INTRODUCTION

Recently adult onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) was proposed as a comprehensive term encompassing hereditary diffuse leukoencephalopathy with...
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 leukencephalopathy 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 leukencephalopathy 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, FMRI, 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 allostynia 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 stimulating hormone (TSH) was mildly elevated to 4.23 μU/ml (normal: 0.1~4.1 μU/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).
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. Vide-oculography 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

---

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

<table>
<thead>
<tr>
<th>Findings from previous report</th>
<th>Our case</th>
</tr>
</thead>
</table>
| **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** | 75% | Present  
Depression → aggressive behavior  
Postural instability, bradykinesia  
(left dominant) → rigidity†  
Hyperreflexia, spasticity |
| **Parkinsonism** | 61% | Present  
Resting tremor, rigidity, bradykinesia, postural instability |
| **Pyramidal signs** | 57% | Present  
Hyperreflexia, spasticity |
| **Seizures** | 32% | Present  
Dysarthria (34%)  
Dysphagia (17%)  
Ataxia (27%)  
Sensory disturbance (10%)  
Peripheral neuropathy (2%) |
| **Other clinical features** | | |
| **MRI findings (prevalence, %)** | | |
| **Bilateral white matter lesions** | 69% | Present |
| **Thining of corpus callosum** | 49% | Present |
| **Calcification in the white matter** | 14% | Absent |

---

<https://doi.org/10.5607/en.2019.28.1.119>
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
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.
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
Adult-onset Leukoencephalopathy 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 Table 3.

Table 2. Primary antibodies used in this case for diagnosis

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

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Transcript ID</th>
<th>AA Δ</th>
<th>Consensus</th>
<th>Zygosity</th>
<th>Disease</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF1R</td>
<td>c.2539G&gt;A</td>
<td>ENST00000515235</td>
<td>E847K</td>
<td>missense</td>
<td>het</td>
<td>Leukoencephalopathy, hereditary diffuse, with spheroids; ALSP</td>
<td>AD</td>
</tr>
</tbody>
</table>

AD, autosomal dominant.

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)-HTRAI-small vessels, vanishing white matter (VWM)-EIF2B family-astrocytes, metachromatic leukodystrophy (MLD)-ARSA-oligodendrocytes, Nasu-Hakola disease-TREM2/TYRBP-microglia, AARS2 mutation-related leukodystrophy (AARS2-L)-AARS2-mitochondria, fragile X-associated tremor and ataxia syndrome (FXTAS)-FMRI-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

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.

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].
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 microglia [22, 23]. ALSP and Nasu-Hakola disease characteristically represent pigmented microglia in addition to leukencephalopathy 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-
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 clinico-pathological heterogeneity and overlap in various other diseases including Parkinson’s disease and some leukoencephalopathies, genetic study is mandatory for confirmation of diagnosis.

ACKNOWLEDGEMENTS

This research was supported by a fund (2018-ER6201-00) by Research of Korea Centers for Disease Control and Prevention.

REFERENCES


8. Marotti JD, Tobias S, Fratkin JD, Powers JM, Rhodes CH

https://doi.org/10.5607/en.2019.28.1.119


26. Ibitoye RT, Renowden SA, Faulkner HJ, Scolding NJ, Rice...


