Neurol Ther (2026) 15:1269–1292
https://doi.org/10.1007/s40120-026-00916-0

ORIGINAL RESEARCH

Natural History of Adult-Onset Leukoencephalopathy with Axonal Spheroids and Pigmented Glia (ALSP): A Retrospective Patient Cohort Study

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Received: November 13, 2025 / Accepted: March 3, 2026 / Published online: March 31, 2026

ABSTRACT

Introduction: Colony-stimulating factor 1 receptor-related adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (CSF1R-ALSP) is a rare, progressive, fatal neurodegenerative disorder caused by pathogenic *CSF1R* gene variants, resulting in microglial dysfunction and neurodegeneration. In patients with CSF1R-ALSP, brain magnetic resonance imaging (MRI) shows early white matter lesions followed by brain atrophy accompanied by progressive cognitive, neuropsychiatric, and motor dysfunction. However, the natural history (NH) of this disease is not yet fully understood.

Prior Publication: This study extends partial data that have been previously published in: Schmitz AS, Raju J, Köhler W, Klebe S, Cheheb K, Reschke F, Biskup S, Haack TB, Roeben B, Kellner M et al.: Novel variants in CSF1R associated with adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP). J Neurol 2024, 271(9):6025–6037; Karle KN, Biskup S, Schule R, Schweitzer KJ, Kruger R, Bauer P, Bender B, Nagele T, Schols L: De novo mutations in hereditary diffuse leukoencephalopathy with axonal spheroids (HDLS). Neurology 2013, 81(23):2039–2044; Bender B, Klose U, Lindig T, Biskup S, Nagele T, Schols L, Karle KN: Imaging features in conventional MRI, spectroscopy and diffusion weighted images of hereditary diffuse leukoencephalopathy with axonal spheroids (HDLS). J Neurol 2014, 261(12):2351–2359; Yska HAF, Golse M, Beerepoot S, Hayer S, Bergner C, de Paiva ARB, Marelli C, Palacios NJ, Osorio YL, Huiban C et al.: Hematopoietic stem cell transplantation in an international cohort of colony stimulating factor-1 receptor (CSF1R)-related disorder. Mov Disord 2025.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40120-026-00916-0.

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Methods: This study examined clinical features, brain MRI findings, and fluid biomarkers in a cohort of 16 symptomatic adults from Germany with genetically confirmed CSF1R-ALSP using a retrospective chart review to evaluate changes in symptoms and imaging during disease progression.
Results: In this cohort, median age of symptom onset was 45 years and disease duration varied widely (2–15 years). The most common presenting clinical symptoms were cognitive impairment (81% of patients) and aphasia (63% of patients), which progressed rapidly over 24 months of observation. Scores for the Montreal Cognitive Assessment and the Barthel Index of independence in performing activities of daily living declined sharply during the observational period. MRI data showed white matter degeneration and brain atrophy accompanied by increased ventricular volume with significant annual progression. Significant correlations were observed between key volumetric MRI measures and clinical outcome assessments of cognition and functional independence.
Conclusion: This retrospective study provides qualitative and quantitative data from the clinical setting for further characterization of the NH of CSF1R-ALSP, a rare neurological disorder with significant unmet medical need. Data will inform the design of and endpoint selection for future NH studies and interventional clinical trials, which will be central to the development of safe and efficacious therapies for CSF1R-ALSP. Further, it will guide clinicians less familiar with the disease in providing patients and caregivers with appropriate support and information about CSF1R-ALSP.

Keywords: Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia; Aphasia; Brain atrophy; Cognitive impairment; CSF1R-related leukoencephalopathy; Dementia; Hereditary diffuse leukoencephalopathy with spheroids; Leukodystrophy; Magnetic resonance imaging; Primary microgliopathy

Key Summary Points

Why carry out this study?

Although the etiology of colony-stimulating factor 1 receptor-related adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (CSF1R-ALSP) has been known since 2011, the natural history of CSF1R-ALSP is not yet fully understood.

This study examined the natural disease course of CSF1R-ALSP based on clinical observations, MRI findings, and characteristics of disease progression in a cohort of 16 symptomatic adults with genetically confirmed CSF1R-ALSP.

What was learned from the study?

In patients with CSF1R-ALSP, presenting symptoms included cognitive impairment, aphasia, and gait disorder, with significant declines in cognitive functioning and functional independence accompanied by significant increases in brain atrophy during the course of the disease.

These results extend the current body of knowledge on the natural history of CSF1R-ALSP and support current thinking on potential clinical, radiological, and fluid biomarkers of the disease that could be used as endpoints in future clinical trials and provide information to clinicians and caregivers.

INTRODUCTION

Colony-stimulating factor 1 receptor-related adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (CSF1R-ALSP) is a rare, progressive, fatal neurodegenerative disorder caused by autosomal dominant inheritance of pathogenic *CSF1R* gene variants, resulting in microglial dysfunction and neurodegeneration [1–5]. Because the genetic basis was only

described in 2011 [2, 4], the natural history remains incompletely characterized. We retrospectively analyzed clinical and MRI features in 16 symptomatic adults with genetically confirmed CSF1R-ALSP.

Most pathogenic variants are missense mutations in the tyrosine kinase domain, although other variant types occur [1, 3, 4]. CSF1R-ALSP is most often inherited in an autosomal dominant manner, although the family history is not always remarkable, which may be explained by de novo gene variants, incomplete penetrance, and/or genetic mosaicism [4, 6, 7]. Although recent population-level research into the frequency of pathogenic and likely pathogenic *CSF1R* variants estimated a rate of 281 per million individuals, fewer than 500 cases have been reported in the literature to date [5, 8]. This discrepancy suggests a high potential for delayed diagnosis and misdiagnosis of CSF1R-ALSP, likely owing to the rarity of the disease as well as a differential diagnosis characterized by a heterogeneous clinical presentation that overlaps with other neurodegenerative and white matter disorders [1, 9–11].

The average age of symptom onset in CSF1R-ALSP is approximately 43 years (range 18–86 years) [9], after which the disease progresses irreversibly, with incapacitation occurring typically within 3–4 years and death within 6–8 years after symptom onset [1, 12]. Characteristic clinical features of CSF1R-ALSP include progressive cognitive decline; neuropsychiatric symptoms such as behavioral or personality changes; motor symptoms such as gait disturbance, parkinsonism, pyramidal signs (e.g., spasticity), bulbar signs (e.g., dysphagia), and ataxia; as well as other neurologic symptoms such as apraxia and epilepsy [1, 13].

Brain MRI of patients with CSF1R-ALSP typically demonstrates white matter abnormalities, including diffusion-restricted lesions, brain atrophy with enlargement of the lateral ventricles, and thinning of the corpus callosum [1, 13]. In patients with white matter abnormalities and atrophy, disease progression can be sensitively detected by volumetric MRI measures (e.g., lesion volume, ventricle volume) and the Sundal MRI severity score [14]. Thus, the objective of this study was to examine clinical features and MRI findings in a cohort of 16 symptomatic adults with genetically confirmed CSF1R-ALSP, as well as to report changes in symptoms and imaging during disease progression.

METHODS

Cohort

Patients were recruited during routine clinical care at the neurological department of the University Hospital Tübingen, Germany. Patients with confirmed pathogenic *CSF1R* variants, signs of leukoencephalopathy on MRI, and clinical symptoms of CSF1R-ALSP were eligible for inclusion. As far as is known, the 16 patients included in this study were not related. The study was conducted in compliance with the principles of the Declaration of Helsinki and adhered to all applicable laws and regulations. Prior to initiation, the study was approved by the Institutional Review Board of the University of Tübingen (approval number 502/2024BO2), which ruled that, because the study was noninterventional and retrospective and all data were deidentified prior to inclusion and analysis, the study posed no risk or burden to patients and did not require participants to provide signed informed consent according to §13(1) 1 Landesdatenschutzgesetz Baden-Württemberg of German law.

Data were retrospectively collected for all patients from clinical charts, including age, sex, age at symptom onset, and *CSF1R* variant data. Clinical manifestations were compiled for all patients based on detailed neurological examinations performed by a neurologist, including ability to perform activities of daily living and cognitive function.

Genetic Analysis

Enrolled patients had undergone whole-exome sequencing or panel analysis at their local institution to identify mutations associated with leukodystrophies. All genetic analyses were conducted according to previously reported standard methods [4, 6]. Identified variants were classified following the recommendations

of the American College of Medical Genetics and Genomics.

Clinical Measures of Disease Severity

Cognitive abilities and dementia severity were determined during clinical care visits on the basis of documented Montreal Cognitive Assessment (MoCA) data. The MoCA is a brief 30-question test that evaluates orientation, short-term memory, delayed recall, abstraction, visuospatial abilities, executive functioning, language, and attention. The test, which is available in German, takes approximately 10 min to complete. Scores range from 0 to 30, and scores of ≥ 26 are generally considered normal [15, 16]. The test includes an adjustment for education level. The German version of the test was applied as all participants were fluent in German.

Ability to perform activities of daily living was based on Barthel Index [17] data obtained from anamnestic information from caretakers (family members or nursing staff for patients living in nursing homes) by two neurologists (S.N.H., L.S.) and documented in patient charts. The Barthel Index captures 10 functional domains: mobility, stairs, transfers, dressing, feeding, grooming, bathing, toilet use, bowel control, and bladder control. Domain scores are combined for an index scale of 0–100, with higher scores indicating greater functional independence.

Additional neurological symptoms were retrospectively rated on a 0–4 scale based on chart review (Supplementary Material, Table S1).

The clinical outcome measures used in this retrospective cohort were not disease-specific for CSF1R-ALSP but are widely applied, validated instruments in neurodegenerative care. In the absence of established ALSP-specific scales, they enable longitudinal comparison and cross-study benchmarking, while offering limited sensitivity to more specific symptoms such as executive dysfunction, processing-speed deficits, and subtle early decline. Accordingly, they should be interpreted as pragmatic severity indicators rather than validated surrogate endpoints for disease progression.

As a result of the retrospective nature of the study, available clinical measures of severity and symptom progression data varied, and visit frequency and time intervals between visits were not standardized. Measurement scores and symptom severity ratings were considered unchanged until the first visit at which a new score or rating was documented regardless of the time interval between visits.

Fluid Biomarkers

Fluid biomarkers (neurofilament light chain [NfL] and chitotriosidase) concentrations were also obtained from patient clinical charts. NfL concentrations had been measured in serum and cerebrospinal fluid (CSF) using a single-molecule array assay (Simoa) according to methods previously described [18]. Chitotriosidase activity had been analyzed according to previously reported methods [19], with subsequently published modifications [20]. Age-dependent reference values for NfL in serum and CSF at our laboratory are provided in Supplementary Material, Table S2. For chitotriosidase, no standardized reference value is available as a result of the limited routine application.

Imaging Data

All available brain MRI data with T1-weighted, T2-weighted, T2-fluid-attenuated inversion recovery (FLAIR)-weighted, T2*-weighted, or diffusion-weighted contrast as well as available head computed tomography (CT) were evaluated. Because patients were transferred to our center from various outpatient centers, imaging parameters, resolution, and magnetic field strength varied. Imaging data were visually inspected by two neuroradiologists (B.B. and E.B.) with 16 and 5 years of clinical experience, respectively. Datasets with severe motion artifacts and obvious mis-segmentations were removed. All available structural MRI data were segmented with WMH_synthSeg (FreeSurfer 8.0.0) [21]. Segmentations that did not cover the whole anatomical region were excluded for that specific region. To account for bias from slice thickness (depending on the acquisition plane

for 2D sequences), different sequences, and field strengths, median values over all anatomical scans at one time point were calculated. Available high-resolution T1 images and CT images were analyzed for periventricular cobblestone calcifications.

Additionally, brain images were analyzed semiquantitatively using an ALSP severity score as previously described [22]. Briefly, scores for white matter degeneration (0–24 points) were determined on the basis of white matter signal intensity presence, distribution, and pattern, focusing on the frontal lobe, parietal lobe, temporal lobe, occipital lobe, pontine base, and cerebellum (0 = none, 1 = patchy, 2 = large patchy, 3 = confluent, 4 = diffuse; if score differed between both hemispheres, the mean value was used). Scores for brain atrophy (0–16 points) reflected lateral ventricle dilatation and cerebral, corpus callosum, and brainstem/cerebellum atrophy (0 = none, 1 = slight, 2 = mild, 3 = moderate, 4 = severe). White matter degeneration and atrophy scores were then summed, for a total ALSP severity score ranging from 0 to 40, with higher scores indicating greater disease severity.

Statistical Analysis

Continuous variables were described using means and standard deviations (SDs) or medians and ranges where appropriate. Categorical variables were summarized using frequencies and percentages. Data imputation methods were not used. Linear mixed models with random slopes for time, clustered by participant, and exchangeable covariance matrices were used to evaluate changes over time in MoCA and Barthel Index scores.

As a sensitivity analysis Tobit mixed-effects models were fitted using time since symptom onset as the timescale to account for potential floor and ceiling effects (MoCA, 0–30; Barthel Index, 0–100). To reflect uncertainty in reported symptom-onset timing, analyses were repeated after applying patient-level onset shifts sampled uniformly within ± 12 months, and predicted mean trajectories were summarized using the 2.5th and 97.5th percentiles across repetitions. Given the small cohort size, leave-one-out influence analyses were performed for the Tobit mixed-effects sensitivity model to assess whether predicted trajectories were driven by any single participant.

For each brain region assessed via MRI, changes from the first available scan per year were summarized by scan among patients with available data for the region at each scan. Changes over time in the volume of each MRI brain region were analyzed using linear mixed models with random slopes for time, clustered by participant, and exchangeable covariance matrices. For data in which MRI, clinical, and/or biomarker observations were available within 90 days of each other, Pearson correlation analyses were conducted between volumetric MRI brain region (white matter hyperintensities, total gray matter, lateral ventricles, lateral plus third ventricles, and brain ventricles) measures, clinical outcome assessments (MoCA and Barthel Index scores), and fluid biomarkers (NfL levels and chitotriosidase activity in serum and CSF). Associations between changes from the first available scan (overall and per year) in MRI brain region volume and clinical outcome assessments, as well as the relationships between clinical measures and fluid biomarkers, were also examined using Pearson correlations.

RESULTS

Patients

Data were summarized for 16 symptomatic patients with genetically confirmed CSF1R-ALSP and data available between 2011 and 2022. This study extends partial data that have been previously published for 11 of the 16 patients (Table 1) [4, 6, 23, 24]. Median age of symptom onset was 45 years (mean ± SD [range], 43.8 ± 7.5 [28–53] years). Disease duration, which was calculated conservatively with whole years (i.e., assuming January 1st for month and day of disease onset and date of death), varied widely from 2 to 15 years, as did age at death among the 6 deceased patients (34 to 61 years among patients with known age of death). The cohort

Table 1 Patient characteristics

Patient	Sex	Age (years)		Received HSCT (age [years])	Deceased (age at death [years])	*CSF1R* variant		Clinical and fluid biomarker assessments						
		At onset	At last visit			Nucleotide[e]	Protein	Age at assessment (years)	MoCA score	Barthel Index score	NfL (pg/mL)		Chito (nmol/h/mL)	
											Serum	CSF	Serum	CSF
Patient 1[a]	F	45	52	No	Unknown	c.2384G > T	p.Gly795Val	48	23	70	–	–	–	–
								49	12	35	251.2	19,080.9	46	76
								52	0	0	–	–	–	–
Patient 2	M	47	59	No	Unknown	c.2606T > G	p.Val869Gly	52	0	0	287.8	43,326.9	13	35
								53	0	0	–	–	–	–
								56	0	0	–	–	–	–
Patient 3[b,d]	M	28	43	No	No	c.2629C > T	p.Gln877*	34	0	0	146.0	4406.7	28	9
								35	0	0	–	–	–	–
								38	0	0	–	–	–	–
								43	0	0	193	–	–	–
Patient 4[b,d]	F	40	47	No	Yes (49)	c.2528T > A	p.Ile843Asn	42	13	100	–	–	–	–
								42 + 4 m	11	100	–	–	–	–
								43	0	85	–	–	–	–
								44	0	15	–	–	–	–
								47	0	0	299.22	5857.18	85	31
Patient 5[b,d]	M	50	53	No	Yes (unknown)	c.2342C > T	p.Ala781Val	51	21	100	–	–	–	–
								52	–	100	–	27,534.8	–	–
								52 + 10 m	–	90	–	–	–	–
								53	–	80	–	–	–	–
Patient 6[a]	F	37	43	No	Unknown	c.2133_2919del[f]	NA	40	21	100	–	–	–	–
								40 + 3 m	16	100	–	–	–	–
								41	8	55	252.2	14,785.9	40	52
								42	8	55	–	–	–	–
								43	3	60	–	–	–	–
Patient 7	F	32	34	No	Yes (34)	c.2345G > T	p.Arg782Leu	33	19	100	–	–	–	–
								34	0	0	890.1	92,070.9	41	185

Table 1 continued

Patient	Sex	Age (years)		Received HSCT (age [years])	Deceased (age at death [years])	*CSF1R* variant		Clinical and fluid biomarker assessments						
		At onset	At last visit			Nucleotide[e]	Protein	Age at assessment (years)	MoCA score	Barthel Index score	NfL (pg/mL)		Chito (nmol/h/mL)	
											Serum	CSF	Serum	CSF
Patient 8	M	51	59	No	Yes (61)	c.2541G > C	p.Glu847Asp	58	13	80	–	–	–	–
								59	8	70	123.6	13,397.4	114	166
								59 + 6 m	3	45	–	–	–	–
								60	0	15	–	–	–	–
Patient 9[a]	M	46	48	No	Unknown	c.1837G > A	p.Val613Met	48	1	75	75.7	8486.2	115	160
Patient 10[a]	M	40	43	No	Unknown	c.2304C > A	p.Phe768Leu	42	27	–	–	–	–	–
								43	17	25	68.3	22,672	42	18
Patient 11[a,c]	M	44	49	Yes (46)	Unknown	c.2642C > T	p.Ala881Val	46	15	90	61.6	12,409.4	28	41
								47	13	80	–	–	–	–
								47 + 7 m	12	–	–	–	–	–
								49	12	90	–	–	–	–
Patient 12[b,d]	M	53	56	No	Yes (59)	c.2512G > C	p.Val838Leu	54	21	–	–	–	–	–
Patient 13[d]	F	38	41	No	Unknown	c.2330G > A	p.Arg777Gln	–	–	–	–	–	–	–
Patient 14[c]	F	46	50	Yes (47)	No	c.2381T > C	p.Ile794Thr	47	23	100	–	–	–	–
								47 + 5 m	17	95				
								47 + 11 m	10	80				
Patient 15	M	52	55	No	Yes (57)	c.2342C > T	p.Ala781Val	55	–	35	–	> 10,000	–	–
Patient 16[a]	F	52	55	No	Unknown	c.1837G > A	p.Val613Met	56	23	–	–	9756	–	–

Chito chitotriosidase, *CSF* cerebrospinal fluid, *CSF1R* colony stimulating factor 1 receptor, *F* female, *HSCT* hematopoietic stem cell transplantation, *m* month, *M* male, *MoCA* Montreal Cognitive Assessment, *NA* not applicable, *NfL* neurofilament light chain

[a]Patient case previously described in Schmitz et al. 2024 [4]

[b]Patient case previously described in Karle et al. 2013 [6]

[c]Patient case previously described in Yska et al. 2025 [24]

[d]Patient case previously described in Bender et al. 2014 [23]

[e]NCBI reference sequence NM_001288705.3

[f]Deletion of exons 15–21

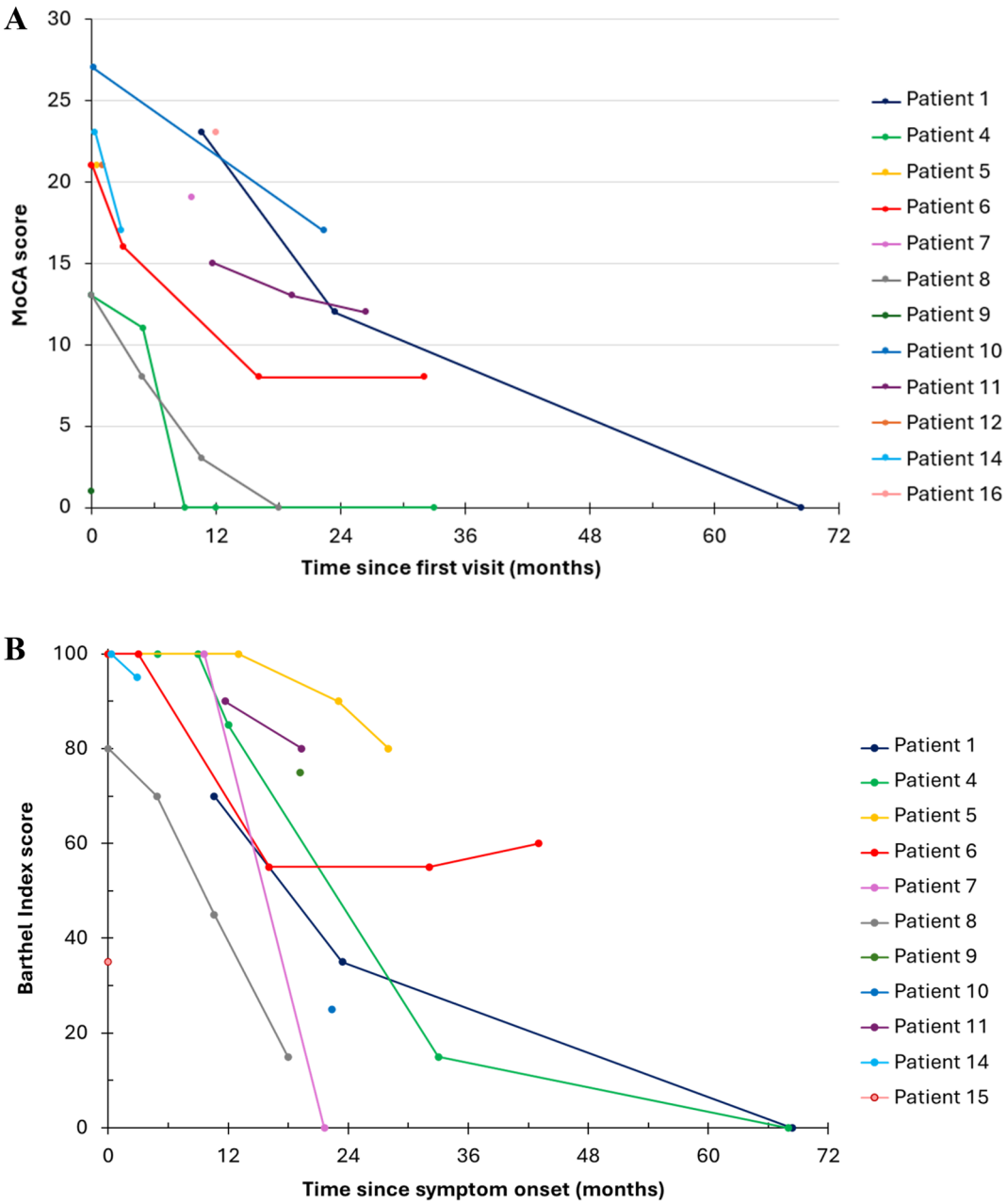


Fig. 1 Longitudinal spaghetti graphs of individual patients' clinical outcome assessments. **a** MoCA scores. **b** Barthel Index scores. *MoCA* Montreal Cognitive Assessment

was approximately evenly split between the sexes (43.8% female; 56.3% male). An overview of disease duration prior to initial assessment and follow-up duration is given in Supplementary Material, Table S3.

Genetic testing revealed heterozygous pathogenic *CSF1R* variants in each of the 16 patients, most of which involve the protein kinase domain of the CSF1R protein and have been previously reported in the literature (Table 1) [2, 4, 6, 25–29]. All variants were missense mutations except for one deletion of exons 15–21 (patient 6) and one nonsense mutation (patient 3).

Variant types for individual patients, including those with severe baseline impairment (e.g., patients 2 and 3) and atypical trajectories (e.g., patient 6, Fig. 1), are detailed in Table 1; given the limited sample size and lack of functional validation, these data are presented descriptively without formal genotype–phenotype analysis.

Neurological Symptoms and Clinical Assessments

MoCA scores of cognitive function were available for 12/16 patients (75.0%) and ranged from 1 (severe cognitive impairment) to 27 (normal cognitive function), with a median score of 21 (Table 1). MoCA scores declined markedly within the first year after initial assessment (Fig. 1a). Data for 24 months and beyond were available for only a limited number of patients ($n=5$) and should therefore be interpreted as exploratory. In this subgroup, cognitive declines continued through 24 months and thereafter. Overall, linear mixed model analysis indicated a significant annual decrease in MoCA scores by −28.5% ($P<0.001$) (Table 2).

In a sensitivity analysis using Tobit mixed-effects models anchored to symptom onset to account for potential floor effects of the MoCA scale, a comparable pattern of decline was observed. Additional simulations incorporating ± 12 months of onset-time uncertainty yielded similar trajectory shapes, with differences primarily reflecting temporal alignment rather than qualitative changes in progression (Supplementary Material, Fig. S1A). Leave-one-out influence analyses of the Tobit model demonstrated limited variation in predicted trajectories across repeated refits, suggesting that the estimated decline was not disproportionately driven by any single participant (Supplementary Material, Fig. S1B).

Barthel Index scores were available for 11/16 patients (68.8%); of these, 10 patients had scores that ranged from 70 to 100, indicating minimal to moderate dependence in performing activities of daily living, and one patient had a score of 35, indicating severe dependence (Table 1). Barthel Index scores indicated that functional independence declined in most patients at varying rates within the first year following initial assessment (Fig. 1b). As in regards to MoCA score, Barthel Index follow-up data extending beyond 12 months were available for only a small subset of patients ($n=4$) and should therefore be considered exploratory. In this subgroup, the decline continued with a significant annual percentage change of −25.2% ($P=0.001$) in Barthel Index scores based on linear mixed model analysis (Table 2).

Sensitivity analyses using Tobit mixed-effects models to account for ceiling effects of the Barthel Index demonstrated a similar overall trajectory of functional decline (Supplementary Material, Fig. S1C). Leave-one-out influence analyses likewise showed only modest variation across refitted models, supporting that the estimated progression pattern was not driven by an individual case (Supplementary Material, Fig. S1D).

The most common presenting clinical symptoms within this patient cohort were cognitive impairment (13/16 [81.3%]) and aphasia (10/16 [62.5%]) (Fig. 2). The proportion of patients with mild cognitive impairment decreased from 7/16 (43.8%) at first visit to 0 at month 24 (Fig. 3a); correspondingly, the proportion of patients with severe cognitive impairment increased from 3/16 (18.8%) at first visit to 4/5 (80.0%) at month 24.

Table 2 Linear mixed models for MoCA and Barthel Index scores, change over time, and percentage change over time

Outcome	*n*	Model	*P* value
MoCA score			
Slope for score over time[a]	5	−2.4	0.02
Annual change	5	−4.7	< 0.001
Annual change, %	5	−28.5	< 0.001
Barthel Index score			
Slope for score over time[a]	4	−10.8	0.04
Annual change	4	−24.5	< 0.001
Annual change, %	4	−25.2	0.001

MoCA Montreal Cognitive Assessment

[a]From linear mixed models with random slope for years of time, clustered by participant, and exchangeable covariance matrices

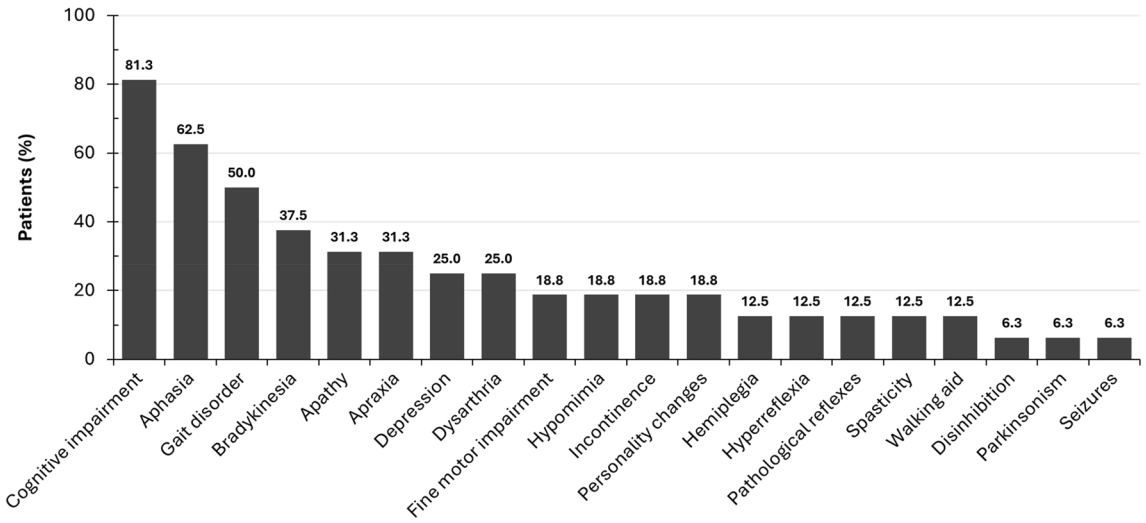


Fig. 2 Percentage of patients with specified symptoms at their first visit ($N = 16$). Pathological reflexes include the Babinski sign

Similar patterns of symptom progression were observed for gait disorder (Fig. 3b) and aphasia (Fig. 3c), with most (gait disorder) or all (aphasia) patients categorized with moderate or severe impairment by 24 months after symptom onset. Symptoms of fine motor impairment (Fig. 3d), apraxia (Fig. 3e), and dysphagia (Fig. 3f) tended to progress more slowly in this cohort, with < 50% of patients affected prior to month 12 (fine motor impairment, apraxia) and clinical symptoms only observed in small numbers of patients through month 24 (dysphagia). Of note, clinical data at 24 months were available for only a small number of patients ($n = 5$); consequently, these findings should be interpreted with appropriate caution.

Two patients in this cohort received hematopoietic stem cell transplantation (HSCT) for CSF1R-ALSP (patients 11 and 14). Outcomes of HSCT in patients with CSF1R-ALSP have been reported in the literature before, amongst others in an international cohort that included these two individuals [24]. Following transplantation, the clinical condition of both patients appeared to stabilize, as evidenced by stable or even increasing MoCA and Barthel Index scores (Supplementary Material, Fig. S2; see also "Discussion"). Follow-up data on these two patients are limited however, and therefore have to be interpreted carefully. To avoid confounding the characterization of the natural disease course, post-transplantation data from these two patients were excluded from the overall analysis in this study.

Magnetic Resonance Imaging

MRI data were available from 14 patients for assessment of white matter changes and brain atrophy (Table 3); for 2 patients (patients 14 and 16), MRI data were not available and are not included in the MRI results presented here. Across all patients' first available MRI scan ($n = 14$), white matter lesion score ranged from 3.0 to 13.5 (median 6.3), atrophy score ranged from 1.0 to 8.0 (median 6.0), and total ALSP score ranged from 4.0 to 19.5 (median 12.8). At the last available time point across all evaluable patients ($n = 13$; spanning 23.2–124.5 months since disease onset), white matter lesion score was between 5.0 and 15.0 (median 10.5), atrophy score was between 7.0 and 15.0 (median 10.0), and total ALSP score was between 13.5 and 29.0 (median 20.0). Periventricular cobblestone calcifications were visible as T1-hyperintense areas on high-resolution T1 MRI or CT scans (Supplementary Material, Fig. S3) in 7 of 9 evaluable patients (Table 3); in 5 patients, presence of calcifications was not evaluable. Diffusion restriction was observed on the

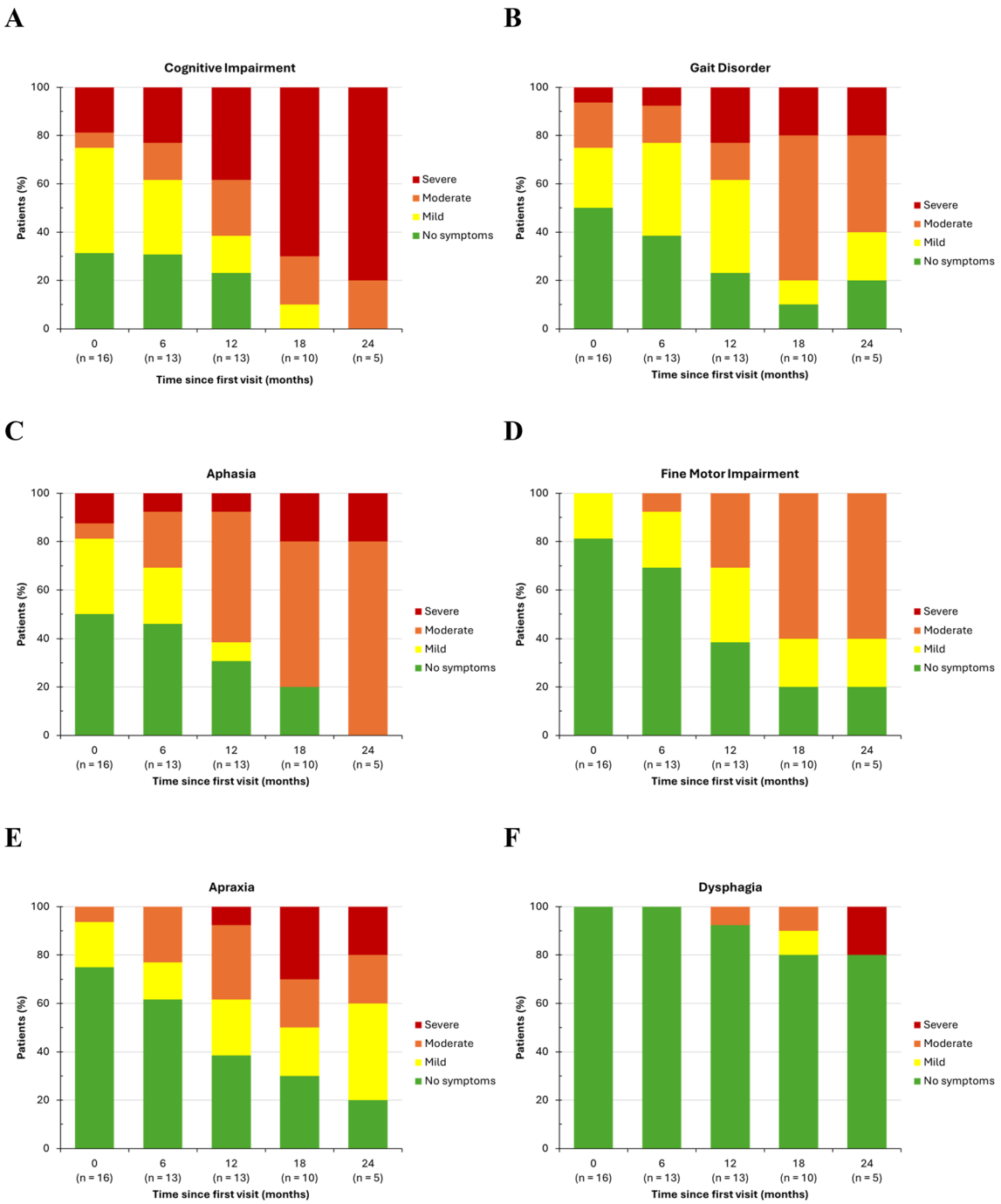


Fig. 3 Proportion of patients by symptom severity over time. **a** Cognitive impairment. **b** Gait disorder. **c** Aphasia. **d** Fine motor impairment. **e** Apraxia. **f** Dysphagia

first available MRI scans in 10 of 13 evaluable patients (< 5 punctuate foci observed in 4 cases, > 5 foci or patchy in 3 cases, and larger confluent areas in 3 cases) and in 12 of 13 patients based on all available scans; a diffusion-weighted sequence was not acquired for patient 5. Diffusion restriction persisted over several months in most patients (Table 3).

Table 3 Key imaging assessments by patient ($n = 14$)

Patient	Imaging	Months from disease onset to imaging[a]	Periventricular calcifications (method)[b]	MRI assessments			
				Diffusion restriction[c]	White matter lesion score[d]	Atrophy score[d]	Total ALSP score[d]
Patient 1	1	23.2	NA	Moderate	7.0	5.0	12.0
	2	24.2	Yes (MRI)	Moderate	7.0	5.0	12.0
	3	30.7	NA	NA	4.0	9.0	13.0
	4	36.8	Yes (MRI)	Moderate	5.0	9.0	14.0
Patient 2	1	26.6	NA	Mild	11.0	6.0	17.0
	2	50.2	NA	None	11.0	8.0	19.0
	3	50.2	NA	Moderate	12.0	13.0	25.0
Patient 3	1	80.9	NA	Severe	6.0	4.0	10.0
	2	92.4	NA	Severe	15.0	11.0	16.0
Patient 4	1	7.8	NA	Moderate	6.0	7.0	13.0
	2	13.3	NA	Moderate	6.0	7.0	13.0
	3	18.2	NA	Moderate	6.0	9.0	15.0
	4	21.9	NA	Moderate	6.0	10.0	16.0
	5	81.6	Yes (CT)	None	14.0	15.0	29.0
Patient 5	1	9.3	No (CT)	NA	8.0	5.0	13.0
Patient 6	1	37.5	NA	Mild	5.0	8.0	13.0
	2	54.1	No (CT)	Moderate	7.0	9.0	16.0
	3	70.3	NA	None	8.0	11.0	19.0
	4	81.8	No (MRI)	None	8.0	12.0	20.0
	5	106.5	No (MRI)	None	8.0	13.0	21.0
	6	124.5	No (MRI)	None	8.0	14.0	22.0
Patient 7	1	19.5	NA	Severe	5.5	3.0	8.5
	2	31.5	NA	Severe	9.5	7.0	16.5
Patient 8	1	89.6	NA	Moderate	13.5	6.0	19.5
	2	102.1	NA	Moderate	13.5	7.0	20.5
Patient 9	1	14.5	No (MRI)	Mild	5.5	6.0	11.5
	2	33.8	Yes (MRI) Yes (CT)	None	10.5	10.0	20.5

Table 3 continued

Patient	Imaging	Months from disease onset to imaging[a]	Periventricular calcifications (method)[b]	MRI assessments			
				Diffusion restriction[c]	White matter lesion score[d]	Atrophy score[d]	Total ALSP score[d]
Patient 10	1	30.6	NA	None	4.0	6.0	10.0
	2	40.8	Probable (MRI) Yes (CT)	Mild	5.5	8.0	13.5
Patient 11	1	17.8	NA	Severe	6.5	7.0	13.5
	2	29.5	NA	Severe	6.5	8.0	14.5
	3	44.2	Yes (MRI)	Moderate	6.5	11.0	17.5
Patient 12	1	3.6	NA	None	8.5	4.0	12.5
	2	4.6	NA	None	9.5	5.0	14.5
	3	5.0	Possible (CT)	None	9.5	5.0	14.5
	4	5.1	NA	None	9.5	5.0	14.5
	5	5.5	NA	None	10.5	5.0	15.5
	6	19.5	NA	None	10.5	6.0	16.5
	7	23.2	Probable (MRI)	None	11.5	7.0	18.5
Patient 13	1	32.2	NA	Mild	6.5	8.0	14.5
	2	36.8	NA	Mild	9.0	8.0	17.0
	3	40.6	NA	Mild	10.0	11.5	21.5
Patient 15	1	−71.3[e]	NA	NA	3.0	1.0	4.0
	2	1.1	NA	Moderate	5.5	4.0	9.5
	3	10.5	NA	NA	7.5	4.0	11.5
	4	11.4	Yes (CT)	NA	7.5	4.0	11.5
	5	14.4	NA	Moderate	8.0	4.0	12.0
	6	16.2	NA	Moderate	8.0	6.0	14.0
	7	34.3	NA	Moderate	11.0	9.0	20.0

ALSP adult-onset leukoencephalopathy with axonal spheroids and pigmented glia, *CT* computed tomography, *MRI* magnetic resonance imaging, *NA* not available

[a]Calculated from January 1st of the year of onset

[b]Visible as T1-hyperintense areas on high-resolution T1 MRI or CT scan

[c]Severity of diffusion restriction were defined as none, mild (< 5 punctate foci), moderate (> 5 punctate foci or patchy), and severe (larger confluent areas)

[d]Total ALSP score was calculated as described in Kinoshita et al. 2024 [22]

[e]Value is negative because an MRI scan had been performed > 5 years prior to disease onset

Table 4 Summary of clinical and fluid biomarker measures at first available scan among patients with available MRI assessments ($n = 14$)

Outcome	*n*	Median	Mean (SD)
Clinical assessments			
MoCA score	9	13	13 (8)
Barthel Index score	8	85	73 (36)
Fluid biomarkers			
NfL, pg/mL			
Serum	6	270	343 (280)[a]
CSF	6	16,933	30,601 (32,938)[b]
Chitotriosidase, nmol/h/mL			
Serum	6	44	57 (37)[c]
CSF	6	64	90 (66)[d]

CI confidence interval, *CSF* cerebrospinal fluid, *MoCA* Montreal Cognitive Assessment, *MRI* magnetic resonance imaging, *NfL* neurofilament light chain, *SD* standard deviation

[a]Mean (SEM) NfL in serum of 26 healthy individuals: 24.3 (3.4) pg/mL, 95% CI 17.4–31.2 [33]

[b]Mean (SEM) NfL in CSF of 17 healthy individuals: 1052 (208.5) pg/mL, 95% CI 609.8–1494 [33]

[c]Mean (SE) chitotriosidase in serum of 10 healthy individuals: 28.7 (3.3) nmol/h/mL, 95% CI 21.6–36.0 [20]

[d]Mean (SE) chitotriosidase in CSF of 10 healthy individuals: 2.0 (0.6) nmol/h/mL, 95% CI 0.7–3.2 [20]

For those patients with available MRI data ($n = 14$), available clinical and fluid biomarker assessments at the first available MRI scan are summarized in Table 4. Longitudinal changes in MRI brain volume by region are summarized in Table 5; based on annual median changes from the first scan, annual rates of white matter degeneration and brain atrophy were fairly consistent across scans 2 and 3 and slightly lower at scan 4, although the number of patients available for scan 4 was low (4 or 5 per MRI region). Because the duration of time between scans varied between patients, a linear mixed model analysis was applied to these data to evaluate change over time at the cohort level (Table 6). On an annual basis, this analysis showed statistically significant brain atrophy (total gray matter volume; −3.8% per year, $P < 0.001$), with corresponding increases observed in lateral ventricle volume (19.5% per year, $P < 0.001$), lateral ventricle + third ventricle volume (19.2% per year, $P < 0.001$), and overall brain ventricle volume (18.7% per year, $P < 0.001$). Unexpectedly, the analysis also revealed annual white matter hyperintensity volume reductions (−8.2% per year, $P = 0.008$).

To examine potential correlations between volumetric MRI measures and clinical and fluid biomarker outcomes, MRI scans that occurred within 90 days of clinical or biomarker assessments were selected to minimize the variable durations of time among data points being correlated from each patient. Based only on a subject's first MRI scan within 90 days of clinical and/or biomarker assessments, lateral ventricle volume ($r = -0.85$, $P = 0.008$), lateral ventricle + third ventricle volume ($r = -0.85$, $P = 0.007$), and overall brain ventricle volume ($r = -0.85$, $P = 0.008$) were statistically and negatively correlated with Barthel Index score (Table 7), indicating that greater ventricular volumes were associated with less independence in performing activities of daily living. Across all MRI scans within 90 days of clinical assessments, white matter hyperintensity was unexpectedly positively and significantly correlated ($r = 0.65$, $P = 0.02$) with MoCA, indicating that greater measured hyperintensity burden was associated with better cognitive performance. However, this finding likely reflects advanced white matter loss in later disease stages, in which progressive atrophy reduces the measurable hyperintense volume, rather than a protective effect (see "Discussion"). No other significant correlations were identified between volumetric MRI measures and clinical or fluid biomarker outcomes (Table 7). Longitudinally, change from the first available scan in total gray matter volume was significantly and positively correlated ($r = 0.90$; $P = 0.04$) with MoCA score, indicating that greater gray matter volume was associated with better cognitive function. Annualized volume changes from the first available scan in lateral ventricle + third ventricle ($r = -0.79$; $P = 0.04$) and overall brain ventricles ($r = -0.78$; $P = 0.04$) were

Table 5 MRI region volume and annual volume change from first available scan

MRI region volume, mL	Volume			Annual volume change from first scan		
	n	Median	Mean (SD)	*n*	Median	Mean (SD)
White matter hyperintensity[a]						
Scan 1 (first available)	9	24.1	21.3 (6.7)	9	NA	NA
Scan 2	9	18.3	17.6 (6.7)	8	− 2.8	− 1.0 (4.9)
Scan 3	5	16.0	17.9 (9.7)	5	− 2.4	− 5.7 (9.3)
Scan 4	5	15.9	20.2 (13.1)	4	− 1.6	− 2.9 (5.9)
Total gray matter						
Scan 1 (first available)	10	462.7	464.4 (34.6)	10	NA	NA
Scan 2	12	430.8	440.5 (38.7)	10	− 32.7	− 29.5 (34.0)
Scan 3	5	405.3	438.0 (56.5)	5	− 33.0	− 94.4 (163.6)
Scan 4	4	456.9	451.4 (62.2)	4	− 29.3	− 72.7 (102.9)
Lateral ventricles						
Scan 1 (first available)	14	71.1	71.5 (28.4)	14	NA	NA
Scan 2	13	78.2	85.5 (48.7)	13	10.1	15.9 (13.3)
Scan 3	9	92.4	105.5 (55.1)	9	13.6	17.6 (13.3)
Scan 4	5	84.3	86.5 (15.0)	5	9.0	11.5 (7.3)
Lateral ventricle + 3rd ventricle						
Scan 1 (first available)	14	73.6	74.0 (29.0)	14	NA	NA
Scan 2	13	81.0	88.3 (49.7)	13	10.5	16.4 (14.5)
Scan 3	9	95.8	108.6 (56.0)	9	14.8	18.3 (13.7)
Scan 4	5	86.3	89.0 (15.0)	5	9.1	11.9 (7.7)
Brain ventricles						
Scan 1 (first available)	14	76.7	76.5 (29.2)	14	NA	NA
Scan 2	13	84.9	91.0 (49.8)	13	10.5	16.1 (13.6)
Scan 3	9	98.4	111.5 (56.3)	9	14.5	19.3 (14.7)
Scan 4	5	88.4	91.5 (15.2)	5	9.1	11.5 (6.6)

MRI magnetic resonance imaging, *NA* not applicable, *SD* standard deviation

[a]Pathologic tissue volume

Table 6 Linear mixed models for brain MRI volume, volume change, and percentage volume change over time

Outcome	*n*	Model	*P* value
White matter hyperintensity volume			
Slope for volume over time[a] mL	10	− 1.29	0.1
Annual change, mL	10	− 1.90	0.02
Annual change, %	10	− 8.2	0.008
Total gray matter volume			
Slope for volume over time[a] mL	11	− 22.8	0.01
Annual change, mL	11	− 18.0	< 0.001
Annual change, %	11	− 3.8	< 0.001
Lateral ventricle volume			
Slope for volume over time[a] mL	13	17.0	0.004
Annual change, mL	13	14.9	< 0.001
Annual change, %	13	19.5	< 0.001
Lateral ventricle + 3rd ventricle volume			
Slope for volume over time[a] mL	13	17.6	0.004
Annual change, mL	13	15.3	< 0.001
Annual change, %	13	19.2	< 0.001
Brain ventricle volume			
Slope for volume over time[a] mL	13	17.8	0.003
Annual change, mL	13	15.5	< 0.001
Annual change, %	13	18.7	< 0.001

From linear mixed models with random slopes for years of time, clustered by participant, and exchangeable covariance matrices

MRI magnetic resonance imaging

significantly and inversely correlated with Barthel Index score (Table 8) (i.e., larger ventricle size was associated with greater dependence on help in activities of daily living). No statistically significant correlations were observed between serum or CSF biomarkers and MRI findings (Table 7) or clinical measures (Table 9).

Figure 4 shows representative MRI brain scans from two individual patients, illustrating the fast progression of cerebral MRI findings in CSF1R-ALSP. MRI scans from both patient 11 (Fig. 4a) and patient 15 (Fig. 4b) demonstrated rapid enlargement of the side ventricles and increasing T2 hyperintensities. In patient 11, T2 hyperintensities were less severe and even decreased at the last time point due to vanishing of white matter (Fig. 4a). This patient had a MoCA score of 15 at first scan and a Barthel Index score of 90, indicating severe cognitive impairment and moderate impairment in activities of daily living, that worsened over time. The MRI observations noted here indicate that atrophy may confound the white matter hyperintensity values, thereby explaining the unexpected reductions and correlations in white matter hyperintensities at the group level. For patient 15, MRI scans indicate that T2 hyperintensities were mainly located around the ventricles in the beginning, but affected areas extended over time to the deep white matter as well as to the subcortical white matter in the frontal and parietal lobes.

DISCUSSION

This study examined the natural disease course of CSF1R-ALSP based on MRI findings and clinical observations and symptoms of disease progression in a cohort of 16 symptomatic adults with genetically confirmed CSF1R-ALSP. In general, we observed rapid worsening of cognitive function and clinical symptoms during the observational period.

Cognitive functioning at the first visit was variable across patients, declined severely over the first year after initial assessment in all patients with available data, and continued to diminish to a similar extent through 24 months in patients with additional data beyond 12 months. Barthel Index scores, which indicate functional independence in performing activities of daily living, were likewise variable at the first visit and tended to decline thereafter: among eight patients with more than one nonzero Barthel Index scores available, four patients sharply declined within 1 year (by 35–100 points), three patients declined to a lesser degree (by 10–15 points), and one patient remained stable with little change in disability (5-point decline). Similarly, neurological

Table 7 Pearson correlation analyses between MRI brain volume measures and clinical and biomarker assessments

MRI region	MoCA score			Barthel Index score			NfL						Chitotriosidase					
	n	*r*	*P* value	*n*	*r*	*P* value	Serum			CSF			Serum			CSF		
							n	*r*	*P* value	*n*	*r*	*P* value	*n*	*r*	*P* value	*n*	*r*	*P* value
For first pair of MRI scans within 90 days of clinical or biomarker assessment																		
White matter hyperintensity	7	0.71	0.07	7	0.31	0.050	6	0.44	0.38	6	0.39	0.44	6	−0.46	0.36	6	0.26	0.62
Total gray matter	7	0.6	0.16	7	0.47	0.29	5	−0.34	0.58	5	−0.1	0.88	5	0.29	0.63	5	0.09	0.89
Lateral ventricles	9	−0.59	0.1	8*	−0.85*	0.008*	6	−0.18	0.74	6	0.13	0.81	6	−0.6	0.21	6	−0.42	0.41
Lateral ventricle + 3rd ventricle	9	−0.59	0.09	8*	−0.85*	0.007*	6	−0.18	0.74	6	0.13	0.81	6	−0.59	0.22	6	−0.42	0.41
Brain ventricles	9	−0.59	0.1	8*	−0.85*	0.008*	6	−0.18	0.74	6	0.13	0.81	6	−0.59	0.21	6	−0.42	0.4
Across all MRI scans within 90 days of clinical or biomarker assessment																		
White matter hyperintensity	12*	0.65*	0.02*	12	0.41	0.19	6	0.44	0.38	6	0.39	0.44	6	−0.46	0.36	6	0.26	0.62
Total gray matter	12	0.47	0.12	12	0.21	0.51	5	−0.34	0.58	5	−0.10	0.88	5	0.29	0.63	5	0.09	0.89
Lateral ventricles	17	−0.27	0.30	15	−0.50	0.06	6	−0.18	0.74	6	0.13	0.81	6	−0.60	0.21	6	−0.42	0.41
Lateral ventricle + 3rd ventricle	17	−0.28	0.28	15	−0.51	0.055	6	−0.18	0.74	6	0.13	0.81	6	−0.59	0.22	6	−0.42	0.41
Brain ventricles	17	−0.27	0.29	15	−0.51	0.055	6	0.74	0.74	6	0.13	0.81	6	−0.59	0.21	6	−0.42	0.40

CSF cerebrospinal fluid, *MoCA* Montreal Cognitive Assessment, *MRI* magnetic resonance imaging, *NfL* neurofilament light chain, *r* Pearson correlation coefficient

*Asterisks indicate statistically significant correlations

Table 8 Pearson correlation analyses for changes from first available scan in MRI brain volume measures versus clinical assessments

MRI region	MoCA score			Barthel Index score		
	n	*r*	*P* value	*n*	*r*	*P* value
Change from first available MRI scan for all subsequent scans within 90 days of clinical assessments						
White matter hyperintensity	5	0.67	0.21	5	− 0.55	0.34
Total gray matter	5*	0.90*	0.04*	5	0.81	0.1
Lateral ventricles	8	0.05	0.9	7	− 0.55	0.2
Lateral ventricle + 3rd ventricle	8	0.04	0.92	7	− 0.57	0.18
Brain ventricles	8	0.05	0.91	7	− 0.58	0.18
Annual change from first available MRI scan for all subsequent scans within 90 days of clinical assessments						
White matter hyperintensity	5	0.66	0.23	5	0.4	0.51
Total gray matter	5	0.54	0.34	5	0.57	0.31
Lateral ventricles	8	− 0.13	0.75	7	− 0.75	0.055
Lateral ventricle + 3rd ventricle	8	− 0.17	0.68	7*	− 0.79*	0.04*
Brain ventricles	8	− 0.17	0.68	7*	− 0.78*	0.04*

MoCA Montreal Cognitive Assessment, *MRI* magnetic resonance imaging, *r* Pearson correlation coefficient

*Asterisks indicate statistically significant correlations

Table 9 Pearson correlation coefficients between fluid biomarkers and clinical assessments

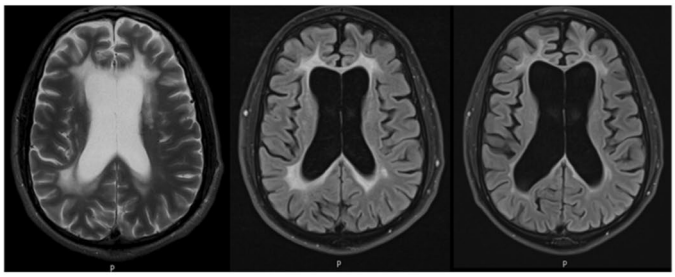
Fluid biomarkers	MoCA score			Barthel Index score		
	n	*r*	*P* value	*n*	*r*	*P* value
NfL						
Serum	6	− 0.29	0.58	6	− 0.62	0.19
CSF	6	− 0.33	0.52	6	− 0.54	0.26
Chitotriosidase						
Serum	6	− 0.21	0.69	6	0.51	0.3
CSF	6	− 0.23	0.66	6	0.25	0.63

CSF cerebrospinal fluid, *MoCA* Montreal Cognitive Assessment, *NfL* neurofilament light chain

impairments such as gait disorder, aphasia, and fine motor impairment worsened rapidly in the patients over the disease course.

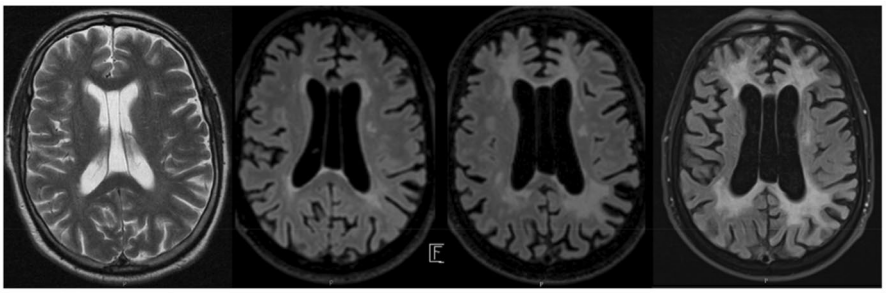
It is important to note, however, that the observed fast disease progression may not accurately represent the early stages of the condition as referral to a university hospital may be delayed as a result of the rarity and general lack of knowledge of the disease. Moreover, given the limited number of patients in this study with follow-up data beyond 12 months, the described disease course after this time point should be regarded as exploratory.

A



	Visit 1	Visit 3	NA
Time point:	First scan	+ 12 months	+ 27 months
Side ventricle volume, mL:	97.0	112.4	152.5
White matter hyperintensity volume, mL:	28.5	32.7	25.2
Total ALSP score:	13.5	14.5	17.5

B



	Historic	Visit 1	Visit 4	Visit 7
Time point:	First scan	+ 72 months	+ 87 months	+ 105 months
Side ventricle volume, mL:	36.7	61.9	79.8	98.8
White matter hyperintensity volume, mL:	9.7	21.9	33.4	37.7
Total ALSP score:	4.0	9.5	14.0	20.0

Fig. 4 MRI findings of rapid enlargement of the side ventricles and increasing T2 hyperintensities in patients with CSF1R-ALSP. **a** For patient 11, enlargement of the side ventricles and increasing T2 hyperintensities are shown from first scan (visit 1; T2) to 12 months (visit 3; FLAIR) and 27 months (FLAIR). **b** For patient 15, rapid enlargement of the side ventricles and increasing T2 hyperintensities are shown from a historic first scan T2 image to 72 months (visit 1; T2), 87 months (visit 4; FLAIR), and 105 months (visit 7; FLAIR). *CSF1R-ALSP* colony-stimulating factor 1 receptor–adult-onset leukoencephalopathy with axonal spheroids and pigmented glia, *FLAIR* fluid-attenuated inversion recovery, *MRI* magnetic resonance imaging, *NA* not available

Treatment of CSF1R-ALSP was not a focus of this study. However, two patients in this cohort underwent HSCT and showed favorable outcomes with respect to disease progression, supporting the potential benefit of HSCT when performed early in the disease course. However, given the limited data as well as the risk of severe adverse effects of HSCT, the decision to pursue this treatment in a patient with CSF1R-ALSP should be made on an individual basis by experienced specialists.

Overall, the prominent signs and symptoms reported herein for CSF1R-ALSP are similar to those reported in existing literature, with some variation likely attributable to the variable size of cohorts. In a large cohort of 291 patients with CSF1R-ALSP identified by literature review, demographics and genetic profile were comparable to those reported here, and cognitive impairment was similarly the most common presenting symptom (47.1%), followed by behavioral/psychiatric symptoms (26.8%), extrapyramidal motor symptoms (16.2%), pyramidal motor dysfunction (11.7%), and speech difficulty (11.3%); white matter hyperintensities, brain atrophy, and ventricular enlargement on brain MRI scans

were also reported [9, 30]. In another study of 65 patients identified by retrospective chart review, behavioral and/or psychiatric signs (43.1%) and cognitive impairment (38.5%) were the most common presenting signs and symptoms, with gait, motor, and speech disturbance reported in 9.2–16.9% of cases [30]. Furthermore, strong, significant correlations were demonstrated between worsening cognition, assessed using Mini Mental Status Exam, and both increasing Sundal MRI severity scores ($n=13$; $r=-0.66$; $P=0.012$) and increasing brain volume loss ($n=18$; $r=-0.48$; $P=0.036$) [30].

In this study, brain MRI scans revealed longitudinal evidence of brain atrophy, with substantial changes occurring in the first 1 to 2 years of available imaging. Gray matter volume loss was associated with declining cognitive function, and corresponding increases in ventricular volume were associated with greater dependency in patients' ability to perform activities of daily living, consistent with previous findings in CSF1R-ALSP [30]. Further, although additional prospective research is needed, correlations between clinical outcome measures and volumetric MRI assessments may serve as valuable markers of disease severity in CSF1R-ALSP and have potential value as endpoints in clinical trials and predictors for disease progression [30, 31]. In contrast, the observed positive association between WMH volume and cognitive performance should be interpreted with caution. In advanced disease stages, progressive white matter atrophy reduces the measurable volume of hyperintense tissue, leading to apparent decline in WMH burden despite worsening clinical status (see Fig. 4a). Thus, this association most likely reflects structural tissue loss and measurement constraints rather than a biologically meaningful relationship.

Other biomarkers have also been proposed for use as endpoints in clinical studies aimed at slowing or halting progression of CSF1R-ALSP. NfL, a polypeptide that is sensitive to neurodegeneration [32], has been proposed as a biomarker for rapidly progressive neurodegenerative diseases such as CSF1R-ALSP and elevated levels have been shown in the serum and CSF of patients with this disease [33, 34]. Similarly, chitotriosidase is an enzyme secreted by microglia that has been proposed as a biomarker of CSF1R-ALSP disease activity based on results of studies demonstrating increased serum and CSF chitotriosidase levels in patients with CSF1R-ALSP [20, 34]; notably, chitotriosidase levels were elevated in the CSF [34] but not serum [20] of presymptomatic carriers of pathogenic *CSF1R* variants compared with healthy controls. Previous studies have been inconsistent in the demonstration of statistically significant correlations between CSF fluid biomarkers (e.g., NfL, chitotriosidase) and clinical outcome measures of cognitive function (MoCA scores) or disability (Barthel Index) [20, 34]. In the current study, the lack of significant correlations observed between fluid biomarkers (serum or CSF) and MRI findings or clinical measures may be due to the small sample size and limited available measurements of NfL and chitotriosidase levels. Larger studies will enable more complex relationships between MRI and biomarker assessments, particularly longitudinal changes in NfL levels. One current hypothesis proposes that elevated NfL levels remain stable while brain atrophy and ventricular enlargement continue to progress; this perspective views NfL level as an actual measure of ongoing axonal damage and MRI as assessing the cumulative effect of the disease process. Because of the association of NfL with axonal integrity and the relationship between chitotriosidase levels and disease progression, these biomarkers may still have a role for disease prognosis and for monitoring the efficacy of treatments in future clinical trials (e.g., NfL levels should decrease and measures of brain atrophy should stabilize with efficacious treatments).

The possibility of incomplete documentation or nonstandard reporting due to the retrospective nature of data collection must be considered when interpreting these results. In addition, the small sample size, the variability in disease duration prior to initial assessment, and the source for all patients being a single treatment center must be considered in terms of the generalizability of these findings. Furthermore, although detailed genetic information was available for all patients, the present study was not designed to establish genotype–phenotype correlations. Given the limited cohort size, the predominance of missense variants, and the absence of

functional validation of individual variants, any apparent associations between variant type and clinical trajectory should be interpreted descriptively and with caution. Larger, prospectively characterized cohorts with functional analyses will be required to rigorously address genotype–phenotype relationships in CSF1R-ALSP. Also, while we consider the applied assessment tools (MoCA, Barthel Index) as pragmatic clinical descriptors that enable cross-study comparability and longitudinal benchmarking, detailed neuropsychological assessments and disease-specific clinical rating scales would be more precise and sensitive in evaluating early symptoms and progression in patients with CSF1R-ALSP.

Although recruiting a sufficient number of patients is challenging in such a rare disease, continued efforts are important for the identification of biomarkers that are informative for clinical trials and drug development. As our findings are generally aligned with previous larger cohorts, these results extend the current body of knowledge on the natural history of CSF1R-ALSP and support current thinking on potential clinical, radiological, and fluid biomarkers of the disease that could be used as endpoints in future clinical trials. In addition, the data generated in this study are intended to support clinicians with limited experience of CSF1R-ALSP to adequately interpret clinical and imaging findings as well as to counsel patients and their caregivers. Further, the field would benefit from the collection of a minimal set of clinical assessments (e.g., a bedside cognitive test, the Barthel Index or the Expanded Disability Status Scale, and a routine neurological examination). Finally, more routine biomarker assessments such as NfL and chitotriosidase levels would further inform our understanding of CSF1R-ALSP.

CONCLUSION

CSF1R-ALSP is a rare neurological disorder with significant unmet medical need, and this retrospective review of patient medical records provides qualitative and quantitative real-world clinical data for further characterization of the natural history of the disease. These data may inform the design of future natural history studies and interventional clinical trials using clinically meaningful endpoints and will guide clinicians less familiar with the disease in providing patients and caregivers with appropriate support and information.

Medical Writing, Editorial, and Other Assistance. Medical writing and editorial support were provided by Morgan C. Hill, PhD, Allyson Lehrman, DPM, and Heidi Reinholdt, MA, MS, of Apollo Medical Communications, part of Helios Global Group, and funded by Vigil Neuroscience, Inc.

Author Contributions. Conceptualization: Stefanie N. Hayer, Benjamin Bender, Ludger Schöls, Donald G. McLaren. Data curation: Stefanie N. Hayer, Benjamin Bender, Donald G. McLaren, Eva Bürkle, Holger Hengel. Formal analysis: Benjamin Bender, Donald G. McLaren, Robin M. Nance. Investigation: Stefanie N. Hayer, Benjamin Bender, Ludger Schöls, Holger Hengel, Benjamin Röben, Melanie Kellner. Methodology: Stefanie N. Hayer, Benjamin Bender, Donald G. McLaren, RMN. Critical review and approval of final version for submission: Stefanie N. Hayer, Benjamin Bender, Ludger Schöls, Donald G. McLaren, Robin M. Nance, Holger Hengel, Benjamin Röben, Melanie Kellner, Eva Bürkle.

Funding. This study was supported by Vigil Neuroscience, Inc., who had a role in the design and conduct of the study; management, and analysis of the data; and the review of the manuscript. The decision to submit the manuscript for publication was made by the authors. The journal's Rapid Service Fee was covered by the Department of Neurology, and the Department of Diagnostic and Interventional Neuroradiology, University Hospital Tübingen, Tübingen, Germany.

Data Availability. The datasets generated during and/or analyzed during the current study will be made available to qualified investigators upon reasonable request to the corresponding author.

Declarations

Conflict of Interest. Stefanie N. Hayer was funded by the Hertie Network of Excellence in Clinical Neuroscience (GHST grant P1200021). Holger Hengel was supported by the Deutsche Forschungsgemeinschaft (DFG, HE 8803/1-1). Benjamin Röben has received a research grant from the University of Tübingen (Clinician Scientist; Project-Nr. 480-0-0). Donald G. McLaren was an employee of Vigil Neuroscience, Inc., and/or held stock/stock options. He is currently an employee of Vigil Neuroscience, Inc., a Sanofi company. Robin M. Nance has received consultancy fees from Vigil Neuroscience, Inc. Melanie Kellner declares that she has no competing interests. Eva Bürkle declares that she has no competing interests. Ludger Schöls is funded by the German Research council (DFG grant SCHO754/6-2), German Ministry of Health (BMG grant ZMVI1-2520DAT94E to LeukoExpert), German Ministry of Education and Research (BMBF grant 01GM1905A to Treat HSP and grant 01GM1907A to Treat ION), European Commission (EU grant 947588 to the ERNRND registry and JPND grant 01ED16028 to ESMI), is a member of the European Reference Network for Rare Neurological Diseases (Project No 739510), and serves as a principal investigator on clinical studies sponsored by Vigil Neuroscience, Inc. (VGL101-01.001; VGL101-01.002). Benjamin Bender is cofounder and shareholder of AIRAmed GmbH and has received consultancy fees from Eisai GmbH, Medtronic, and Novartis.

Ethical Approval. The study was conducted in compliance with the principles of the Declaration of Helsinki and adhered to all applicable laws and regulations. Prior to initiation, the study was approved by the Institutional Review Board of the University of Tübingen (approval number 502/2024BO2), which ruled that, because the study was noninterventional and retrospective and all data were de-identified prior to inclusion and analysis, the study posed no risk or burden to patients and did not require participants to provide signed informed consent according to §13(1) 1 Landesdatenschutzgesetz Baden-Württemberg of German law.

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