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Case Report

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An Autopsy Proven Case of CSF1R-mutant Adult-onset Leukoencephalopathy with Axonal Spheroids and Pigmented Glia (ALSP) with Premature Ovarian Failure

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Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a progressive degenerative white matter disorder caused by mutations in the tyrosine kinase domain of the *CSF1R* gene. ALSP is often misdiagnosed as other diseases due to its rarity and various clinical presentations such as Parkinsonism, pyramidal signs, cognitive impairment and/or psychiatric symptoms. We describe an autopsy case of ALSP with a *CSF1R* mutation. A 61-year-old woman presented insidious-onset gait difficulty for 12 years since her age of 49, and premature ovarian failure since her age of 35. At initial hospital visit, brain magnetic resonance imaging revealed hydrocephalus. Initially, Parkinson's syndrome was diagnosed, and she was prescribed L-dopa/carbidopa because of spasticity and rigidity of extremities, which had worsened. Subsequently, severe neuropsychiatric symptoms and cognitive impairment developed and radiologically, features of leukoencephalopathy or leukodystrophy were detected. She showed a down-hill course and died, 12 years after initial diagnosis. At autopsy, the brain showed severe symmetric atrophy of bilateral white matter, paper-thin corpus callosum, thin internal capsule, and marked hydrocephalus. Microscopically, diffuse loss of white matter, relatively preserved subcortical U-fibers, and many eosinophilic bulbous neuroaxonal spheroids were noted, but there was no calcification. Pigmented glia with brown cytoplasmic pigmentation were readily found in the white matter, which were positive for Periodic acid-Schiff, p62, and CD163 stains, but almost negative for CD68. Whole-exome and Sanger sequencing revealed a *CSF1R* mutation (c.2539G>A, p.Glu847Lys) which was reported in prior one ALSP case. This example demonstrates that ALSP could be associated with premature ovarian failure.

Key words: Autopsy, *CSF1R*, Leukoencephalopathy, Neuroglia, Whole exome sequencing

INTRODUCTION

Recently adult onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) was proposed as a comprehensive term encompassing hereditary diffuse leukoencephalopathy with

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spheroids (HDLS) and pigmentary orthochromatic leukodystrophy (POLD), because of similar clinical and pathological features, and presence of *CSF1R* gene mutations [1, 2]. POLD was firstly described in a Swedish family with leukoencephalopathy as an autosomal dominant disease in 1936 [3]. Since then, many sporadic cases have been reported [4]. HDLS was reported in 17 members of a Swedish family in 1984 [5]. Even before the *CSF1R* mutation was discovered by whole-exome sequencing in 2012 [6], there were reports that the two diseases may be a single entity, because of the similar clinical and pathological features [7]. Indeed, familial cases of HDLS and POLD show similar neuropsychiatric symptoms (depression, behavioral change, frontal release signs, etc.) and neurological symptoms (Parkinsonism, pyramidal signs, epilepsy, ataxia, etc.), and similar pathological features such as widespread loss of myelinated nerve fibers with frontal or frontotemporal predominance [7]. Additionally, several HDLS families and POLD families demonstrated pigmented macrophages and numerous spheroids, respectively, on brain autopsy [8].

More than 120 cases of ALSP have been reported to date based on confirmation of the *CSF1R* mutation [9]. Five Korean cases of ALSP with CSF1R mutation have been reported, but autopsy was not carried out in these five cases [10, 11]. Diagnostic criteria [12] and staging scheme for disease progression of ALSP were established through review of autopsy cases [13, 14]. However, the rarity of the disease and the variety of phenotypic presentations cause difficulties in reaching accurate diagnosis; thus, this disease is often confused with other diseases such as primary progressive multiple sclerosis, central nervous system (CNS) vasculitis, Alzheimer's disease, frontotemporal dementia, corticobasal degeneration, and atypical Parkinsonism [7, 15, 16].

In 2014, another type of leukoencephalopathy termed as the *AARS2* mutation-related leukodystrophy (AARS2-L) was reported by Dallabona et al. [17]. This disease shares several clinical and radiological features with ALSP, but is associated with premature ovarian failure (ovarioleukodystrophy) in female patients and no *CSF1R* mutation [17-19].

Based on genetic alterations which can be autosomal dominant or recessive, or X-linked, eleven subtypes of adult onset leukoencephalopathy have been identified so far [20]. Ikeuchi et al. [20] have summarized the driver genes for the disease subtypes, which include *CSF1R*, *NOTCH3*, *LMNB1*, *GFAP*, *HTRA1*, the *EIF2B family*, *ARSA*, *TREM2/TYROBP*, *AARS2*, *FMR1*, and *ABCD1*, and have described the primary cellular involvement of these 11 disease subtypes.

If a genetic study is not carried out, ante-mortem diagnosis of these diseases are difficult. Here we report an ALSP case diagnosed based on findings from an autopsy and whole-exome sequencing; the patient had a history of a highly suspicious premature ovarian failure.

CASE REPORT

The patient was a 49-year-old Korean woman who presented with gait disturbance and slurred speech that had started 5 months prior to the first hospital visit. Her medical history included complaints of depressive moods two years before the visit, for which she was prescribed antidepressant medication (nortriptyline). She was diagnosed with premature ovarian failure at the age of 35 and subsequently received hormone replacement therapy. Clinical manifestations of this patient are summarized and compared with previously reported cases in Table 1 [9].

On neurological examination in the initial visit (12 years before death), her Korean Mini-Mental State Examination (K-MMSE) score was 25/30 points which was consistent with mild cognitive impairment. Physical examination revealed ataxic gait, postural instability, and left dominant bradykinesia. Deep tendon reflexes were 3+ in the both upper and lower extremities, and the Babinski sign was observed on the left side. Abnormal rapid alternating movement was observed on her left side on cerebellar function test.

Family history revealed that her father had symptoms of dementia and gait disturbance at the age of 80 and that her sister also showed gait disturbance. However, according to the patient, no accurate neurological diagnosis was provided.

Over time, dysarthria and dysphagia became worse, and rigidity and spasticity increased, leading to a bed-ridden state that began at around 52 years of age. At that time, the patient showed intermittent psychotic features and aggressive behavior, and complained of self-voiding difficulty. At the age of 53, generalized allodynia developed. She died at the age of 61. She had been diagnosed with premature ovarian failure at an outside hospital at the age of 35 and had received hormone replacement therapy. However, serum hormone levels such as follicle stimulating hormone (FSH) and / or estradiol (E2) and ultrasonographic findings necessary for the diagnosis of premature ovarian failure were not available.

Medications to reduce Parkinson's disease-like signs and symptoms like L-Dopa/carbidopa were prescribed only during the first year, and were ineffective. Later, the spasticity increased and baclofen was given, which was partially effective.

Laboratory test results including electrolyte levels, and adrenal function, were within normal range. Thyroid stimuating hormone (TSH) was mildly elevated to 4.23 μIU/ml (normal: 0.1~4.1 μIU/ml) and anti-TSHR and anti-microsomal antibodies were elevated to 11.0% (normal: 0~1%) and 659 U/ml (normal: 0~60 U/ml).

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Table 1. Clinical manifestation and brain imaging features presented in previously reported cases [9] and our patient

	Findings from previous report	Our case
Age of onset for female and male patients (mean±SD)	40±10 years in female 47±11 years in male	49 years / female
Disease duration (mean±SD)	6.8±5.4 years	12 years
Mode of inheritance	Autosomal dominant inheritance or sporadic occurrence	Not definite Father: dementia and gait disturbance Sister: gait disturbance
Clinical features (prevalence, %)		
Cognitive impairment	94%	Present K-MMSE; 25/30 initially
Psychiatric symptoms Anxiety, depression, apathy, indifference, abulia, irritability, disinhibition, distraction, etc.	75%	Present Depression → aggressive behavior
Parkinsonism Resting tremor, rigidity, bradykinesia, postural instability	61%	Present Postural instability, bradykinesia (left dominant) → rigidity↑
Pyramidal signs Hyperreflexia, spasticity, increased tone in extremities, pseudobular palsy	57%	Present Hyperreflexia, spasticity
Seizures	32%	Present
Other clinical features	Dysarthria (34%) Dysphagia (17%) Ataxia (27%) Sensory disturbance (10%) Peripheral neuropathy (2%)	Present Present Present Allodynia History of premature ovarian failure Voiding difficulty
MRI findings (prevalence, %)		
Bilateral white matter lesions	69%	Present
Thining of corpus callosum	49%	Present
Calcification in the white matter	14%	Absent

Anti-thyroglobulin antibody was <25 U/ml (normal: 0~60 U/ml), however, T3, Free T4, cortisol and ACTH were within normal limits. Genetic tests for spinocerebellar ataxias (*SCA1*, *SCA2*, *SCA3*, *SCA6*, and *SCA7*) and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (*NOTCH3*) were all negative. A spectrophotometric assay using peripheral blood revealed that there was no increase in arylsulfatase A enzymatic activity. Galactosylcerebrosidase enzymatic activity as assayed by liquid chromatography-tandem mass spectrometry was also in the normal range. Plasma levels of very long chain fatty acids (VLCFAs) and vitamin E were normal. Although serum ceruloplasmin level was slightly higher than the normal value (45 mg/dL, normal: 15~40 mg/dL), it was not considered as clinically relevant. Lumbar puncture and *CSF* analysis revealed 92 mg/dL of protein (normal: 15~45 mg/dL), 59.1 mg/dL of albumin (normal: 10~30 mg/dL), and an IgG level of 7.1 mg/dL. The IgG index was 0.286, but the causes of the elevation of protein and albumin were unknown.

No electrophysiologic abnormalities suggestive of peripheral neuropathy or widespread denervation were observed. Videooculography revealed no abnormality, and both eyes had normal visual evoked potential (VEP). Magnetic resonance imaging (MRI) performed at the ages of 49 and 52, (Fig. 1 and 2), which did not include diffusion weighted images (DWIs), and the MRI performed at age 52 did not include a fluid attenuated inversion recovery (FLAIR) image. The first MRI scan was performed five months after the beginning of the gait disturbance and two years after the onset of the depressive mood, when the patient was aged approximately 47 years (Fig. 1A~C), which showed relative symmetrical confluent atrophy of the white matter mainly involving the frontoparietal lobes. The T2 hyperintense white matter lesion was confluent from the subcortical area to the periventricular area. Ventricular dilatation and mild cerebral atrophy were also observed. In 2008, all the findings had progressed to the occipital lobe (Fig. 1D~F). The subcortical U-fibers was preserved. Gadolinium enhancement showed no enhancing lesion.

Autopsy findings

The weight of the brain before formalin fixation was 1020 g. Grossly, the brain showed global atrophy, which was prominent in

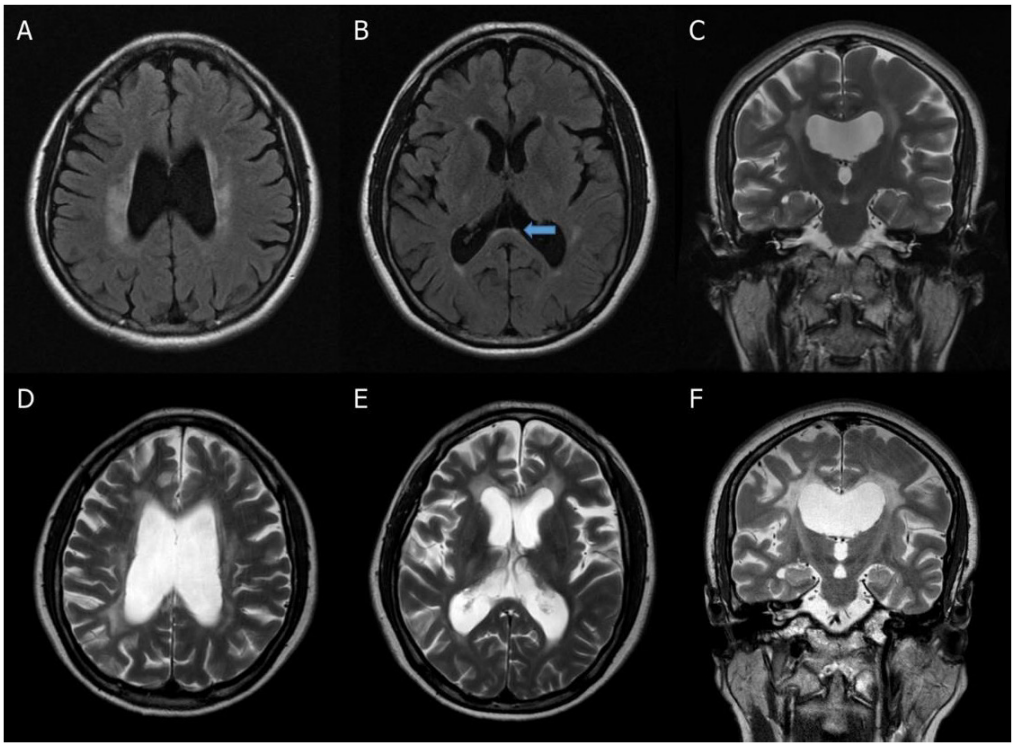

Fig. 1. Magnetic resonance imaging (MRI) findings from T2 FLAIR images (A,B) and T2-weighted image (C) obtained in 2005 and T2-weighted images obtained in 2008 (D~F). There is an interval of about 3 years between A~C and D~F. A and D show images from almost the same level, and so are B;C and E;F respectively. (A~C) White matter lesion with T2 hyperintensity is observed. Splenium of corpus callosum (arrow) is also involved (B). Sagittal image shows internal capsule involvement and corpus callosal thining (C). Mild cerebral atrophy and lateral ventricular dilatation is present. (D~F) In 2008, definite disease progression was detected, and the cerebral atrophy and ventricular dilatation worsened. Despite progression of the disease, the subcortical U-fibers are relatively preserved.

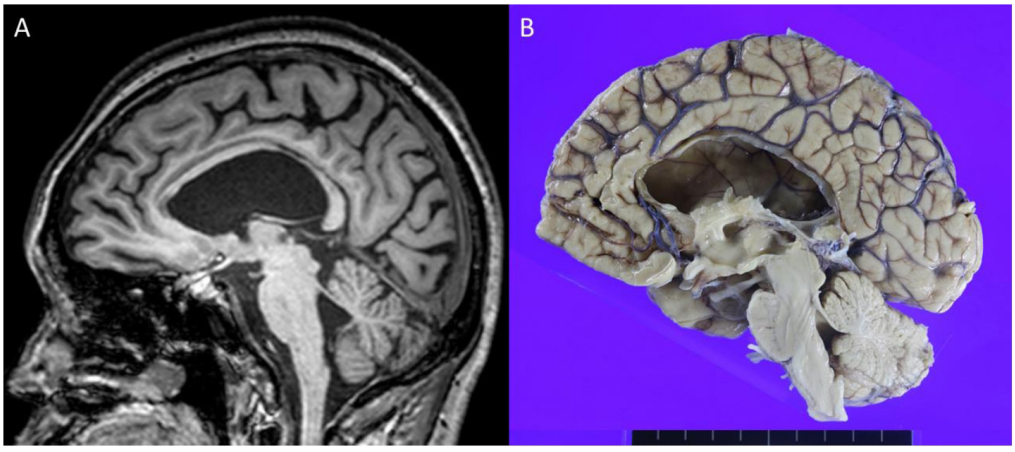

Fig. 2. (A) The Sagittal T1 image obtained in 2008 shows a thin white matter with severe hydrocephalus and atrophic cortex, cerebellum, pons, medulla and spinal cord. This section of the MRI is 0.5 mm lateral from the midline. (B) The formalin-fixed autopsy hemibrain obtained in 2017 shows a much thinner corpus callosum than that of MRI image obtained in 2008 and shows marked hydrocephalus. In this view, cortical atrophy does not seem severe.

the frontal area. The crus cerebri and the pons were also atrophic. The corpus callosum showed a paper-thin appearance (Fig. 2B).

Through coronal section, severe atrophy of the centrum semiovale and the internal capsule with severe ventriculomegaly was detected (Fig 3A). The basal ganglia and the thalamus were also atrophic (Fig. 3A). The skin, muscle, and the ovaries were not taken during autopsy.

In order to exclude other neurodegenerative diseases, the brain tissue was stained for α-synuclein, β-amyloid, 3 repeat (3R) tau, 4 repeat (4R) tau, p-Tau (AT8), and p-TDP43; the tissue did not yield any positive finding.

The luxol fast blue (LFB) and the myelin basic protein (MBP) staining revealed a marked loss of myelin sheath in the white matter of the frontal and parietal lobes, though the white matter of the

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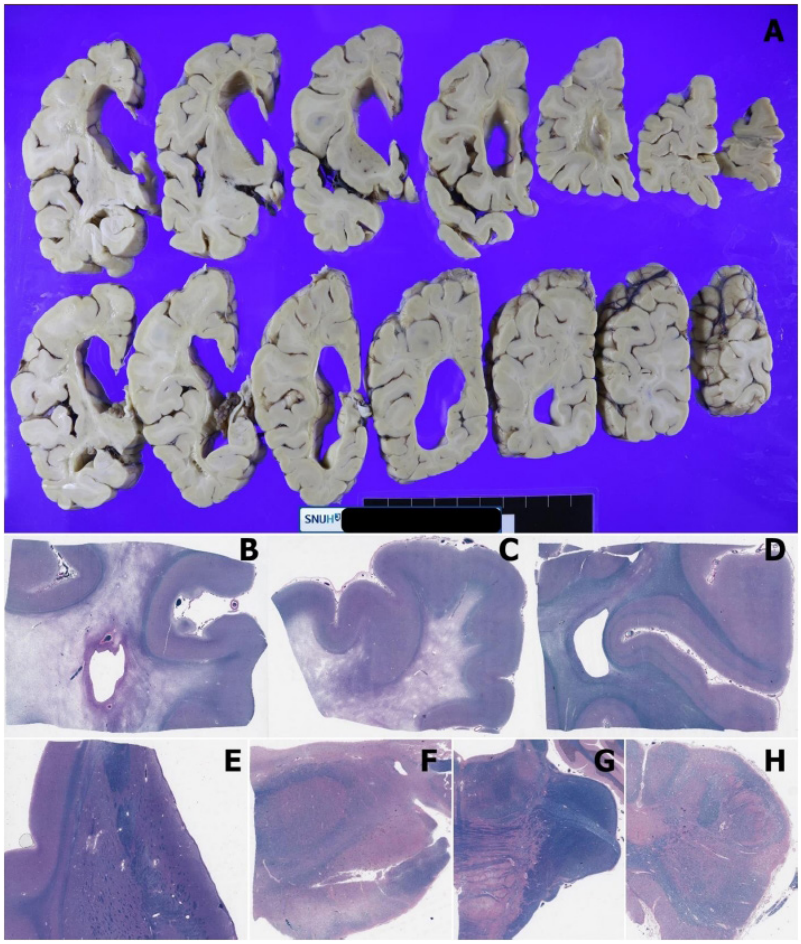

Fig. 3. Gross image from a coronal section of the cerebral hemisphere (A) and a low-power microscopic view of Luxol fast blue (LFB) staining slides from the frontal lobe (B), parietal lobe (C), occipital lobe (D), basal ganglia (E), midbrain (F), pons (G), and the cervical spinal cord (H). (A) White matter with brown-gray discoloration is observed. Corpus callosum is paper-thin and atrophied. Ventriculomegaly is evident. (B~D) LFB staining reveals severe confluent myelin loss in the frontal lobe (B) and the parietal lobe (C). The occipital lobe is relatively preserved. Subcortical U-fibers are mostly preserved. (E) The caudate nucleus, putamen, claustrum and the nigrostriatal pathway are somewhat atrophic. The anterior limb of internal capsule is relatively intact. (F~H) Pyramidal tract fiber shows loss of myelin in the crus cerebri (F), basis pontis (G), and in the anterior and lateral corticospinal tract (H). Pigmented neurons in the substantia nigra are well preserved (F).

occipital lobe was relatively preserved (Fig. 3B~D). The subcortical U-fibers are relatively preserved in the frontal and parietal lobes (Fig. 3B, C). The pyramidal tract appeared to be atrophic and demyelinated from the cerebral white matter to the cervical spinal cord (Fig. 3F~H). Neurofilament (NF) and Bielschowsky silver staining revealed that most axons were lost and the remaining axons were localized in the periphery of the centrum semiovale. Neuroaxonal spheroids (Fig. 4A~C) and reactive gliosis (Fig. 4I) were occasionally observed at these white matter. Neuroaxonal spheroids were also observed in parts of the neocortex including the frontal, parietal, and occipital lobes, and in the cerebellar white matter (Fig.4D and 4J). Therefore, spheroids were found in the cerebral gray and white matter, but they were more commonly seen in the gray matter. A few spheroids were observed in the midbrain and cerebellum, but not in pons, medulla oblongata and spinal cord. The maximal diameter of the axon at each location was 17.5 μm in the cerebral cortex, 31.6 μm in the cerebral white matter, and 40.1 μm in the cerebellar white matter.

Scattered pigmented macrophages were characteristically observed mainly in the periphery of the devastated white matter (Fig. 4E). Occasionally, pigmented astrocyte-like cells were also observed. Positive staining for CD68 was found only in a small number of pigmented cells, but CD163 staining was robustly present in the pigmented cells (Fig. 4F, G). No calcium deposits were found in the brain. Primary antibodies used in this case were summarized in Table 2.

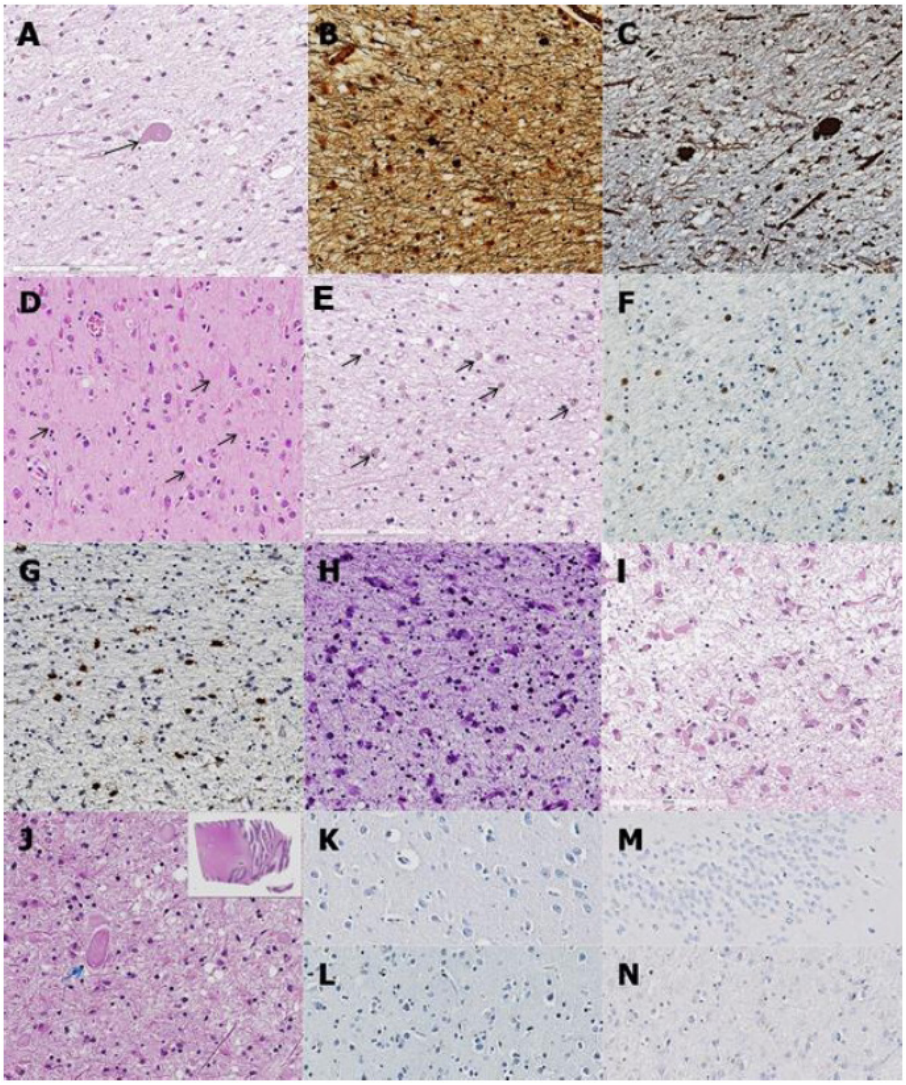

Fig. 4. High-power microscopic view of tissue stained with various stains. (A~C) A spheroid (arrow) is rarely observed in the white matter of the cerebral hemisphere (A). Spheroids are emphasized via Bielschowsky silver staining (B) and neurofilament staining (C); axonal loss can also be observed with these stains. (D) The spheroids (arrows) are occasionally observed in the cortex of the parietal lobe and the density is higher than that of spheroids in the white matter. (E~H) Macrophage shows hyperchromatic nuclei with brown-pigmented cytoplasm and round cellular contour (arrows) (E). CD68 immunohistochemistry shows a mostly negative status in pigmented cells (F). CD163 staining shows pigmented glial component with a ramification pattern. (G) Periodic acid–Schiff (PAS) also stained pigmented cells (H). (I) Reactive gliosis is observed in the white matter of the frontal lobe. (J) The white matter of the cerebellum is also involved and is accompanied by parenchymal vacuolation. The inset is a low-power view of the image in J., and the lesion is located in the subcortical area of the vermis. (K~L) p-Tau (AT8) was negative in both hippocampal formation (K) and disease affected cerebral neocortex (L). (M~N) p-TDP43 was also negative in both hippocampal formation (M) and disease affected cerebral neocortex (N). (A, D, E, I, J: Hematoxylin and eosin, B: Bielschowsky silver, C: Neurofilament, F: CD68, G: CD163, H: Periodic acid–Schiff, K, L: p-Tau (AT8) M, N: p-TDP43).

Genetic study

Genomic DNA was extracted from autopsy brain tissue using the Promega Maxwell® instrument and the PROMEGA DNA extraction kit. Samples were prepared according to the Agilent SureSelect Target Enrichment Kit preparation guide. The libraries were sequenced using the Illumina platform sequencer. The SureSelect Target Enrichment workflow is a solution-based system utilizing ultra-long 120-mer biotinylated cRNA baits to capture regions of interest, and enriching these regions of interest from a NGS genomic fragment library.

A total of 16,237,450,840 bp were read, and the total number of reads was 160,766,840; the GC% was 51.2%, the Q20 was 98.0%, the Q30 was 94.5%, the percentage of on-target reads was 79.1%, and the mean depth of target region was 164.1. Among the Variant Call, only the variants corresponding to the patient's clinical manifestations were listed. Therefore, an annotation process was

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Table 2. Primary antibodies used in this case for diagnosis

Antibodies	Dilution	Company	Findings in this case
GFAP	1:300	DAKO, Glostrup, Denmark	+ in reactive astrocytes
NeuN	1:500	Millipore, Temecula, USA	+ in neurons
Neurofilament (NF)	1:2000	DAKO, Glostrup, Denmark	+ in axons and axonal spheroids
Phosphorylated NF	1:10,000	Millipore, Temecula, USA	+ in axons and axonal spheroids
Synaptophysin	1:100	Novocastra, Newcastle, UK	+ in gray matter
CD163	1:200	ABCAM, Bristol, UK	+ in pigmented microglia
CD68	1:2000	DAKO, Glostrup, Denmark	+ in a few of the pigmented microglia
α-synuclein	1:200	ABCAM, Bristol, UK	Negative in entire brain
β-amyloid	1:500	Covance, Dallas, USA	Negative in entire brain
3 repeat (3R) tau	1:100	Millipore, Ontario, Canada	Negative in entire brain
4 repeat (4R) tau	1:1000	Millipore, Ontario, Canada	Negative in entire brain
p-Tau (AT8)	1:100	ThermoFisher, Waltham, USA	Negative in entire brain
p-TDP43	1:1,000	Cosmobio, Tokyo, Japan	Negative in entire brain

GFAP, Glial fibrillary acidic protein; NeuN, Neuronal nuclei; CD, Cluster of differentiation; p-Tau, phospho-Tau; p-TDP43, phosphorylated TAR DNA binding protein.

Table 3. List of variants found by whole exome sequencing carried out in this patient's autopsy brain tissue

Gene	Mutation	Transcript ID	AA Δ	Consensus	Zygosity	Disease	Inheritance
CSF1R	c.2539G>A	ENST00000515235	E847K	missense	het	Leukoencephalopathy, hereditary diffuse, with spheroids; ALSP	AD

AD, autosomal dominant.

performed on the selected variants. Combined Annotation Dependent Depletion (CADD), dbSNP, gnomAD, SIFT, PolyPhen, and ClinVar were used for performing the annotation. The report obtained information on CADD (Phred-like score) of 15 or more, Novel Variant or Minor Allele Frequency of less than 1%, and VUS (Variant of Uncertain Significance) results; an interpretation of the results was carried out. The variant calling of the genes considered to be related to the patient's symptoms is shown in the Table 3.

Whole-exome sequencing using brain tissue obtained during autopsy revealed a *CSF1R* mutation, which was confirmed by Sanger sequencing (Fig. 5). A heterozygous missense mutation in exon 19 of the *CSF1R* gene (c.2539G>A) was found (Table 3); this mutation, which results in the substitution of glutamic acid with lysine (p.E847K), was previously reported by Di Donato et al. [21].

DISCUSSION

ALSP is a subtype of a rare autosomal dominant, inherited leukoencephalopathy caused by a mutation in *CSF1R* that progressively involves white matter in the adult CNS [2]. Diseases previously diagnosed with HDLS and POLD are known to have the same genetic mutation. Therefore, ALSP is defined to include HDLS and POLD [7]. This entity may manifest as Parkinsonism and cognitive impairment. In present case, clinical manifestations started with gait disturbance and bradykinesia followed by cognitive decline, psychiatric deterioration, and severe motor impairment as summarized in Table 1.

There are eleven subtypes of adult onset leukoencephalopathy defined so far, based on genetic alterations which may be autosomal dominant or recessive or X-linked [20]. The detailed features of the eleven subtypes with their driver genes, and primary cell involvement have been summarized by Ikeuchi et al. as follows: ALSP-*CSF1R*-microglias, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)-*NOTCH3*-small vessels, adult-onset autosomal dominant leukodystrophy (ADLD)-*LMNB1*-oligodendrocytes, Alexander disease-*GFAP*-astrocytes, cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL)-*HTRA1*-small vessels, vanishing white matter (VWM)-*EIF2B family*-astrocytes, metachromatic leukodystrophy (MLD)-*ARSA*-oligodendrocytes, Nasu-Hakola disease-*TREM2/TYROBP*-microglias, *AARS2* mutation-related leukodystropy (AARS2-L)-*AARS2*-mitochondria, fragile X-associated tremor and ataxia syndrome (FXTAS)-*FMR1*-unknown, and adrenoleukodystrophy (ALD)-*ABCD1*-oligodendrocytes [20].

Although the predominant pathology of each subtype has been reported, it may be difficult to accurately identify subtypes unless we identify the genetic variation through DNA sequencing. Each

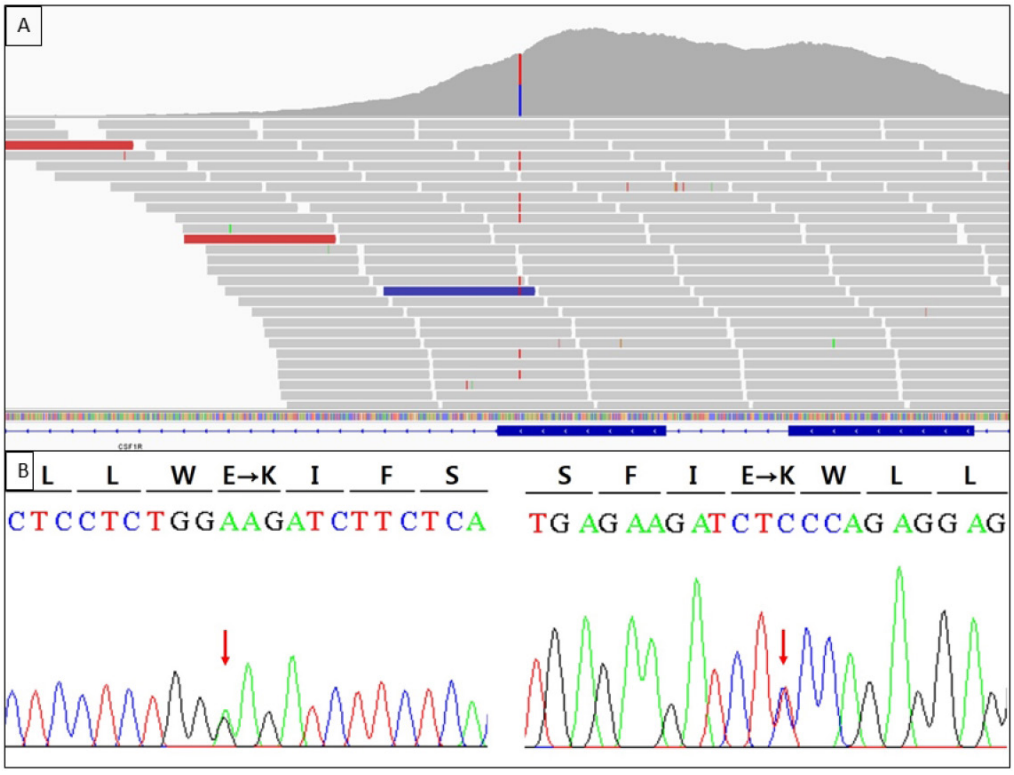

Fig. 5. Heterozygous c.2539G>A missense mutation of the *CSF1R* gene. (A) An integrative genomic viewer (IGV) display of a BAM (binary alignment/map) file in the *CSF1R* gene region. Coverage plot and aligned short reads mapped to the reference sequence are seen. (B) Sequence chromatogram of the *CSF1R* exon 19 in forward (left) and reverse (right) direction. This is a highly conserved region. Mutated nucleotide is indicated by an arrow.

subtype exhibits significant heterogeneity and there is a significant overlap of clinical, radiological and pathological features among the subtypes. For example, ALSP shares pathological features with Nasu-Hakola disease and the primary pathology of these two entities is microglias [22, 23]. ALSP and Nasu-Hakola disease characteristically represent pigmented microglia in addition to leukoencephalopathy and axonal spheroids. Nasu-Hakola disease is an autosomal recessive disorder and show relatively well preserved internal capsule and pontine base (crus cerebri), distinct from ALSP [13]. ALSP also shares clinical and radiological features with AARS2-L [17, 18]. Although some authors suggest that ALSP and AARS2-L are similar in histopathology [19], the histopathology of AARS2-L was not fully described because of the lack of autopsy-proven AARS2-L.

AARS2-L usually presents with childhood- to adulthood-onset neurological deterioration such as ataxia, spasticity, cognitive decline, and frontal lobe dysfunction, which are common findings in ALSP [17]. The previous reports have emphasized premature ovarian failure in female patients, periventricular white matter rarefaction with suppression of the FLAIR signal, and absence of periventricular calcification, an important features of AARS2, distinct from ALSP [18, 19]. However, these findings are not entirely exclusive to AARS2.

The ALSP case we report here included a history of premature ovarian failure. The most common causes of amenorrhea in women after normal secondary sexual characteristics and normal pelvic anatomy are polycystic ovarian syndrome, hyperprolactinemia, primary ovarian insufficiency, and hypothalamic dysfunction [24]. Overt primary ovarian insufficiency is defined as the presence of amenorrhea for more than 4 months with a menopausal serum FSH levels for a woman who is less than 40 years of age [24]. Thus, if women with normal secondary sexual characteristics present with amenorrhea, assessment of serum prolactin, thyroid stimulating hormone (TSH) levels, and FSH levels is helpful for accurate diagnosis. However, these hormone levels and sonographic finding of the ovary at the time of diagnosis of premature ovarian failure could not be obtained from this patient. Therefore we could not concluded the etiology of ovarian failure. However, considering the fact that the patient had received hormone replacement therapy and that there was no history of long-term hospital visits or remedies for other symptoms related to the above listed abnormalities during the following period, the possibility of premature ovarian failure is high.

Ovarioleukodystrophy, which is defined as the co-occurrence of leukodystrophy and premature ovarian failure, is a genetically heterogenous syndrome; to date, VWM and AARS2-L have been linked to premature ovarian failure [25, 26]. Therefore, if leukodystrophy is suspected in the patients with premature ovarian fail-

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ure, genetic testing should cover evaluation of *EIF2B family* and *AARS2*. Our patient showed wildtype *EIF2B family* and *AARS2*.

Although there is a report that POLD can be linked to premature ovarian failure [27], as far as we know, an association between premature ovarian failure and genetically confirmed ALSP has not been previously reported.

Another unique feature of this case is the allodynia, a common feature of the neuropathic pain, which is abnormal perception of pain by non-painful mechanical or thermal stimuli [28]. The allodynia can be generalized or focal. Cord injury induced pain may involve the diffuse body region below the level of injury [29]. Peripheral neuropathy can also induce allodynia in the distal part of the limbs. However, the generalized pattern of this patient's allodynia suggests central origin, which is also supported by normal electromyography (EMG) and nerve conduction study (NCS). Allodynia is usually not a symptom of leukoencephalopathy, however, it is rarely reported in the patients with leukoencephalopathy, including ALSP [30, 31]. Therefore, central origin allodynia is suggested in this patient, but fibromyalgia or somatic allodynia cannot be ruled out.

Leukoencephalopathy can involve any part of the white matter and may manifest with a variety of symptoms. From the viewpoint of clinico-pathologic correlation, a depressive mood since the age of 47 may be related to ALSP. The extrapyramidal symptoms exhibited by the patient may have been related to the pathology of the basal ganglia, because this region is atrophic. Ataxia is can be associated with cerebellar lesions of ALSP, but can also be caused by factors other than cerebellar lesions [7].

Several previous studies have reported on the temporal and spatial sequence of ALSP [13, 32]. Our patient's disease duration was about 12 years, with severe white matter loss extending to the occipital lobe, dominance of CD163-positive over CD68-positive microglial subsets, and involvement of the cerebellum and the spinal cord; these features suggest late-stage disease, as compared to that reported in previous studies (Supplementary Table 1).

A previous study reported that calcifications are known to be present in the white matter in about 3~54% of ALSP cases [9, 33], however our case did not show any calcification.

Over 60 mutation foci of the *CSF1R* gene have been identified in ALSP [1]. The mutations in exon 19 of the *CSF1R* gene (c.2539G>A, p.Glu847Lys) found in our patient have been reported in one previous case of ALSP as a novel mutation [21]. Di Donato et al. [21] reported on an ALSP case in which peripheral neuropathy on electrophysiological examination and parieto-occipital predominant nature were apparent, but these features were not observed in our case.

Here we report a case of CSF1R gene-mutant and autopsy-proven ALSP with a history of premature ovarian failure. Autopsy findings are pathognomonic for this disease, however, due to clinicopathological heterogeneity and overlap in various other diseases including Parkinson's disease and some leukoencephalopathies, genetic study is mandatory for confirmation of diagnosis.

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