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Clinical spectrum of adult-onset leukoencephalopathy with axonal spheroids and pigmented glia in individuals of Korean ancestry

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Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a rare white matter disease characterized by axonal and glial injury. Although its clinical characteristics have been described in case reports, the prevalence of *CSF1R* mutations in clinically suspected ALSP cases remains unclear. Herein, we analysed the frequency of *CSF1R* mutations in patients with probable or possible ALSP and describe the genetic, clinical, radiological, and pathological findings of ALSP cases in individuals of Korean ancestry. Twenty-eight patients with probable or possible ALSP diagnosed at Samsung Medical Center, Seoul, between January 2014 and August 2020, were retrospectively reviewed. All participants underwent brain magnetic resonance imaging (MRI) and *CSF1R* genetic testing. Overall, 9 of the 28 patients (32.1%) [5/6 (83.3%) of probable ALSP and 4/22 (18.2%) of possible ALSP] were confirmed to have pathogenic or likely pathogenic variants in *CSF1R* gene. Additionally, one patient without *CSF1R* mutation exhibited histopathological findings consistent with ALSP on brain biopsy. All patients with *CSF1R* mutation presented with cognitive impairment and/or psychiatric symptoms. Brain MRI revealed bilateral white matter hyperintensities in all patients, and 5/8 (62.5%) showed diffusion-restricted lesions. Notably, patients with *CSF1R* mutation had younger age at onset, rapidly progressive course, and diffuse hyperintensity in the splenium compared to patients without *CSF1R* mutation. Our findings suggest that for definite diagnosis, *CSF1R* genetic testing is recommended in patients who meet the diagnostic criteria for possible or probable ALSP. Our findings provide insights into the genetic, clinical, radiological, and pathological dimensions of ALSP in individuals of Korean ancestry.

Keywords Adult-onset leukodystrophy with axonal spheroids and pigmented glia, *CSF1R* gene

Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a rare autosomal dominant white matter degenerative disease, including both axonal and glial damage[1]. ALSP is characterized by the presence of axonal spheroids and pigmented glia, and generally presents as a spectrum of neurological symptoms, including cognitive impairment, psychiatric manifestations, and motor dysfunction. This condition

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encompasses two previously recognized diseases which share similar pathological and clinical features; hereditary diffuse leukoencephalopathy with axonal spheroids and pigmentary orthochromatic leukodystrophy[2]. The global prevalence of ALSP remains unclear[3]. Estimates indicate that there are approximately 10,000 cases in the United Stated[4]. Additionally, case series from various regions have been documented, including 16 cases in France, 122 cases in Japan, and 5 cases in Taiwan[5–7]. In South Korea, approximately ten patients have been reported to have ALSP, signifying an increased need for awareness of this disease[8–11].

Several case reports and studies have previously explored the clinical and imaging characteristics of ALSP[5,12–14]. Clinically, most patients exhibit impaired cognition or neuropsychiatric symptoms. Imaging studies have identified characteristic magnetic resonance imaging (MRI) findings in ALSP, including bilateral white matter hyperintensities (WMH), affecting the corticospinal tract and splenium, as well as persistent hyperintense lesions on diffusion-weighted imaging (DWI), and punctuate calcifications on computed tomography (CT)[5].

Above all, the identification of causative mutations in the *colony-stimulating factor 1 receptor* (*CSF1R*) gene has helped to uncover the etiology of ALSP[15,16]. The *CSF1R* gene encodes a tyrosine kinase receptor that plays a pivotal role in regulating cytokines like CSF-1 and interleukin-34. Patients carrying one mutant *CSF1R* allele typically present with ALSP, while those with two mutant alleles may experience brain abnormalities, neurodegeneration, and dysosteosclerosis at an earlier age[17]. The penetrance of ALSP associated with *CSF1R* mutations is high, although it is often not complete, as asymptomatic carriers were reported[3,6,18,19]. Because the *CSF1R* gene is the primary causative gene for ALSP, genetic testing is essential for a definitive diagnosis[3]. To screen for the necessity of genetic testing, Konno et al. proposed diagnostic criteria specifically for ALSP with *CSF1R* mutations[16]. However, the positive predictive value (PPV) of these criteria has not been well established due to the rarity of the disease. A study in Japan reported a PPV of 15.3%, but it needs to be validated[20].

In this study, we aimed to (1) report the prevalence of *CSF1R* mutations in patients clinically suspected of having ALSP and (2) provide a detailed description of the clinical, imaging, genetic, and pathological characteristics of definite ALSP cases obtained from a single referral center in Seoul, Korea.

Results

Of the 28 possible or probable ALSP patients, nine (32.1%) were diagnosed with definite ALSP based on the identification of *CSF1R* mutations, while one patient who tested negative for *CSF1R* mutation was definitively diagnosed with ASLP based on pathological findings compatible with the disease. Specific details of *CSF1R* variants, clinical features, initial diagnoses, and radiological findings of the 10 ALSP cases were summarized in Table 1.

Identification of *CSF1R* variants

Among the 28 possible or probable ALSP patients screened for *CSF1R* variants, nine (32.1%) from eight unrelated families were identified as having a pathogenic variant (PV) or likely pathogenic variant (LPV) in the *CSF1R* gene. Therefore, 5/6 (83.3%) cases previously classified as probable ALSP, and 4/22 (18.2%) cases previously classified as possible ALSP were finally diagnosed as definite ALSP according to Konno et al.[16] Specifically, we identified one PV and seven LPVs within the *CSF1R* gene. One PV (p.Phe849del) was detected in case 3. LPV (p.Arg782His) was found in cases 1 and 2, who were the siblings of a previously reported ALSP case[8]. Other identified LPVs included p.Pro878Ser in case 4, p.Ile794Thr in case 5, p.Ala823Val in case 6, p.Gly7470* in case 7, and p.Gly589Arg in cases 8 and 9. Variant of uncertain significant (VUS) (p.Phe971Serfs*7) was also identified in case 7. All detected variants were located within the tyrosine kinase domain (TKD) of the *CSF1R* protein, which is encoded by exons 12–21 (Table 1).

In case 10, although no PV or LPV was detected in the *CSF1R* gene, pathological evaluation of a brain biopsy revealed axonal spheroids and pigmented macrophages with CD68-immunopositive macrophages and microglia, compatible with ALSP. Whole exome sequencing (WES) was performed to investigate other potential genetic factors contributing to the patient's clinical and pathological presentation. However, despite extensive analysis, no additional PVs or LPVs were identified in the genes typically associated with autosomal dominant dementia, dementia with severe white matter changes, or dementia with motor symptoms. Consequently, this case was classified as possible ALSP according to Konno et al.[16].

Clinical and imaging findings

The mean age at symptom onset in the 10 above cases was 47.5 years (range, 37–63 years). The most prevalent initial symptom was cognitive impairment, noted in 90% (9/10) of cases. Neuropsychological assessments conducted in eight cases showed that the frontal/executive and memory domains were commonly impaired. Psychiatric symptoms, such as abulia, depression, and irritability, were observed in 70% (7/10) of cases. Pyramidal signs and parkinsonism were each observed in 50% (5/10). Epilepsy was noted in 20% (2/10) during the later stages of the disease. A further 90% (9/10) of cases presented with rapidly progressive course, progressing to bedridden status within five years of symptom onset. Autosomal dominant inheritance patterns were observed in three of the eight unrelated families, as illustrated in Fig. 1.

All cases exhibited bilateral WMH on brain MRI (Fig. 2), while 70% (7/10) showed symmetric WMH. High signal intensity was noted along the corticospinal tract in 50% (5/10) and splenium in 80% (8/10) of the cases. Specifically, in splenium, focal signal changes were present in 2 cases, while diffuse signal changes were noted in 6 cases. In addition, 90% (9/10) showed diffuse cortical atrophy, predominantly affecting the frontal regions. Among the eight cases who underwent DWI, five showed multiple small diffusion-restricted lesions in the deep white matter, and calcifications were observed in one case (case 8) on brain CT.

Pedigrees of 10 patients carrying a *CSF1R* pathogenic or likely pathogenic variant. Open symbol: unaffected; filled symbol: affected; symbol with a diagonal line: deceased; arrow: proband; square: male; circle: female.

Case no.	Sex	Onset age	*CSF1R* gene			Family history	Core clinical features	Initial MMSE	Initial diagnosis	Imaging findings on brain CT and MRI	Pathological findings
			Variant	Nucleotide	Amino acid						
1	M	61	LPV	c.2345G > A	p.Arg782His	+	Cognitive impairment Psychiatric symptoms Pyramidal sign Parkinsonism Rapidly progressive course	27	ALSP	Diffuse brain atrophy Bilateral WMH affecting splenium, Thinning of corpus callosum DWI restriction	Not investigated
2	M	44	LPV	c.2345G > A	p.Arg782His	+	Cognitive impairment Psychiatric symptoms Pyramidal sign Parkinsonism Epilepsy Rapidly progressive course	25	Vascular dementia	Diffuse brain atrophy Bilateral WMH	Autopsy
3	F	44	PV	c.2546_2548delTCT	p.Phe849del	+	Cognitive impairment Rapidly progressive course	10	EOAD	Diffuse brain atrophy Bilateral WMH affecting splenium	Not investigated
4	F	45	LPV	c.2632 C > T	p.Pro878Ser	–	Cognitive impairment Psychiatric symptoms Parkinsonism	17	bvFTD	Diffuse brain atrophy, Bilateral WMH affecting splenium Thinning of corpus callosum	Not investigated
5	F	51	LPV	c.2381T > C	p.Ile794Thr	–	Cognitive impairment Psychiatric symptoms Rapidly progressive course	4	Vascular dementia	Diffuse brain atrophy Bilateral WMH affecting splenium Thinning of corpus callosum DWI restriction	Not investigated
6	M	46	LPV	c.2467G > A	p.Ala823Val	+	Cognitive impairment Pyramidal sign Rapidly progressive course	28	Demyelinating disease	Diffuse brain atrophy Bilateral WMH affecting corticospinal tract and splenium Thinning of corpus callosum	Not investigated
7	M	46	LPV VUS	c.2239G > T c.2906_2909dupATCA	p.Gly7470* p.Phe971Serfs*7	–	Cognitive impairment Psychiatric symptoms Parkinsonism Rapidly progressive course	14	EOAD	Diffuse brain atrophy Bilateral WMH affecting corticospinal tract and splenium Thinning of corpus callosum DWI restriction	Not investigated
8	F	37	LPV	c.1765G > A	p.Gly589Arg	–	Pyramidal sign Parkinsonism Rapidly progressive course	30	Demyelinating disease	Calcification on CT Bilateral WMH along corticospinal tract Thinning of corpus callosum DWI restriction	Biopsy
9	M	40	LPV	c.1765G > A	p.Gly589Arg	–	Cognitive impairment Psychiatric symptoms Pyramidal sign Rapidly progressive course	13	Demyelinating disease	Diffuse brain atrophy Bilateral WMH along corticospinal tract and splenium Thinning of corpus callosum DWI restriction	Not investigated
10	F	63	Not detected			–	Cognitive impairment Psychiatric symptoms Epilepsy Rapidly progressive course	19	Epileptic dementia	Diffuse brain atrophy Bilateral WMH along corticospinal tract and splenium DWI restriction	Biopsy

Table 1. Clinical, genetic, and radiological findings of the 10 ALSP cases. *LPV* likely pathogenic variant, *PV* pathogenic variant, *VUS* variant of uncertain significant, *MMSE* Mini–mental state examination, *ALSP* adult-onset leukoencephalopathy with axonal spheroids and pigmented glia, *EOAD* Early-onset Alzheimer's disease, *bvFTD* Behavioural-variant frontotemporal dementia, *WMH* white matter hyperintensities, *DWI* diffusion-weighted imaging.

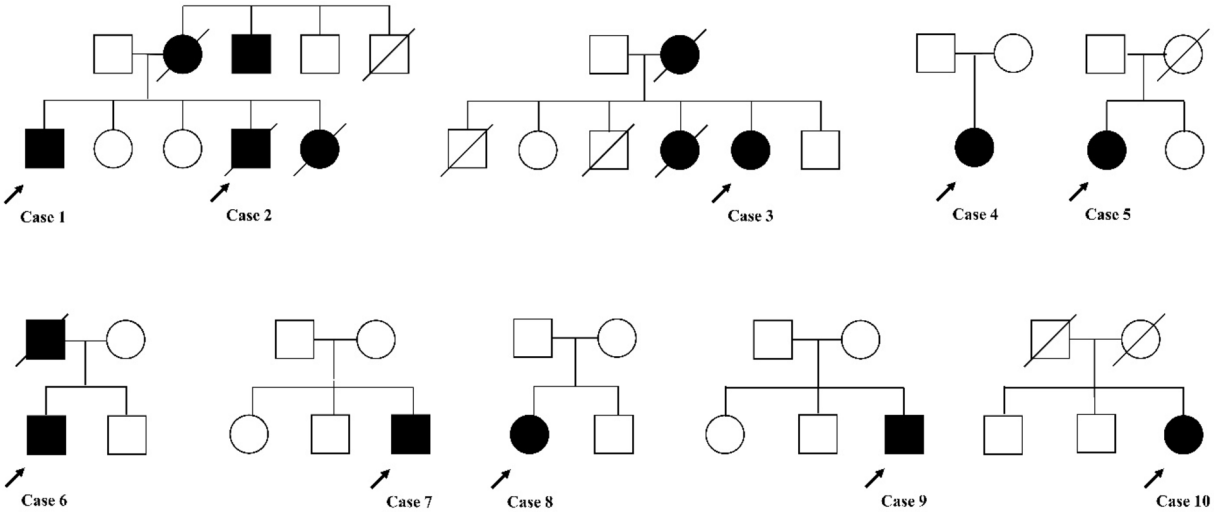

Fig. 1. Pedigrees of the ALSP patients.

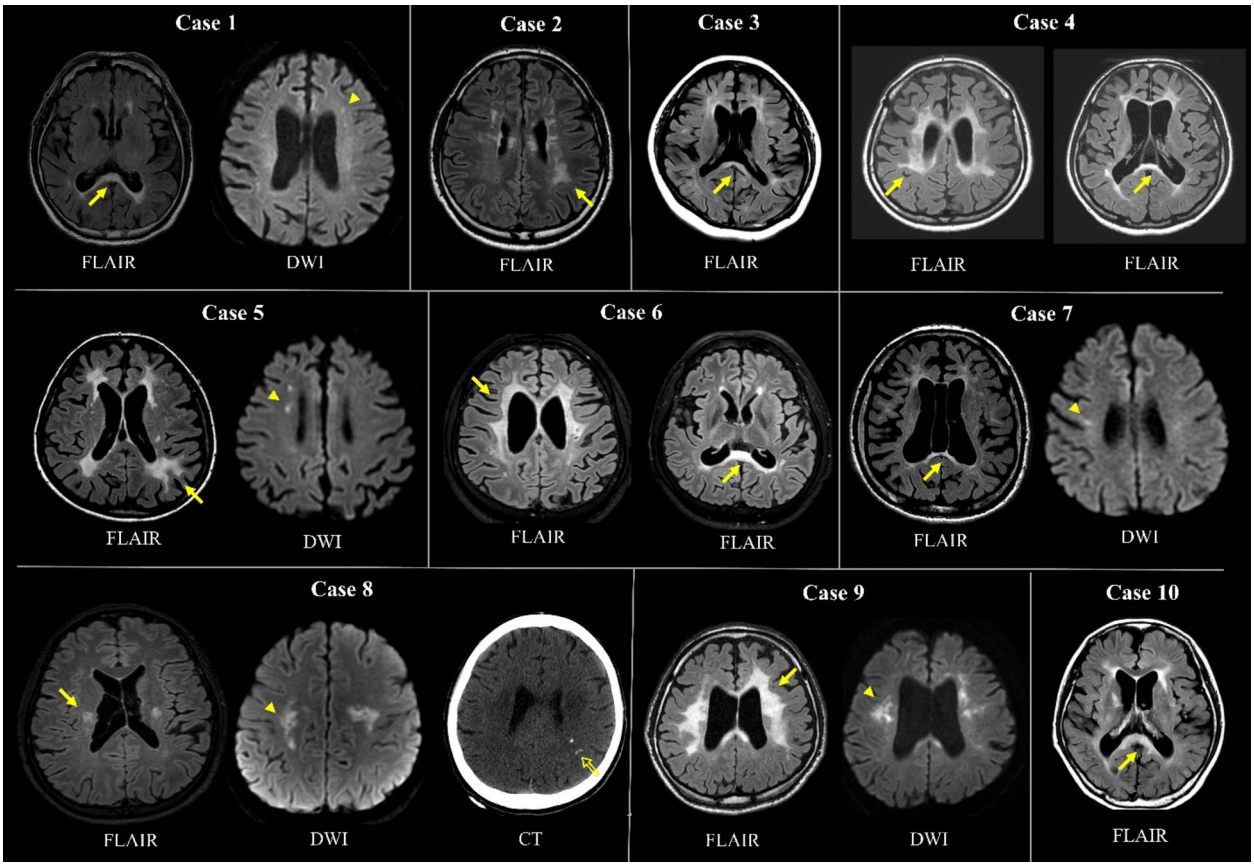

Fig. 2. Neuroimaging findings of 10 ASLP cases.

Fluid-attenuated inversion recovery (FLAIR) images showing bilateral white matter hyperintensities prominently involving the splenium and corticospinal tract (arrows). Diffusion-Weighted Imaging (DWI) revealed persistent, multifocal, and restricted lesions (arrow heads). In addition, brain CT imaging in case 8 showed calcifications (empty arrow).

Histopathological findings

The histopathological diagnosis of ALSP was confirmed in three cases. An autopsy was conducted in one case (case 2) and brain biopsies were performed in two cases (cases 8 and 10), as shown in Fig. 3. Haematoxylin and eosin staining (HE) revealed that the cerebral white matter in these cases contained numerous eosinophilic axonal spheroids and pigmented macrophages (Fig. 3a, d). Immunohistochemical staining for Cluster of Differentiation 68 (CD68) was positive in macrophages and microglia (Fig. 3b, e). Additionally, neurofilament (NF) staining revealed a few positive spheroids (Fig. 3c, f).

Haematoxylin and eosin (HE)-stained sections of white matter lesions from cases 8 (a) and 10 (d) displaying axonal spheroids and pigmented macrophages (100x). Axonal spheroids and pigmented macrophages were immunohistochemically positive for CD68 (100x) (b and e) (arrows) and phosphorylated neurofilaments (NF) (100x) (c and f) (empty arrows), respectively. Scale bars represent 100 μm in all figures.

Comparison of features between patients with and without *CSF1R* mutation

The differences in clinical and brain imaging features between patients with and without *CSF1R* mutation were presented in Table 2. The age at onset was significantly younger in the *CSF1R* mutation-positive group (median [interquartile range, IQR] 45.0 [44.0,46.0]) than in the *CSF1R* mutation-negative group (median [IQR] 63.0 [58.0,70.0], $p = 0.006$). Rapidly progressive course was significantly more prevalent in the *CSF1R* positive group ($p = 0.004$). Diffuse hyperintensity in the splenium was more prevalent in the *CSF1R* mutation positive group ($p = 0.008$). There was a significant difference in the distribution of probable and possible ALSP according to Konno's criteria, with a higher proportion of *CSF1R* mutation-positive patients classified as probable ALSP ($p = 0.007$).

Discussion

This study showed that 9 (32.1%) of the 28 probable or possible ALSP patients were confirmed to have PVs or LPVs in the *CSF1R* gene. In addition, one patient without known *CSF1R* mutations was determined to be pathologically compatible with ALSP. Subsequently, we noted that all patients presented with cognitive and/or psychiatric symptoms and characteristic ALSP imaging findings. Notably, the age at onset, rapid progression of the disease, and diffuse hyperintensity in the splenium emerged as significant discriminative characteristics, differentiating probable or possible ALSP with *CSF1R* mutation-positive patients from mutation-negative patients.

Our findings indicate that *CSF1R* mutations were present in 9 patients among 28 who met the probable or possible criteria for ALSP, as proposed by Konno et al.[16]. Thus, the PPV of Konno's criteria for predicting *CSF1R* mutation was 32.1%. More specifically, the mutations were identified in 5 patients (83.3%) among 6 probable ALSP, while only in 4 patients (18.2%) among 22 possible ALSP patients. Confirmation of *CSF1R* gene mutation elevates the diagnosis to definite ASLP. Our data suggests that while Konno's criteria for probable ALSP provide supportive information in diagnosing ALSP (PPV 83.3%), genetic testing is mandatory for a definite diagnosis.

According to Konno et al., the diagnostic criteria for ALSP emphasizes early onset, defined as age ≤ 60 years. In our study, eight out of nine *CSF1R* mutation carriers had early onset, whereas only one patient presented with symptoms at age 61. Our data supports the validity of using 60 years of age as a cutoff for the diagnostic criteria. The prevalence of adult-onset leukodystrophy was reported to be approximately 300 cases per million[21], with

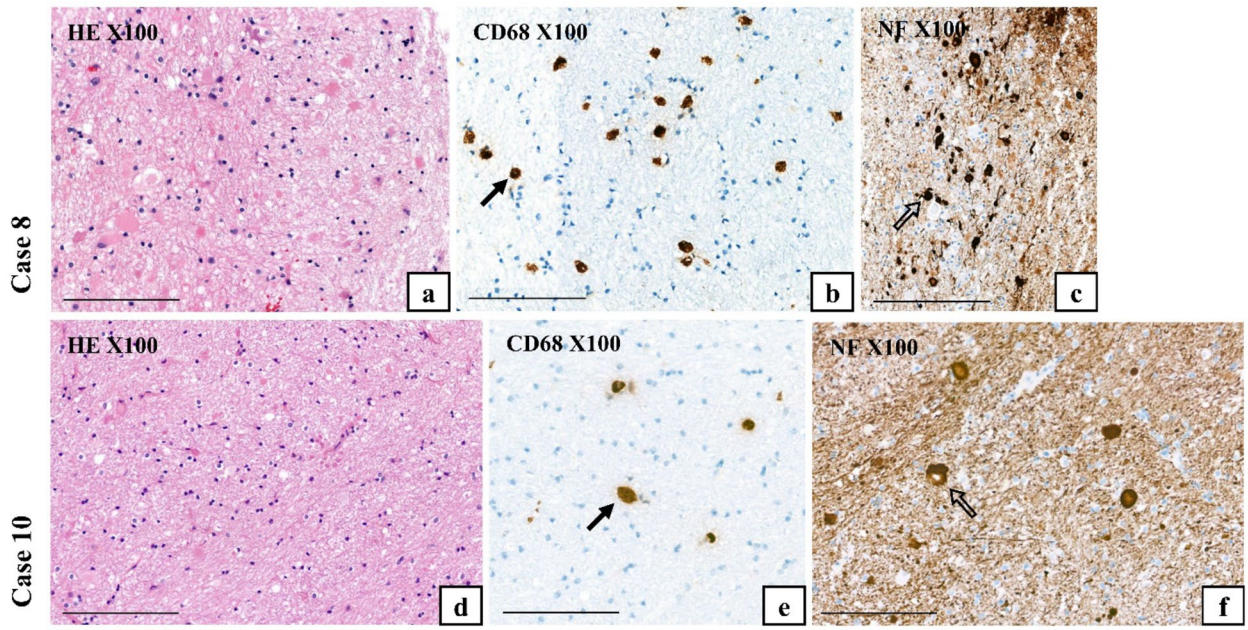

Fig. 3. Histopathological features of the ALSP cases.

	CSF1R gene mutation (*N* = 9)	*CSF1R* gene mutation negative (*N* = 19)	*p*-value
Sex (male)	5 (55.6%)	7 (36.8%)	0.432
Onset age	45.0 [44.0;46.0]	63.0 [58.0;70.0]	0.006
MMSE	17.0 [13.0;27.0]	19.0 [14.5;27.0]	0.805
Family history	3 (33.3%)	9 (47.4%)	0.687
Clinical features			
Cognitive impairment	8 (88.9%)	19 (100.0%)	0.321
Psychiatric symptoms	6 (66.7%)	18 (94.7%)	0.084
Pyramidal signs	5 (55.6%)	9 (47.4%)	1.000
Parkinsonism	5 (55.6%)	10 (52.6%)	1.000
Seizure	1 (11.1%)	7 (36.8%)	0.214
Rapidly progressive course*	8 (88.9%)	5 (26.3%)	0.004
Brain imaging features			
Diffuse brain atrophy	8 (88.9%)	13 (68.4%)	0.371
Bilateral cerebral WMH	9 (100.0%)	19 (100.0%)	1.000
Thinning of the corpus callosum	7 (77.8%)	10 (52.6%)	0.249
Hyperintensity in splenium			0.008
None	2 (22.2%)	6 (31.6%)	
Focal	1 (11.1%)	11 (57.9%)	
Diffuse	6 (66.7%)	2 (10.5%)	
Hyperintensity in corticospinal tract	4 (44.4%)	2 (10.5%)	0.064
DWI restriction	5/8 (62.5%)	6/19 (31.6%)	0.206
Spotty small calcifications	1/8 (12.5%)	2/19 (10.5%)	1.000
Konno's diagnostic criteria			0.007
Possible	4 (18.2%)	18 (81.8%)	
Probable	5 (83.3%)	1 (16.7%)	

Table 2. Comparison of clinical and radiological features according to *CSF1R* gene mutation status. Median [Interquartile range] for continuous variable. N (%) for categorical variable. *MMSE* Mini-Mental State Exam, *WMH* white matter hyperintensity, *DWI* diffusion-weighted imaging. *Become bedridden within 5 years after onset.

10–25% attributed to *CSF1R*-related ALSP[22,23]. We identified *CSF1R* mutations in nine patients over a seven-year period. Give these data, *CSF1R*-related ALSP should be considered in patients with adult-onset leukodystrophy with onset age before 60.

Mutations in the *CSF1R* gene within TKD is the cause of ALSP[15]. To date, at least 106 mutations in *CSF1R* have been identified worldwide[3]. In our study, we identified one PV and seven LPVs in the *CSF1R* gene[1,24,25]. A novel LPV (p.Gly7470*) with VUS (p.Phe971Serfs*7) was also identified in case 7. The identified LV or LPVs in our patients were located across various exons within the TKD of the *CSF1R* gene, including exon 13 (cases 8 and 9), 16 (case 7), 18 (cases 1, 2, and 5), 19 (cases 3 and 6), and 20 (case 4). This distribution is consistent with findings that the majority of *CSF1R* gene mutations in ALSP are located within the TKD, with a predominance in the distal part[3,7,26]. *CSF1R* plays a crucial role in regulating microglial proliferation and survival, as well as the differentiation of neural progenitor cells[1]. Microglia are integral to the myelination process, and can induce spontaneous remyelination following injury[27]. As such, in *CSF1R* mutation carriers, a reduced number of microglia, leading to impaired repair mechanisms, *CSF1R* may contribute to axonal degeneration and the development of ALSP.

In this study, we included one patient (case 10) who, despite testing negative for *CSF1R* mutations, was pathologically compatible with ALSP. This patient, who developed symptoms at the age of 63 years, met the clinical criteria for possible ALSP[16]. Pathological evaluation of a brain biopsy revealed axonal spheroids and pigmented macrophages with CD68-immunopositive macrophages and microglia, consistent with ALSP. Despite the relatively late age of onset, the clinical and radiological features were compatible with ALSP. Our findings align with those of other reports of pathologically confirmed ALSP cases lacking *CSF1R* mutations[14,24,28,29]. Typically, genetic testing for *CSF1R* mutation focuses on exon 12–22, which encode intracellular TKD. However, mutations in other areas can also lead to ALSP. For example, Miura et al. reported a novel frameshift mutation in exon 4 (c.310delC) located outside the TKD[24]. Additionally, Leng et al. reported a splicing mutation in intron 16 of the *CSF1R* gene[28]. Moreover, biallelic mutations in the *AARS* gene, which encodes a mitochondrial enzyme, have been associated with the clinical manifestations and neuropathology of ALSP, further complicating the genetic landscape of this disease[3,30]. Furthermore, there have been cases for which no mutations were found even after extensive genetic analysis. For example, Kimura et al. reported a case of pathologically confirmed ALSP in which no mutations were found in the *CSF1R*, *TYROBP*, or *TREM2* genes across all exons[14]. In addition, Dulski et al. reported two familial ALSP cases where no mutations were identified in the *CSF1R*, *AARS1*, or *AARS2* genes[29]. In our patient, we conducted WES in which no PVs or LPVs were identified in the 24 genes (including

the *AARS* gene) known to be responsible for autosomal dominant dementia, dementia with severe white matter change, or dementia with motor symptoms (genes listed in the Methods). Collectively, these findings suggest that unknown genetic factors may contribute to ALSP.

Clinical features of our cases and previously reported ALSP patients in Korea align with those reported in other countries[5–10,12,13,25]. Most Korean patients present with cognitive impairment and psychiatric symptoms, which are more common than motor dysfunction or epilepsy[1,3]. Our study further supports the rapidly progressive nature of ALSP, noted in supportive findings of the diagnostic criteria by Konno et al., as most of our cases progressed to a bedridden state within five years of symptom onset. Given the rapid progression, particularly in patients exhibiting core features of the disease, genetic testing for *CSF1R* gene is highly recommended. Intriguingly, six of the 10 patients had no family history. This may be explained by the incomplete penetrance of several variants or de novo mutations in these patient[23].

Radiological features in the ALSP cases presented herein were bilateral WMH, predominantly affecting the pyramidal tract or splenium, and diffuse brain atrophy. Additionally, diffusion-restricted lesions were noted in five cases, while intracranial calcifications were detected on CT scans in one case, aligning with findings reported in previous studies[31]. These bilateral WMH and thinning of the corpus callosum are recognized as core diagnostic criteria for ALSP. Additionally, we propose that hyperintensity in the splenium should also be recognized as a key indicator of ALSP, as this prevalence differs between the *CSF1R* mutation-positive and negative groups. Consistent with our findings, a previous study reported that abnormal signal changes were significant, albeit at a borderline level ($p = 0.05$)[20].

Overall, Konno et al.'s criteria are useful for screening ALSP and assessing the need for genetic testing[16], particularly when probable ALSP is met (PPV, 83.3%). Although the criteria for possible ALSP demonstrated relatively low PPV (18.2%), it remains crucial for clinicians to assess for supportive findings. As the rapidly progressive course is often undetectable in the early stages, it is important to reassess the potential for ALSP during the follow-up. Notably, as Konno et al. developed the criteria for a screening tool, *CSF1R* gene test is necessary for confirmation of ALSP.

This study has some limitations. First, all clinical, radiological, and genetic data were collected retrospectively from patients with suspected ALSP. This retrospective study design may have led to the exclusion of patients who met the diagnostic criteria for ALSP but were not initially recognized or documented. Second, positive or negative control for pathologic data was not available for publication. Third, the sample size was relatively small. However, this study represents the largest cohort of patients with ALSP collected in South Korea. We believe that documenting the prevalence of *CSF1R* mutations and providing a detailed comparison between *CSF1R* mutation positive and negative patients among probable or possible ALSP will broaden our understanding of ALSP, particularly in Korea.

In conclusion, we described the genetic, clinical, radiological, and pathological findings of ALSP cases of Korean ancestry with ALSP. We recommend performing *CSF1R* gene testing, particularly for patients who fulfil the diagnostic criteria for possible or probable ALSP. Additionally, a brain biopsy can provide diagnostic insights in cases in which *CSF1R* mutations are not detected.

Methods

Participants

We retrospectively reviewed 37 patients with suspected ALSP who were treated at the Samsung Medical Center in Seoul, Korea between January 2014 and August 2020. Patients were eligible if they showed (i) deteriorating neurological symptoms, (ii) bilateral cerebral white matter lesions, and (iii) symptoms not attributable to other known causes of leukoencephalopathies. Patients were classified as probable (fulfil core features 1–5) or possible (fulfil core features 2a, 3, and 4a) ALSP according to Konno et al.'s diagnostic criteria for ASLP[16]. The core features are (1) age of onset ≤ 60; (2) clinical signs and symptoms (2a-cognitive impairment or psychiatric symptoms, 2b-pyramidal signs, 2c-parkinsonism, 2d-epilepsy); (3) autosomal dominant inheritance or sporadic occurrence; (4) brain CT/MRI findings (4a-bilateral cerebral white matter lesion, 4b-thinning of the corpus callosum); (5) exclude other causes of leukoencephalopathy. After applying the diagnostic criteria, 28 patients with probable ALSP ($n = 6$) or possible ALSP ($n = 22$) were included and underwent genetic testing for *CSF1R* mutations. This study was approved by the Institutional Review Board of Samsung Medical Center (2021-05-087). The requirement for informed consent was waived due to the retrospective design of the study. All research was performed in accordance with relevant guidelines.

Genetic analysis

Genomic DNA (gDNA) was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification Kit, in accordance with the manufacturer's instructions (Promega, Madison, WI, USA). Sanger sequencing was further performed to analyze *CSF1R*. For the *CSF1R* gene, exons 12–22 (in which the intracellular TKD is located), were analysed. Sequence alignment and variant identification were performed using Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, MI, USA). NM_005211.3 was used as the reference transcript for the *CSF1R* gene.

In one patient (case 10) with no PV or LPV in the *CSF1R* gene, but whose pathological findings confirmed ALSP, we further performed WES to identify other genetic variants responsible for autosomal dominant dementia (*GFAP*, *LMNB1*, *NOTCH3*, *MAPT*, *CHMP2B*, *GRN*, *APP*, *PSEN1*, and *PSEN2*), dementia with severe white matter change (*EIF2B1*, *EIF2B2*, *EIF2B3*, *EIF2B4*, *EIF2B5*, *AARS2*, *GLA*, *PLG*, and *TYMP*), or dementia with motor symptoms (*TREM2*, *TYROBP*, *ARSA*, *GALC*, *DARS2*, and *ABCD1*)[22]. For WES, we used the SureSelect Human All Exon V6 (Agilent Technologies, Santa Clara, CA, USA) on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Sequence reads were aligned to the hg19 human reference sequence using Burrow-Wheeler Aligner (BWA version 0.7.17). Local realignment and recalibration were performed using the

Genome Analysis Tool Kit (GATK version 4.1.2, https://gatk.broadinstitute.org/). Variant calling was performed using the GATK software.

Pathology

Pathological assessments were conducted in three patients to further characterise the disease manifestations of ALSP. Two neuropathologists (Y.L.S. and H.J.K.) reviewed all HE slides of the three cases. The gross findings of the autopsy case were also examined. The white matter of each case showed scattered axonal spheroids and pigmented macrophages on HE staining. Immunohistochemical stain for NF and CD68 was performed on one representative section of the cases to clearly confirm axonal spheroids and pigmented macrophages. Loss of myelinated axons was confirmed by Luxol-fast blue and Bielschovsky silver staining. The detailed findings of these evaluations have been previously documented[11].

Statistical analyses

Participants were categorized into two groups based on the presence or absence of *CSF1R* gene mutations. We conducted the Mann-Whitney U test and Fisher's exact test to compare demographic, clinical, and radiological features between two groups, as appropriate. All statistical analyses were performed using R studio. Statistical significance was defined as $p < 0.05$.

Data availability

The datasets generated and/or analysed in the current study are available from the corresponding authors upon reasonable request.

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Design and conceptualization of the study: J.R.K., H.J., and H. J. K. Collection of the clinical data: J.R.K., S.L, J.J., Y.L.S, J.H.P., H.J.K., and H.J.K.; Analysis and interpretation of the data: J.R.K, S.L, S.L, H.J.S., E.K., S.W.S., D.L.N., H.J., and H.J.K.; Drafting and revising the manuscript: J.R.K., H.J.K, H.J., and H.J.K.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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