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Progression of Cortical Dysfunction in CSF1R-related Leukoencephalopathy Detected Using Single Photon Emission Computed Tomography

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Short title
Cortical Progression in HDLS
Abstract

**Background:** Hereditary diffuse leukoencephalopathy with spheroids (HDLS) is a rare autosomal dominant disease progressively affecting cognitive and motor functions, most often caused by mutations in the colony-stimulating factor 1 receptor gene (CSF1R).

**Aim:** To elucidate the mechanism of disease progression, changes in white matter lesions and cortical cerebral blood flow (CBF) were evaluated in cases during various stages of the disease.

**Methods:** All patients were diagnosed with HDLS by confirming mutations in CSF1R. Regional CBF was evaluated using single photon emission computed tomography and was analyzed semiquantitatively.

**Results:** Three cases (2 males and 1 female, ages 51, 53 and 48 years old on admission, disease duration from 1 to 8 years) were registered. All cases exhibited different CSF1R mutations and progressive frontal dysfunction. Scores of the Frontal Assessment Battery and time in the Trail Making Test worsened as the disease progressed, whereas the Mini-Mental State Examination score remained relatively stable. MRI revealed progressive white matter lesions in the frontal lobe and atrophy of the anterior body of the corpus callosum. Regional CBF was low in the medial frontal cortex in the early case, and the area of hypoperfusion spread to the lateral frontal cortex and parietal cortex as the disease progressed. CBF was maintained in the basal ganglia, thalamus and occipital lobes.

**Conclusions:** Hypoperfusion was initially observed in the medial frontal lobe and spread to the lateral frontal lobe and parietal lobe with disease progression. Spreading of accumulated abnormal proteins induced by mutation in CSF1R may be involved as a molecular mechanism of disease progression.

**Keywords**

Hereditary diffuse leukoencephalopathy with spheroids (HDLS), cerebral blood flow, medial frontal lobe, CSF1R, white matter lesion
**Introduction**

Hereditary diffuse leukoencephalopathy with spheroids (HDLS) is a rare autosomal dominant disease progressively affecting both cognitive and motor functions\(^1\,2\). Since most cases are caused by mutations in the protein tyrosine kinase domain of the *colony-stimulating factor 1 receptor (CSF1R)* gene on chromosome 5q32\(^3\), the disease was recently renamed CSF1R-related leukoencephalopathy\(^4\). CSF1R is an essential factor for the development and maintenance of microglia in the brain, and aberrant microglia, either through toxic gain of function or loss of protective function, may trigger axonopathy with spheroid formation\(^4.5\).

Pathologically, the spheroids in HDLS contain abnormal proteins, such as phosphorylated neurofilament and amyloid precursor protein\(^3.4\). The accumulation and spread of abnormal proteins, including amyloid β, tau and α-synuclein, is currently considered a cardinal mechanism involved in the development of neurodegenerative diseases, such as Alzheimer’s disease and Parkinson’s disease\(^6.7\). A similar spreading mechanism may be involved in HDLS.

A few case reports have suggested that lesions initially develop in the localized white matter and spread to periventricular and subcortical regions, finally becoming confluent and generalized\(^8.9\). However, the detailed longitudinal development of the disease is not known. To elucidate disease progression, cerebral blood flow (CBF) was evaluated in cases
during various stages of CSF1R-related leukoencephalopathy.

**Materials and Methods**

**Ethical approval.** All procedures performed in studies involving human participants were approved by the Institutional Research Ethics Committee of Osaka City University Graduate School of Medicine (IRB# 3009) and were performed in accordance with the 1964 Declaration of Helsinki and its later amendments.

**Informed consent.** Written informed consent was obtained from all participants or from close family members when the participants were cognitively impaired.

**HDLS cases.** From 2010 to 2020, all patients who were referred to the Osaka City Cognitive Disorder Center were cognitively evaluated and underwent magnetic resonance imaging (MRI) if they had no contraindication. Young age at onset, family history, frontal lobe dysfunction and rapid progression were regarded as high-risk factors for HDLS\(^3,4\). On MRI, white matter changes predominantly affecting the frontal lobes and the corpus callosum with atrophy may strongly suggest HDLS\(^9\). After exclusion of other diagnoses, all patients suggestive of HDLS were evaluated for mutations in the *colony-stimulating factor 1 receptor*
gene (CSF1R).

**Cognitive function tests.** The Mini-Mental State Examination (MMSE)\(^{10}\) and the revised Hasegawa Dementia Scale (HDS-R)\(^{11}\) were administered to assess general cognitive function, whereas the Frontal Assessment Battery (FAB)\(^{12}\) and Trail Making Test (TMT)\(^{13}\) were administered by a qualified clinical psychologist (MA) to evaluate frontal function.

**MRI acquisition.** Magnetic resonance images were obtained using a 1.5 Tesla magnetic resonance scanner (MAGNETOM Avanto, Siemens Healthcare, Erlangen, Germany or Ingenia, Philips Healthcare, Best, The Netherlands). Lesions in the white matter and the corpus callosum and cortical atrophy were evaluated using FLAIR-, T2- and T1-weighted images. The ratio of the area of white matter lesions to the brain was calculated in coronal sections of FLAIR images at the level of the anterior horn of the lateral ventricle. ImageJ software (the National Institutes of Health, Bethesda, Maryland, USA, version 1.52a) was used to measure dichotomized areas. Similarly, the ratio of the area of the anterior body of the corpus callosum to the whole brain was calculated in the midline sagittal section of the FLAIR image. The anterior region of the whole corpus callosum was defined as the anterior half based on the horizontal length, and the area above the horizontal line that crossed the anterior peak of the corpus callosum was measured as the anterior body.
SPECT data acquisition. CBF was evaluated using single photon emission computed tomography (SPECT) with $^{99m}$Tc-ethyl cysteinate dimer ($^{99m}$Tc-ECD) as a tracer for cerebral blood flow (CBF). After an injection of 600 MBq $^{99m}$Tc-ECD, images were acquired with a GCA9300A gamma camera (Toshiba, Tokyo, Japan) for 6 min with 95 rotations. Images were reconstructed using a ramp filter for the filtered back projection. Attenuation was corrected using Chang’s method. Scatter correction was not applied. For the 3D-stereotaxic regions of interest template analysis of $^{99m}$Tc-ECD, eZIS software (FUJIFILM RI Pharma, Tokyo, Japan) was used.

Results

Demographic data. Table 1 shows the demographic data. Three cases (2 males and 1 female, mean age 50.7 years old, disease duration 1 to 8 years, consanguinely unrelated) were registered. All three cases presented different types of CSF1R mutations. Cases 1 and 2 had a family history of young-onset dementia.

Neurocognitive function and tests: Initial symptoms included attention deficit and speechlessness, suggestive of frontal dysfunction (Table 1). A lack of empathy and severe
apathy developed in the advanced cases (cases 2 and 3). HDS-R and MMSE scores showed similarly mild low scores in all cases. FAB scores were lower in the advanced cases, and the time of TMT was beyond the cutoff level in these cases.

**Other lab tests:** Total tau level in the cerebrospinal fluid of case 1 was 363 pg/ml, which was significantly higher than the cutoff level of 300 pg/ml for subjects between 21–50 years old\(^4\). Corresponding data were not available for the other two cases.

**MRI.** Figure 1 shows brain MRI of case 1 at 12 months after onset, case 2 at 15 months and case 3 at 96 months. Coronal FLAIR images at the level of the anterior horn of the lateral ventricle show that white matter lesions were localized mostly in the corpus callosum and spread into the deep white matter of the bilateral frontal lobes as the disease progressed. Moreover, white matter lesions spread from deep to subcortical, and axial T2-weighted images at the level of the body of the lateral ventricle showed that high-intensity lesions were first localized in the genu of the corpus callosum and the frontal lobes but later spread into the parietal lobes. Sagittal T2-weighted median sections show dotted high-intensity lesions and progressive atrophy of the anterior half of the body of the corpus callosum. Extension of the lesion from the anterior to the posterior part of the corpus callosum was not observed.
The area of high-intensity white matter lesion in a coronal FLAIR image at the level of the anterior horn of the lateral ventricle was measured, and the ratio to the brain cross section was calculated. As shown in Figure 2, white matter lesions increased with disease duration; i.e., 12 months in case 1, 15 months in case 2 and 96 months in case 3. In contrast, the area of the anterior half of the body of the corpus callosum decreased with disease duration, revealing progressive atrophy of the anterior corpus callosum (Figure 2).

**SPECT.** CBF data obtained using SPECT were visually analyzed (Figure 3). Case 1 presented decreased CBF in the medial frontal lobes 12 months after onset. An additional decrease in CBF was found in the lateral frontal lobe on the right at 15 months in case 2. The largest area in the bilateral frontal lobes showed decreased CBF at 96 months in case 3.

The decrease in cerebral blood flow in each case was statistically compared to the already established control group, and a z-score map of the cerebral cortex was reconstructed using cortical three-dimensional projection with eZIS mapping software (Figure 4). Case 1 at 12 months after onset showed a localized area of hypoperfusion in the medial frontal lobes. Additionally, case 2 at 15 months showed a larger area of hypoperfusion extending from the medial to the right lateral frontal lobe. Finally, case 3 at 96 months showed that the largest area in the medial frontal lobes and bilateral lateral frontal lobes had
decreased CBF. Furthermore, parietal lobes were also involved in cases 2 and 3.

**Discussion**

Clinically, HDLS is known to induce frontal dysfunction, such as apathy, depression and speechlessness\(^1\). The present study is the first to demonstrate that in addition to white matter lesions in the frontal cortex, cortical dysfunction starts at the medial frontal lobe, spreads laterally in the frontal lobe and finally reaches the parietal lobe, while the clinical symptoms of frontal dysfunction progress correspondingly.

In the present cases, initial symptoms included attention deficit, speechlessness and disorientation. In the early case (case 1), apathy was not observed, and specific tests for the frontal lobe were relatively normal. In this phase, CBF was low only in the limited area of the medial frontal lobe. As the disease progressed, frontal dysfunction became prominent and worsened (cases 2 and 3). The area of hypoperfusion in the medial frontal lobe became larger and spread laterally. HDLS is clinically characterized by 2 major components: neuropsychiatric and motor symptoms\(^15\). The former includes depression, apathy, anxiety and nonfluent aphasia, resembling behavioral variant frontotemporal dementia. The latter represents parkinsonism, pyramidal signs, bulbar signs and ataxia\(^4\). In our case series, motor symptoms were not prominent at the time of initial neuroimaging. This may explain why
cortical symptoms corresponded well with the area of hypoperfusion in our study. Similar case with dominant neuropsychiatric symptoms and low cerebral blood flow in the frontal lobe assessed with SPECT was previously reported although the progression of the lesion was first reported in the present study\textsuperscript{16}.

Progressive atrophy of the corpus callosum and enlargement of white matter lesions were also observed with disease progression in the present study. White matter lesions were localized in the corpus callosum in the early case, extended into the frontal white matter as a continuous lesion and finally reached just below the lateral frontal cortex, sparing U-fibers. The extension of white matter lesions from medial to lateral in the frontal lobe and from the frontal to parietal lobe in MRI may correspond well with the progression of the hypoperfused area on SPECT. Although the distribution of white matter lesions in MRI is well documented\textsuperscript{4,9}, the present study is the first to demonstrate that extension of white matter lesions corresponds well with cortical dysfunction in the frontal lobe.

A key pathological finding is widespread axonal spheroids in the white matter with a loss of myelin\textsuperscript{4}. Cortical neurons are relatively spared, whereas ballooned neurons are frequently observed in the overlying cortex\textsuperscript{2,4}. The spatial correlation of white matter extension and distribution of low CBF found in the present study may reflect this pathological characteristic.
Spheroids can be immunostained with phosphorylated neurofilament, amyloid precursor protein and ubiquitin. Recently, aberrant proteins, including amyloid β, tau and α-synuclein, were found to accumulate and spread as a mechanism for the disease progression of Alzheimer’s disease, Parkinson’s disease and other neurodegenerative diseases. The continual expansion of white matter lesions in HDLS shown in the present study may suggest that aberrant proteins in the spheroid may spread and accumulate repeatedly as a mechanism to enlarge neurodegenerative lesions. CSF1R is an essential factor for the development and maintenance of microglia. Mutation in the CSF1R gene is reported to impair microglial activity in the brain, reducing the ability to scavenge abnormal proteins. Once insidiously accumulated aberrant proteins in the white matter exceed the threshold, the vicious cycle of spread and accumulation of the proteins may start and progressively accelerate, rapidly inducing the development of disease. There are several candidate proteins involved in the spread of the lesion. In an autopsy-confirmed cohort, Reimand et al. reported a case of HDLS in a 49-year-old patient who was positive for amyloid β on positron emission tomography and presented elevated amyloid β_{42} in cerebrospinal fluid. In this case, the neuropathological status for Alzheimer’s disease was A1B1C0, suggesting no confounding effect of Alzheimer’s disease. Similar to a case in the present study, Spitzer et al. also reported a 35-year-old woman with HDLS whose tau levels in the cerebrospinal fluid
were moderately elevated\textsuperscript{22}. We previously reported that tau accumulation spreads with disease progression in a large series of Alzheimer’s disease\textsuperscript{6}. Further studies are warranted to confirm the current hypothesis.

Limitation of the present study include the small number of cases involved. Moreover, the phenotype of the cases is limited mostly to neuropsychiatric symptoms. Apparent expansion of the lesion with disease progression may be enhanced by this limitation. Furthermore, no longitudinal studies on the same case were performed. Larger case numbers with various types of HDLS phenotypes may further confirm the pathomechanism of this disease.

**Acknowledgments:** The authors thank Ms. M. Ando (Osaka City University) for psychological tests.

**Conflict of Interest:** The authors declare no conflicts of interest for this article.

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† Father developed dementia and died at the age of 48.
‡ Mother developed dementia and died at 47.
§ Beyond the cutoff level
Figure legends

Figure 1
Brain MRI of case 1 at 12 months after onset (a), case 2 at 15 months (b) and case 3 at 96 months (c). R: right, L: left. Coronal FLAIR images at the level of the anterior horn of the lateral ventricle (top row) show that white matter lesions were first found in the corpus callosum (a) and then spread into the deep white matter of the bilateral frontal lobes from deep (b) to subcortical (c). In addition, axial T2-weighted images at the level of the body of the lateral ventricle (middle row) show that high-intensity lesions were first localized in the genu of the corpus callosum (a) and frontal lobes (b) but later spread into the white matter of parietal lobes (c). Sagittal T2-weighted median sections (bottom row) showed expanding high-intensity lesions and progressive atrophy of the anterior half of the body of the corpus callosum. Extension of the lesion from the anterior to the posterior part of the corpus callosum was not observed.

Figure 2
(a) The area ratio of white matter lesion to the brain cross section, calculated using
a coronal FLAIR image at the level of anterior horn of the lateral ventricle, which increased with disease duration; i.e., 12 months in case 1, 15 months in case 2 and 96 months in case 3. (b) The area ratio of the anterior half of the body of the corpus callosum to the brain cross section, calculated using sagittal T2-weighted median sections, decreased with disease duration.

Figure 3

Single-photon emission computed tomography (SPECT) with $^{99m}$Tc-ethyl cysteinate dimer ($^{99m}$Tc-ECD) was used to evaluate cerebral blood flow (CBF). (a) Case 1 presented decreased CBF in the medial frontal lobes 12 months after onset. (b) An additional decrease in CBF was found in the lateral frontal lobe on the right at 15 months in case 2. (c) The largest area in the bilateral frontal lobes showed decreased CBF at 96 months in case 3. Arrow heads indicate regions of reduced CBF. R: right, L: left.

Figure 4

A decrease in cerebral blood flow was shown as the z-score by eZIS, a mapping software. (a) Case 1 at 12 months after onset showed a localized area of
hypoperfusion at the medial frontal lobes. (b) Case 2 at 15 months showed a larger area of hypoperfusion extending from the medial to the right lateral frontal lobe. (c) Case 3 at 96 months showed that the largest area in the medial frontal lobes and bilateral lateral frontal lobes had decreased CBF. Additionally, a decrease was found in the parietal lobes in cases 2 and 3.
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