13) vs 2.58 (n = 9) [$P = 0.5731$]
LI Ambulant vs LI non-Ambulant (*from birth*)	10.17 (n = 13) vs 9.42 (n = 9) [$P = 0.5768$]		2.62 (n = 13) vs 2.12 (n = 9) [p = 0.1791]	2.42 (n = 13) vs 1.92 (n = 9) **[$P = 0.0482$]**	4.08 (n = 11) vs 4.75 (n = 9) [$P = 0.6845$]	3.33 (n = 13) vs 3.58 (n = 9) [$P = 0.9598$]
LI vs EJ	8.42 (n = 22) vs n.r.[a] (n = 14) **[$P = 0.0134$]**	0.23* (n = 13) vs 0.58 (n = 14) [$P = 0.0552$]	1.12 (n = 22) vs 2.7 (n = 14) **[$P = 0.0013$]**	0.88 (n = 22) vs 1.58 (n = 14) **[$P = 0.0105$]**	3.75 (n = 20) vs 9.83 (n = 14) [$P = 0.0835$]	2.17 (n = 22) vs 1.75 (n = 14) [$P = 0.9430$]
EJ: Motor symptoms at onset vs only cognitive symptoms	n.r.[a] (n = 9) vs n.r.[b] (n = 5) [$P = 0.8020$]	0.57 (n = 9) vs 0.58 (n = 5) [p = 0.1279]	3.29 (n = 9) vs 0.91 (n = 5) **[$P = 0.0360$]**	1.83 (n = 9) vs 0.75 (n = 5) [$P = 0.0939$]	12.17 (n = 9) vs 1.50 (n = 5) [$P = 0.0509$]	2.00 (n = 9) vs 1.50 (n = 5) [$P = 0.1289$]
EJ vs LJ	n.r.[a] (n = 14) vs n.r.[c] (n = 5) [$P = 0.2482$]	0.58 (n = 14) vs n.r.[d] (n = 5) **[$P = 0.0003$]**	2.7 (n = 14) vs n.r.[e] (n = 5) **[$P = 0.0031$]**	1.58 (n = 14) vs n.r.[d] (n = 5) **[$P = 0.0036$]**	9.83 (n = 14) vs n.r.[e] (n = 5) **[$P = 0.0433$]**	1.75 (n = 14) vs 13.83 (n = 5) **[$P = 0.0071$]**

Note: Survival analysis (in bold when *P* value <0.05). When comparing Time to GMFC>1, only patients who achieved independent ambulation were considered (**only Ambulant LI).* All analyses are performed from disease onset, unless otherwise specified.

Abbreviations: AD, adults; EFS = event-free survival; EJ, early-juvenile; GMFC, gross motor function classification; LI, late-infantile; LJ, late-juvenile; n.r. = not reached; PEG, percutaneous gastrostomy, OS, overall survival.

[a]15y-OS = 68.6%.

[b]15y-OS = 66.7%.

[c]15y-OS = 100%.

[d]15y-EFS = 80%.

[e]15y-EFS = 100%.

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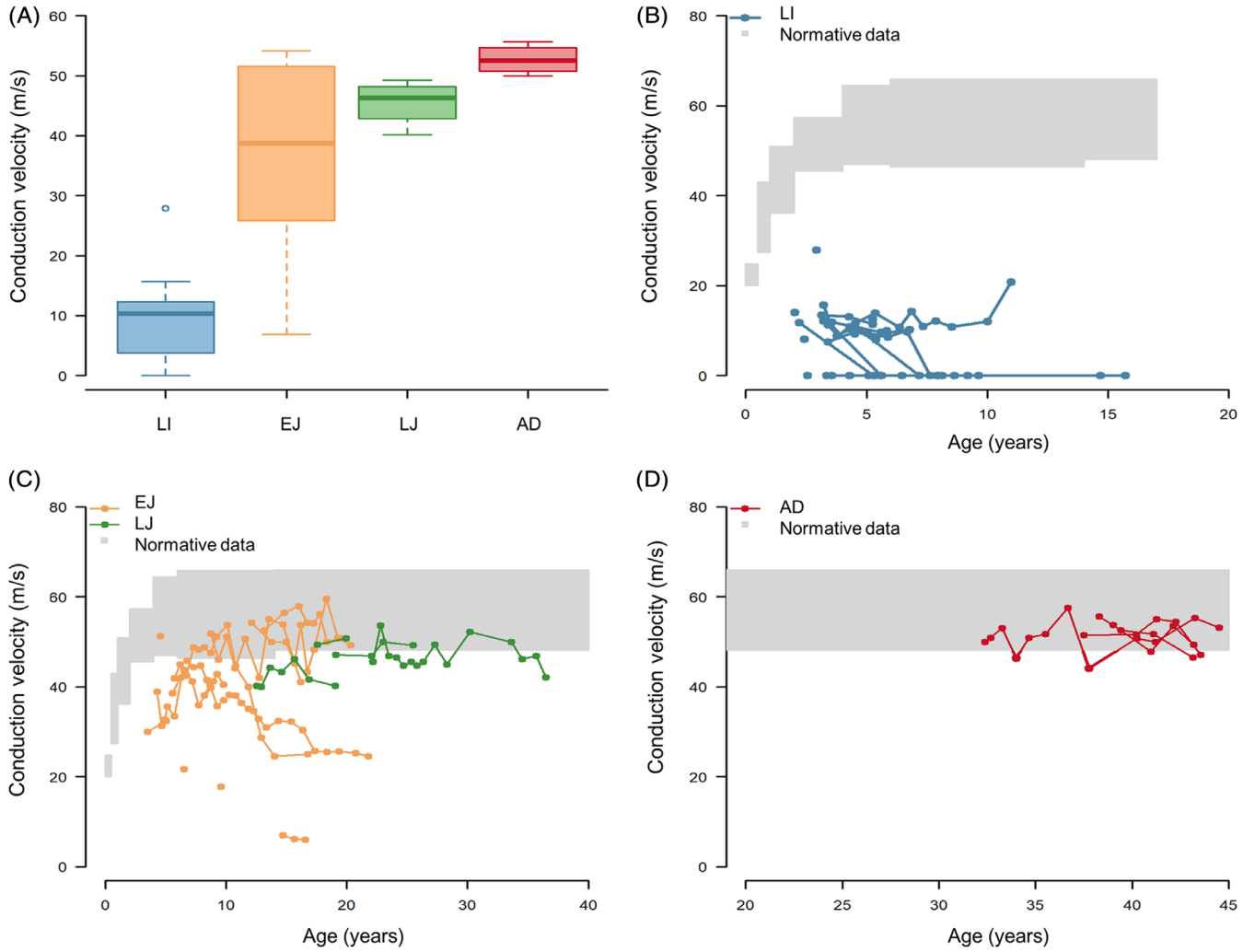


FIGURE 2 Nerve conduction studies. Right deep peroneal nerve conduction velocities (m/s) of MLD patients. (A) Boxplots displaying comparison of conduction velocities measured at first evaluation between the four different groups. Longitudinal trend in relation to age of (B) LI, (C) J, and (D) adult patients. Juvenile patients are further divided in patients with onset of symptoms before (EJ) and after (LJ) the seventh birthday (orange and green, respectively). AD, adults; CV, conduction velocity; EJ, early-juvenile; LI, late-infantile; LJ, late-juvenile. Normative values for age (mean ± 2.5 SD) are pictured in gray

J06, J04**, and J11**), who carried the c.256C>T; p.(Arg86Trp) variant in one of the alleles, showed mild reduction of NCVs, and additional 4 subjects (J01, J07, J08*, and J14*), who carried the c.608A>G; p.(Tyr203Cys) variant either in one or both alleles, showed no impairment of NCS at all tested time points. All LJ and AD patients in this cohort had mild or no PNS damage.

3.3.2 | Brainstem auditory evoked response

Kaplan-Meier curves and single observations of I-V interpeak latencies are displayed in Figure 3A-C. The I-V interpeak latency, which reflects integrity of central pathways, could not be plotted for LI patients due to the early disappearance of the V wave. Time to development of a pathological BAER from disease onset differed between groups (Figure 3A), especially between LI and EJ ($P = 0.019$). The probability of having a normal BAER 4 years after disease onset was 0% in LI pts, 36.36% in EJ, 100% in LJ, and 75% in AD pts (Figure 3A). In LI, the complete disappearance of both peripheral and central recordable components was observed in 37.5% of recordings within 5 years from onset. The probability of having a BAER score >2 at 10 years from onset was 100% in LI, 15.38% in EJ, and 0% in LJ and AD groups.

3.3.3 | Brain magnetic resonance imaging

The involvement of specific brain areas in the different subgroups at first examination and within the first

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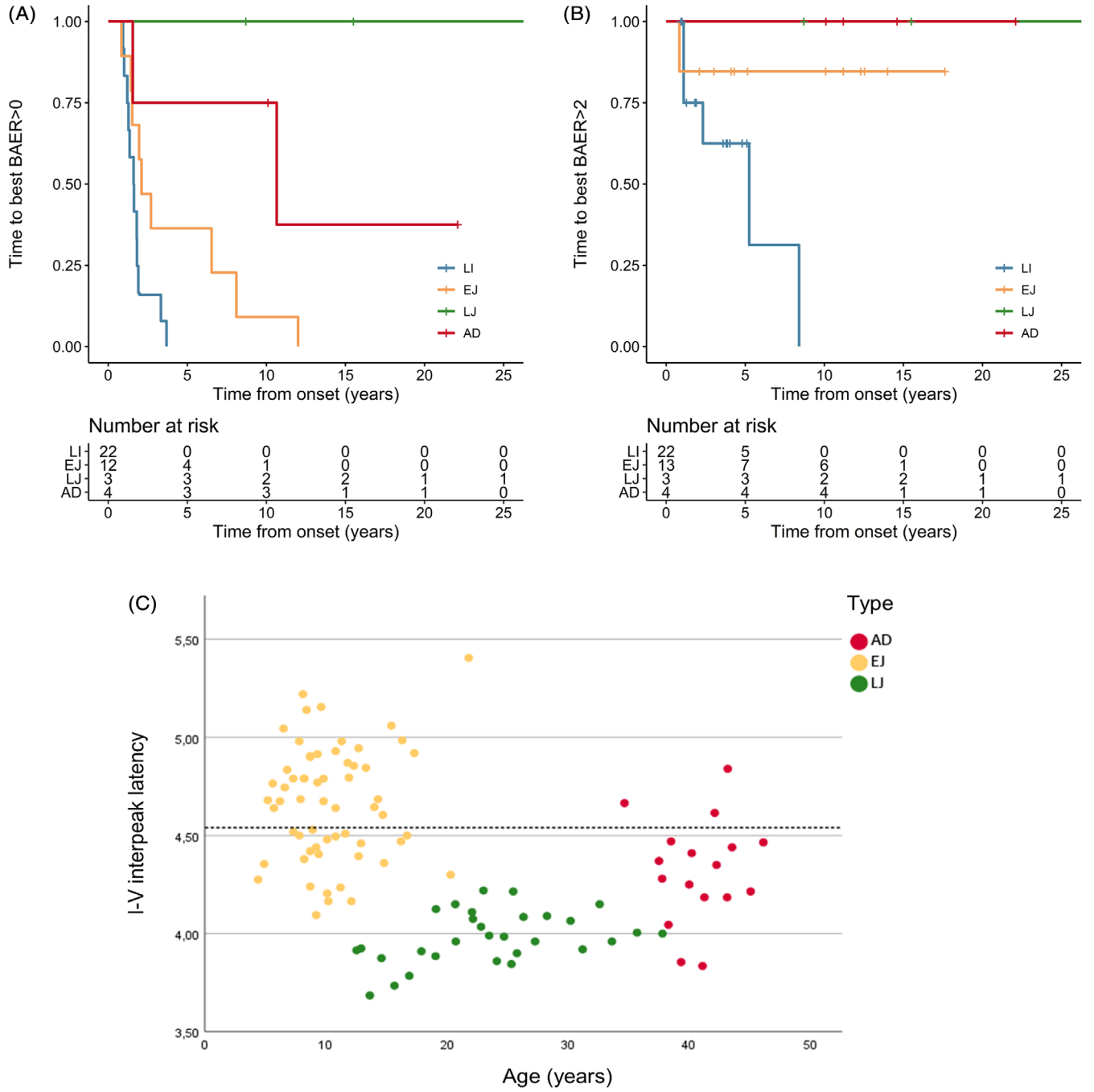


FIGURE 3 Brainstem auditory evoked response (BAER). (A, B) Kaplan-Meier estimates for time interval of BAER score entry (A) into level >0 (abnormal exam) and (B) into level 3 (complete absence of peripheral [Wave I] and central [Wave III and V] responses). The best measurement between the two ears was considered when scoring was discordant. (C) Scatter plot of wave I-V interpeak latencies when measurable in both ears (mean between right and left ear). Dotted line represents the upper limit of normal according to internal lab reference values (4.54 ms). AD, adults; BAER, Brainstem auditory evoked response; EJ, early-juvenile; LI, late-infantile; LJ, late-juvenile

12 months after disease onset is presented in Table S2. When considering MRIs performed within 12 months from onset, LI subjects showed a partial sparing of antero-temporal and frontal supratentorial white matter (WM) regions in 4/8 and 3/8 cases, respectively. Only one LI showed deep cerebellar white matter alterations and atrophy at this stage. In EJ subjects, all the MRI scans performed within 12 months from onset showed involvement of supratentorial WM areas, while only one out of 10 showed deep cerebellar white matter alterations and atrophy.

The total MRI score (according to both patient age and time from onset) and subscores of demyelination and

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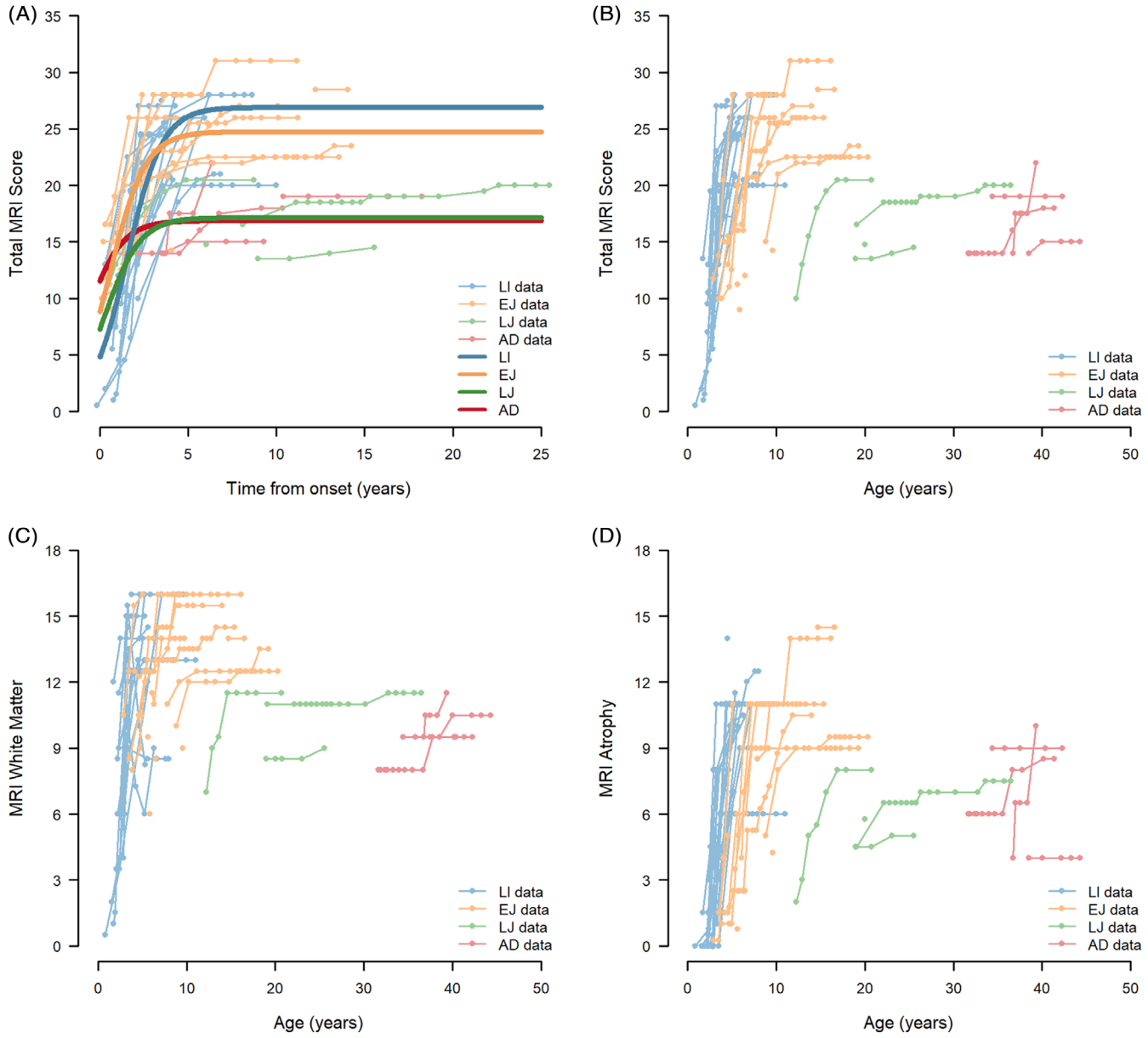


FIGURE 4 Brain MRI. (A) Total MRI severity score progression of patients with late-infantile, juvenile and adult MLD in relation to time from onset of symptoms. The thicker solid lines represent the curves estimated with the NLME model. Longitudinal trend in relation to age of (B) total MRI score and the sub scores (C) white matter involvement and (D) atrophy. AD, adults; EJ, early-juvenile; LI, late-infantile; LJ, late-juvenile

atrophy according to patient age were longitudinally evaluated (Figure 4). The mean plateau values (estimated by the NLME model) of the total MRI score (maximum 31.5) were 27, 24, 17 and 17 for LI, EJ, LJ, and AD patients, respectively (Figure 4A).

LJ and AD patients had significant lower plateau values compared to LI subjects ($P < 0.001$ for both groups); no significant differences were observed between LI and EJ ($P = 0.207$). Using the same statistical model, time from onset to plateau was significantly shorter in AD group ($P = 0.005$), LJ ($P = 0.002$), and EJ ($P < 0.001$), when compared to LI.

As outlined above, the cerebellum was mostly spared regardless MLD group at onset. In LI subjects, the probability of maintaining normal cerebellar WM was 61 and 33% at 2 and 3 years from onset, respectively. The probability of preserving a normal cerebellar volume at the same time points was 29 and 5%, respectively.

The probability of showing normal cerebellar WM in EJ was 45 and 18% at 2 and 3 years from onset, respectively. The probability of preserving a normal cerebellar volume at the same time points was 47 and 11%, respectively. None of the LJ and AD patients had changes in cerebellar white matter or volume at both time points,

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and only a mild cerebellar atrophy was later observed in these subgroups (Figure S6B-D). In fact, for LJ, the median time to cerebellar atrophy was 4.3 years from onset, while the median time to severe cerebellar atrophy was not reached during the follow-up. For AD, the same events were reached at a median time from onset of 5.5 and 9.85 years, respectively.

4 | DISCUSSION

Despite a common pathogenic mechanism, MLD is characterized by a wide spectrum of clinical severity.[13,30,31] Promising experimental treatments, whose efficacy can be difficult to interpret in such a complex disorder, have reached clinical testing in the past few years and Hematopoietic stem cell transplantation (HSC-T) gene therapy[16] has been recently approved for marketing by the European Medicines Agency.

Valuable prognostic factors need to be identified, as there is an unmet need to manage expectations of families and caregivers, and there is also a demand for the best outcome variables to be identified in order to assess treatment-related effects in the setting of present and future therapeutic trials. These are likely to vary across different MLD subtypes.

As previously described,[11,13,30] LI subjects in our cohort rapidly experienced psychomotor deterioration, bulbar dysfunction and seizures, with loss of trunk control and dysphagia occurring concomitantly, and resulting in a substantially reduced survival (Figure S2). Interestingly, the insertion of percutaneous gastrostomy did not prolong survival in this cohort, which is valuable information for counseling. While historically considered a rather homogeneous group of patients, we found that the attainment of independent walking before disease onset was associated with an older age at bulbar dysfunction in our LI population. Similarly, a later loss of trunk control was also observed, but only descriptively. Despite the recorded difference (~6 months) might seem modest, in reality could be very relevant for predicting the outcome of therapies that require months to exert an effect (eg, hematopoietic cell transplantation or HSC-T gene therapy).

A key question is whether there could be other subtle clinical or instrumental signs already detectable in the first 15 months of life that can predict prognosis. Unfortunately, a limitation of this study is that we lacked sufficient data regarding nerve conduction studies, brainstem evoked responses and brain MRI scans to establish the prognostic value at disease onset of each of these assessments.

Despite a number of recent interventional studies considering juvenile patients as a single group,[17,21,32] we have confirmed that major differences between early-juvenile and late-juvenile subgroups exist. Interestingly, Kehrer and colleagues recently showed that not only the age at onset (and therefore, MLD subtype) is important, but also the quality of symptoms should be considered, such as the presence of motor symptoms at onset being a negative prognostic factor.[14] In our cohort, LI and EJ show a similar rapid disease progression, particularly for time to loss of ambulation and onset of seizures. In contrast, EJ lost trunk control and displayed feeding difficulties later then LI.[13] Also, MRI score at plateau was comparable between LI and EJ. All five LJ subjects included in this study presented instead with isolated cognitive impairment not followed by gait abnormalities in the first year, and displayed both radiologic and neurophysiologic features more similar to AD rather than EJ/LI. Notably, EJ exhibiting isolated cognitive regression as the first symptom progressed similarly compared to those who had gross-motor involvement, with loss of ambulation occurring at a median of 6 months from disease onset. Surprisingly, these patients had an earlier loss of trunk control than those presenting with motor symptoms, possibly due to a different role played by peripheral versus central involvement.

Considering the above, we reckon that the definition of isolated cognitive phenotype should require a follow-up of at least 1 year, and a more detailed characterization. In keeping with the clinical findings of Kehrer et al., we hypothesize that attributes including a preserved or minimally damaged peripheral nervous system, a less pronounced white matter involvement on MRI, and a lesser degree of cerebellar involvement in the first stages could be the features defining late-onset patients with a "cognitive profile." However, we acknowledge that our LJ cohort has a very small sample size and might not be representative of the overall late-onset MLD population. In addition, rapid deterioration and severe MRI involvement has been reported in late-onset MLD patients.[6] Further studies on larger cohorts are needed to confirm our findings.

One of the aims of this study was to identify instrumental tools that could serve as reliable biomarkers to establish the efficacy of new treatments. The long-term prospective follow up of our cohort of patients outlined important differences across MLD subtypes that need to be considered. While 100% of LI subjects had a severe demyelinating neuropathy with CVs rapidly dropping <20 m/s, we confirm that both the presence and the severity of PNS involvement is extremely variable in older patients.[33-36] Therefore, while the preservation/improvement of NCVs in LI subjects is a strong endpoint for experimental treatments, it is not obvious to establish the effects on PNS by group analysis in EJ, LJ, and AD patients.

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In these groups, we found that patients carrying the at least one of copy of the R variants c.256C>T (both J and AD) and c.608A>G (J) showed mild or no PNS involvement over time. In keeping with descriptions by Rauschka and colleagues, who demonstrated a more severe reduction of CVs in p.Pro428Leu homozygotes than in p.Ile181Ser heterozygotes, we think that further studies to explore genotype-phenotype correlation, particularly regarding R alleles, are warranted.[15]

Notably, we have provided first evidence that BAERs (a cheap and non-invasive technique) could be employed as exploratory outcome measures for therapeutic trials in MLD.[37] Indeed, BAER longitudinal monitoring in LI demonstrated early disappearance of central waves, at odds with expected progressive shortening of the I-V interpeak latency that mirrors brainstem myelination in healthy children in the first years of life. In contrast, the use of BAER might be less straightforward in EJ and LJ/AD patients, in whom close to normal recordings or stable I-V interpeak latencies can persist despite psychomotor deterioration in some cases. As previously demonstrated, Brain MRI is a powerful tool to investigate CNS involvement in MLD. We have historically applied a qualitative score developed to increase sensitivity and detect atrophic changes.[16] We confirm that periventricular WM signal alteration is the earliest pathological hallmark of the disease, associated with an initial sparing of the anterior periventricular WM in some LI patients.[31,38]

Although some differences were noted within the first 12 months from disease onset (Table S2), we lacked statistical power to perform inter and intra group comparisons and to evaluate associations with clinical parameters.

As anticipated above, the NLME model showed no significant differences between LI and EJ when considering the plateau of Total MRI score. On the contrary, LJ and AD patients had significant lower plateau values compared to younger patients.

When exploring the different involvement of specific brain areas over time, we confirmed previous findings suggesting an initial sparing of the cerebellum,[38] notwithstanding the early onset of truncal ataxia in many children with MLD. Contrarily to what has previously been described[39] and differently from what happens in LI and EJ, cerebellum remained relatively spared in the first years from disease onset in LJ and AD, with few subjects progressing to severe atrophy and only after more than 5 years from onset.

Such discrepancies could be related either to the peculiar phenotype of our late onset cohort or to the timing of MRI scans. To our knowledge, this is the first report that highlights the differences in cerebellar involvement across clinical variants when assessed in a longitudinal manner. The use of advanced MRI techniques and semi-automated software is being implemented for a more accurate evaluation of demyelination load and brain volume loss in the future.[40]

In conclusion, this study represents a unique and valuable source of quantitative, long-term, longitudinal data from a large cohort of untreated MLD patients. The data suggest that ENG, BAER and MRI all represent valuable parameters ancillary to functional/neurodevelopmental scales for monitoring disease progression, but their application and interpretation should be tailored according to the MLD subtypes. Parameters that could serve as prognostic factors at baseline for patients amenable to experimental treatments (eg, attainment of independent walking in LI) need to be confirmed in larger cohorts of patients.

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CONFLICT OF INTEREST

FF, AA, VC, and MGNS are investigators of gene therapy clinical trials for MLD sponsored by Orchard Therapeutics, the license holder of investigational medicinal product OTL-200. FF and VC act as consultant for ad hoc Advisory board of Orchard Therapeutics. AB is a member of the scientific advisory board and scientific consultant of Orchard Therapeutics. MC is currently an employee of AGC Biologics S.p.A, the company which holds a cGMP MSA with Orchard Therapeutics. PR and CDS have a

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contract with Orchard Therapeutics to perform statistical analyses of gene therapy clinical trials for MLD. All other authors have no financial interest related to the work described in the manuscript.

AUTHOR CONTRIBUTIONS

FF, AA, AB, and MS designed the study, collected and interpreted data, and wrote the manuscript. AZ, CB, SC, and RP collected and interpreted data and wrote the manuscript. PR performed statistical analyses, interpreted the data, and wrote the manuscript. FC and CDS contributed to statistical analyses. IS, SM, FC, VC, and UDC collected and interpreted data. MF, MSa, LL, MC, GA, and SR collected data. MGNS helped with interpretation of data.

ETHICS STATEMENT

Patients were enrolled in the Metachromatic Leukodystrophy observational clinical trial LDM/1, a retrospective and prospective natural history study approved by the Institutional Review Board (IRB) of San Raffaele Hospital July 15th 2004, and performed in accordance with the ethical standards of the above mentioned institutional responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from both parents of all the patients included in the study and it is available upon request. This article does not contain any studies with animal subjects performed by any of the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors, upon request.

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SUPPORTING INFORMATION

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