Hum Mol Genet. 2011)アストロサイトが分泌する因子の制御異常が強く疑われる。

E.結論

CARASIL 原因遺伝子; HtrA1 の機能喪失は、CARASIL 類似の病態を in vivo で誘発することが結論付けられた。また、CARASIL の病態背景に強く関係していることが疑われているTGFβファミリーシグナルの異常が、同様にCARASIL 病態を再現することが示された

F.健康危険情報

なし

G.研究発表

4. 論文発表

なし

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H.知的財産権の出願・登録状況（予定を含む）

1.特許取得

2.実用新案登録

3.その他

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）

遺伝性脳小血管病およびその類縁疾患の診断基準の確立と治療法の研究班
（分担）研究報告書

軸索腫大を伴う遺伝性びまん性白質脳症（HDLS）の拡散強調像の多様性と末梢血単球由来マクロファージを用いた細胞生物学的検討

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研究要旨（10ポイント程度）

興味深いMRI拡散強調像（DWI）所見を呈したHDLS自験例を報告した。うち2例は運動障害で初発し、一次進行型多発性硬化症（PPMS）が疑われた若年発症例であり、いずれも前頭葉～頭頂葉優位に広範なDWI高信号病変を示した。また、臨床的にPPMSが疑われた弧発例23例に対して、*CSF1R*検査を施行したところ3例で*CSF1R*変異を認めた。HDLSのDWI/ADC map所見は多様であり、その多様性は脳内病変の病理過程や病態の違いを反映している可能性があると考えられた。HDLS患者の末梢血単球由来マクロファージを用いた検討では、CSF1Rリガンド刺激で形態的に成熟したマクロファージが得られず、健常対照者に比較して生存率も低かった。このことからHDLS患者末梢血単球ではマクロファージへの分化・成熟、および生存に障害があることが示唆された。

A.研究目的

軸索腫大を伴う遺伝性びまん性白質脳症（HDLS）の診断において脳MR画像所見の評価は不可欠であるが、拡散強調像（DWI）の意義付けについては不明な点が多い。興味深いDWI/ADC map所見を呈した自験例について考察する。

HDLS患者における既報のコロニー刺激因子1受容体遺伝子（*CSF1R*）変異は細胞内チロシンキナーゼ部位に集中していることから、本症の白質変性には同部位のリン酸化阻害による細胞内シグナル伝達異常が深く関与していることが想定される。また、CSF1Rは主にマクロファージ・ミクログリアなどの単球系細胞に発現していることから、結果的に上記のシグナル伝達異常はこれらの細胞の機能障害をもたらすことが推察される。HDLS患者のマクロファージ・ミクログリアの機能障害を明らかにすることを目的に末梢血単球由来マクロファージを用いた細胞生物学的検討を

行った。

B.研究方法

当科ではこれまでに白質異常症46名の*CSF1R*検査の依頼を受け、うち10名で*CSF1R*のエクソン12-22に変異を見出した。これらの患者の中で特徴的なDWI/ADC map所見を呈した3例の脳画像を、文献的考察を加えて提示する。

遺伝学的にHDLSと診断した2例（58歳女性、44歳男性）および健常対照者（54歳女性、40歳男性）の末梢血から採取した単球（CD14陽性末梢血単核球）を用い、これらをCSF1Rのリガンドであるcsf-1およびIL-34、またCSF2Rのリガンドであるcsf-2の存在下で培養し、細胞の形態、および免疫組織化学法による蛋白発現の検討を行った。（倫理面への配慮）HDLSの遺伝学的検査、および末梢血単球由来マクロファージを用いた検討に関しては、それぞれ学内の倫理委員会の承認を得た。

C.研究結果

一次進行型多発性硬化症（PPMS）が疑われた22歳女性例では頭頂葉を中心に広範なDWI高信号、かつADC低信号を呈する病変が見られた。同様に運動障害で初発し、前頭葉を中心に広範なDWI高信号病変が見られた38歳女性例では同部はADC等信号を呈した。この2例（いずれも*CSF1R*変異あり）を含めて、臨床的にPPMSが疑われた弧発性の白質異常症23例を検討したところ、さらに1名に*CSF1R*変異を確認した。また、*CSF1R*のエクソン12-22に変異が見られなかった65歳女性例では側脳室前角近傍の深部白質から前頭葉皮質下に拡がるDWI high signal rimが見られた。

健常者2例から採取した単球を用いた培養では、csf-1、IL-34、およびcsf-2の存在下いずれにおいても正常形態の培養マクロファージを得た。一方、HDLS患者2例から採取した単球を用いた培養では、健常者と比較し、培養日数を経るごとに細胞密度は顕著に減少した。また、csf-1、IL-34の存在下でのHDLS患者由来単球の培養では、spindle type、round typeのマクロファージの出現は見られず、小突起を有する非常に小型の細胞のみを得た。また、これらの細胞は健常者の細胞と比較し、CSF1R、マクロファージの表面抗原として知られるCD163、CD203の免疫染色性が低下していた。csf-2の存在下でのHDLS患者由来単球の培養では、健常者と比べて数は少ないながらもspindle type、round typeの培養マクロファージを得た。またCSF1R、CD163、CD203の免疫染色性も明瞭に認められたが、健常者と比較すると全体に低下していた。

D.考察

HDLS弧発例では、臨床的にも脳画像的にもPPMSとの鑑別が問題になるが、PPMS様の臨床像を呈するHDLS患者ではしばしば頭頂葉を中心に真の拡散障害を示す病変が広範に見られる。この所見はPPMSとの鑑別上、重要である。また、DWI high signal rimはオリジナルのスウェーデン家系の患者でも報告されているが、ともに*CSF1R*変異は確認されていない。HDLS患者の中にはDWI高信号病変を呈さない患者もあり、DWI/ADC map所見の差異が何かしらの脳内病変の病理過程や病態の違いを反映している可能性がある。

HDLS 患者の末梢血単球由来マクロファージでは、健常者に比べて CSF1R リガンド刺激による生存率の低下、形態変化が明らかであった。このことは HDLS 患者末梢血単球では CSF1R 機能異常によりマクロファージへの分化・成熟、および生存に障害を来していることを示唆するものと考えた。

E.結論

HDLS において MR 画像の DWI/ADC map は脳内病変の病理過程や病態を考察する上ではきわめて興味深い。また、HDLS では末梢血単球由来マクロファージにも明らかな機能異常が存在することが示唆されたが、このことが脳におけるマクロファージ・ミクログリアの異常といかに関連するのか、が今後の課題である。

F.健康危険情報

特記すべきことなし。

G.研究発表

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H.知的財産権の出願・登録状況（予定を含む）

1.特許取得　なし

2.実用新案登録　なし

3.その他　なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
遺伝性脳小血管病およびその類縁疾患の診断基準の確立と治療法の研究班
（分担）研究報告書

スフェロイド形成を伴うびまん性白質脳症（HDLS）の診断基準案の策定

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研究要旨

スフェロイド形成を伴う遺伝性びまん性白質脳症（HDLS）は、認知障害を中核症状とする成人発症の遺伝性白質脳症である。確定診断を行うには脳組織を用いた病理診断が従来まで必要であったため、HDLSの診断例は少なく、そのため疾患概念は十分には確立されていなかった。2012年にHDLSの原因遺伝子としてCSF-1Rが同定されて以来、遺伝子解析による確定診断が生前に可能になり、HDLSの確定診断例が本邦を中心に増えている。本研究は、遺伝子解析により確定診断されたHDLS症例について、自験例および既報例をレビューすることにより、HDLSの診断基準案の策定を行った。CSF-1R遺伝子変異が陽性例を、本診断基準案に適応すると、probable HDLSと診断される感度は90%、特異度は75%となった。本診断基準は成人発症の白質脳症の鑑別診断に有用である可能性がある。

A.研究目的

スフェロイド形成を伴う遺伝性びまん性白質脳症（HDLS）は、認知障害を中核症状とする成人発症の遺伝性白質脳症である。本研究は、遺伝子解析により確定診断されたHDLS症例について、自験例および既報例をレビューすることにより、HDLSの疾患概念の確立を目指し、診断基準草案を作成することを目的とする。

B.研究方法

遺伝子解析によりCSF-1R遺伝子に変異が同定された自験例10家系10症例、既報例73症例54家系について、発症年齢、経過年数、初発症状、および経過中に認める臨床症状を明らかにした。

(倫理面への配慮)

当施設における遺伝子解析は、遺伝子倫理委員会の承認を受け行った。

C.研究結果

既報例54家系中、日本からの報告は15家系（22%）と多い。平均発症年齢は45±12歳（18～78歳）。死亡時年齢は54±11歳。死亡時までの罹患期間は8±6年。遺伝形式は77%が常染色体優性遺伝形式を呈し、23%は

孤発例であった。臨床症状としては、認知障害（97%）、性格変化や行動異常（97%）、パーキンソン徴候（67%）、錐体路徴候（28%）、うつ状態（51%）、けいれん（41%）であった。CSF-1R 遺伝子変異・陽性例を本診断基準案に適応すると、probable HDLS と診断される感度は 90%、特異度は 75%であった。

D.考察

自験例および文献的な報告例をレビューすることにより HDLS の臨床的な特徴を抽出した。本診断基準案を CSF-1R 遺伝子変異陽性例に適応されると、高い診断感度が得られた。

E.結論

HDLS に特徴的な臨床的および画像所見を抽出し、その知見を基に HDLS の診断基準案を作成した。

F.健康危険情報

特記すべきことなし

G.研究発表

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H.知的財産権の出願・登録状況（予定を含む）

1.特許取得

2.実用新案登録

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Increasing Microbleeds in CADASIL

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Following left thalamic hemorrhage and several ischemic episodes, a 45-year-old male with mild hypertension and diabetes mellitus was diagnosed with CADASIL by genetic testing (fig. 1a). Despite less-characteristic evidence of CADASIL in the external capsules and the poles of the temporal lobes (fig. 1b), cerebral microbleeds increased yearly on T2* MRI (fig. 1c), indicating the pathogenesis of microbleeds in CADASIL may be independent of white matter hyperintensity lesions [1] and cerebrovascular risk factors may modulate the progression of microbleeds in CADASIL [2].

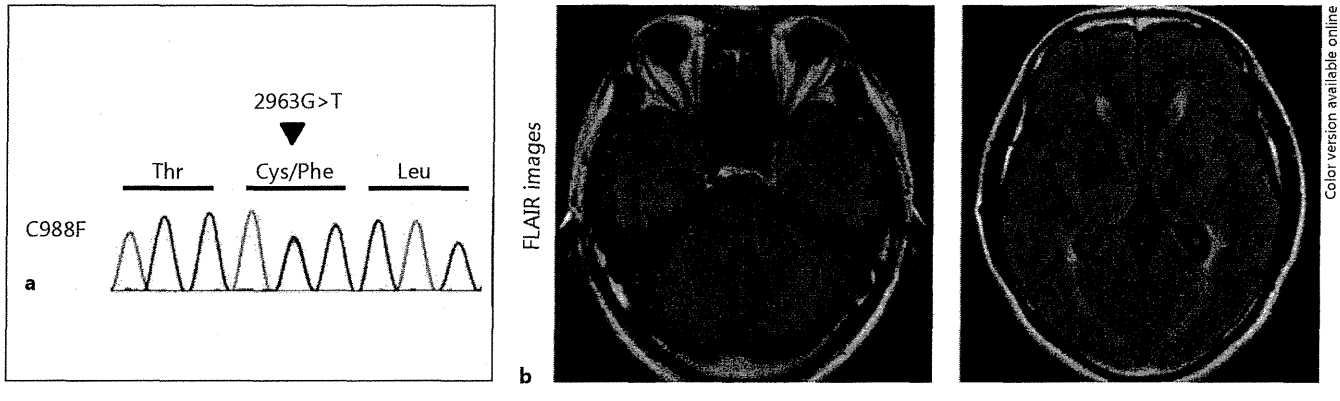

Fig. 1. a Genetic testing revealed p.Cys988Phe (C.2963G>T) mutation in exon 18 of the patient's *NOTCH3* gene. **b** FLAIR imaging at 51 years of age showed the anterior temporal poles and the external capsules appeared relatively sparse in this case as compared with typical findings of CADASIL.

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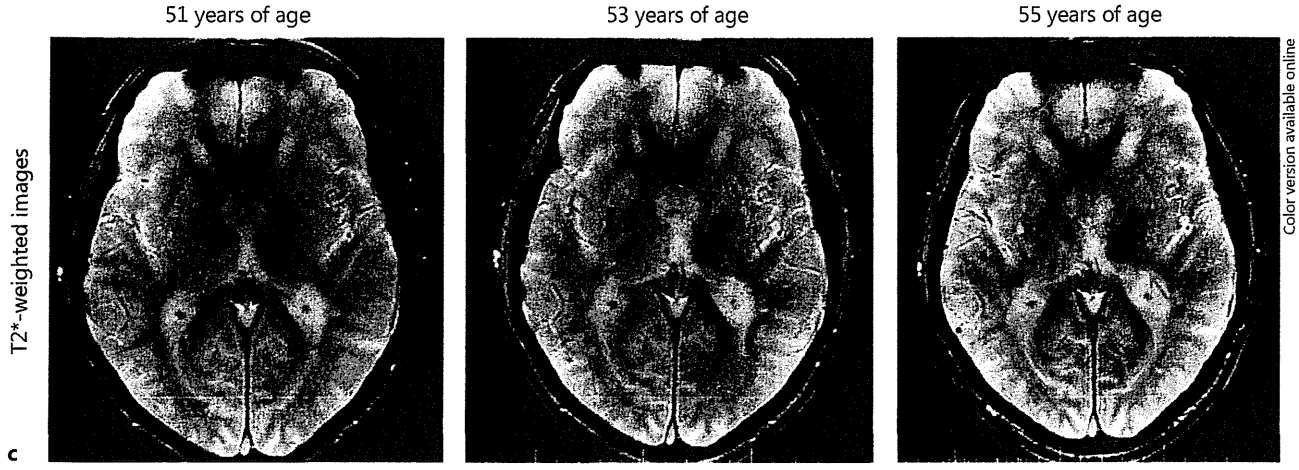

Fig. 1. c T2*-weighted imaging revealed prominent cerebral microbleeds that increased in number from year to year. Arrows point to the new microbleed lesions.

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INTERNAL MEDICINE

□ CASE REPORT □

Early Involvement of the Corpus Callosum in a Patient with Hereditary Diffuse Leukoencephalopathy with Spheroids Carrying the *de novo* K793T Mutation of *CSF1R*

Yasufumi Kondo[1], Michiaki Kinoshita[1], Kazuhiro Fukushima[1], Kunihiro Yoshida[2] and Shu-ichi Ikeda[1]

Abstract

We herein report the case of a 41-year-old Japanese man with hereditary diffuse leukoencephalopathy with spheroids (HDLS) who carried the *de novo* K793T mutation in the colony-stimulating factor 1 receptor gene (*CSF1R*). He showed a gradual decline of his cognitive and mental functions over the following six months. On brain MRI, a thin corpus callosum with T2- and FLAIR-high signal intensity in the splenium was conspicuous, whereas cerebral deep and periventricular white matter lesions were mild. We propose that a diagnosis of HDLS should be considered in patients with presenile dementia presenting with corpus callosum lesions on MRI, even in cases with a lack of any apparent family history.

Key words: HDLS, *CSF1R*, *de novo* mutation, corpus callosum

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Introduction

Hereditary diffuse leukoencephalopathy with spheroids (HDLS) is an autosomal-dominant white matter disease caused by mutations in the colony-stimulating factor 1 receptor gene (*CSF1R*) (1). It is characterized clinically by adult-onset behavioral, cognitive and motor dysfunction and pathologically by a widespread loss of axons and myelin sheaths and the appearance of axonal spheroids and pigmented macrophages (2-4). Several sporadic patients who fulfill the clinical and pathological criteria for HDLS have been reported (5-8). However, it remains unknown whether these sporadic cases reflect phenotypic copies, reduced disease penetrance or are caused by *de novo* mutations in the genes responsible for the HDLS phenotype (4).

We herein describe the case of an HDLS patient who carried a novel *de novo* K793T mutation in *CSF1R*. This is the second family, next to the Norwegian family described by Rademakers et al. (1), in which both parents have been shown to not carry the causative *CSF1R* mutation. The cases of these families clearly suggest that a subset of sporadic cases of HDLS are caused by *de novo CSF1R* mutations. Furthermore, we propose that involvement of the corpus callosum on MRI may be an early diagnostic clue indicating a diagnosis of HDLS.

Case Report

A 41-year-old Japanese man had been well until 40 years of age when he began to show a decline in his cognitive function. He was first noticed to have difficulty performing occupational tasks and handling a cell phone in October 2011. In January 2012, he twice developed confusion and unresponsiveness while during drinking alcohol. His wife encouraged him to visit the hospital in February 2012, where he was suspected of having presenile dementia. Thereafter, he became unable to use a personal computer or drive a car and had to quit working. His wife noted that his speech and gait had become awkward. His previous medical history was unremarkable. There was no particular family history of psychiatric or neurological diseases. His parents

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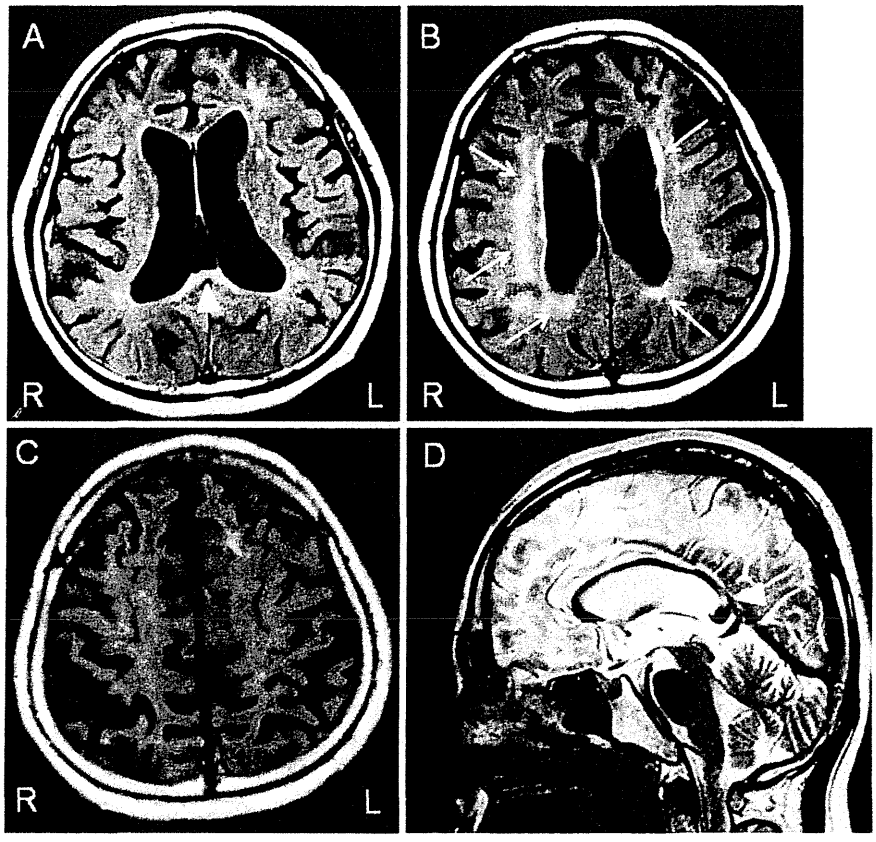

Figure 1. Brain MRI images. Brain MRI images of the patient (at 41 years of age) are shown. Dilatation of the lateral ventricles and hyperintense foci are observed in the cerebral deep and periventricular white matter on FLAIR-weighted images (arrow, A-C). The corpus callosum is atrophic and shows high signal intensity in the splenium (arrowhead) on FLAIR-weighted (A) and T2-weighted images (D).

and two sisters were healthy.

On admission in April 2012, the patient was found to be normotensive and the general findings were not particular. He was cooperative, although he did not appear to have any awareness of being ill. A neurological examination showed a slow initiation of speech, dysarthria, slight left hemiplegia and an unstable gait. The patient's deep tendon reflexes were slightly exaggerated; however, his plantar responses were flexor. No rigidity or involuntary movements were observed. The Mini-Mental State Examination score was 23 points and the Frontal Assessment Battery (FAB) score was 10 points. These tests demonstrated poor attention, calculation and comprehension and a disturbance in verbal fluency; however, the patient's short-term memory was relatively well preserved.

The routine laboratory findings were unremarkable. Tests with negative or normal results were as follows: vitamin B1, vitamin B12, anti-nuclear antibody, syphilis, HIV, soluble interleukin 2 receptor and angiotensin converting enzyme. A cerebrospinal fluid examination showed a slight increase in the levels of total protein (51 mg/dL) and tau protein (290 pg/mL, normal value: 0-200 pg/mL). Brain MRI disclosed frontoparietal lobe atrophy and ventricular dilatation with high-intensity lesions in the cerebral deep white matter (Fig. 1). Hyperintense foci were more evident in the right hemisphere than in the left. The corpus callosum was atrophic and showed high signal intensity in the splenium (Fig. 1). No microbleeds were detected in the white matter or basal ganglia on T2*-weighted images (data not shown). There was no atrophy or signal changes in the cerebellum or brainstem. Cerebral angiography showed no findings of atherosclerosis. The findings of a nerve conduction study were normal.

The clinical and neuroradiological findings reminded us of the possibility of frontotemporal dementia, leukoencephalopathy or leukodystrophy of several genetic causes, vascular dementia (VaD) (including Binswanger disease), cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), multiple sclerosis (MS) or progressive multifocal leukoencephalopathy (PML). Immediately before this patient was referred to us, we experienced another patient with leukoencephalopathy who exhibited presenile dementia and a thin corpus callosum on MRI, similar to our patient, and was diagnosed as having HDLS based on a brain biopsy and a *CSF1R* analysis (9). Therefore, we first searched for the *CSF1R* mutation after obtaining informed consent from the patient's wife and parents.

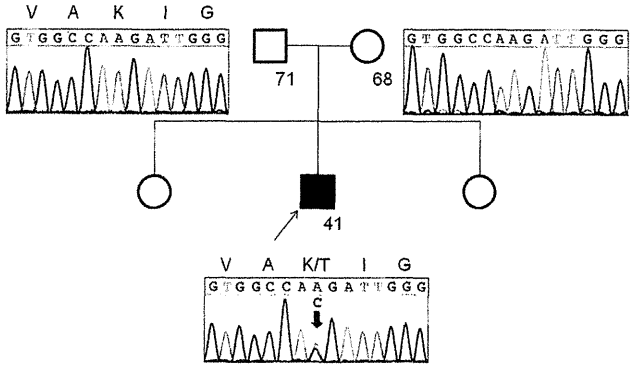

Figure 2. Molecular analysis of *CSF1R*. The sequencing results of exon 18 of *CSF1R* indicate a heterozygous c.2378A>C (p. Lys793Thr) substitution in the patient (indicated by the red arrow). The cDNA and protein numberings are relative to NM_005211.3 starting at the translation initiation codon and NP_005202.2, respectively. Neither parent has the mutation.

Genomic DNA was extracted from peripheral blood samples obtained from the patient and his parents using the Gentra Puregene Blood Kit (QIAGEN). Exons 12-22 of *CSF1R*, the mutation 'hot spots' in HDLS patients, were PCR-amplified according to a previous report (1), purified and subjected to direct sequencing. We found the p.K793T (c.2378A>C) mutation in the patient, but not in his parents (Fig. 2). Similar to the *CSF1R* mutations reported previously (1, 9), this mutation is involved in a highly conserved amino acid in the intracytoplasmic tyrosine kinase domain of *CSF1R*. An in silico analysis using PolyPhen-2 indicated that the change was probably damaging. This mutation was not detected in 80 Japanese control individuals.

Discussion

Previously, a definitive diagnosis of HDLS was made based on postmortem, neuropathological examinations only. Owing to the availability of molecular analyses of *CSF1R*, however, our patient was diagnosed as having HDLS in the early stage when he was still self-supporting at home approximately six months after the onset of symptoms.

Brain MRI, which disclosed lateral ventricle enlargement with corpus callosum lesions, was an important tool in the diagnosis of our patient. Sundal et al. reported the MRI characteristics of 15 HDLS patients with *CSF1R* mutations (10). Of these 15 patients, 14 exhibited corpus callosum involvement (the disease duration at the initial MRI study ranged from 0.5 to 5.0 years). Corpus callosum involvement included atrophy (eight patients), T2- and FLAIR-high signal intensity (11 patients) and both atrophy and signal changes in the corpus callosum (five patients) (10). In our patient, MRI performed at a disease duration of approximately six months showed atrophy and signal changes in the corpus callosum. At this stage, the cerebral deep and periventricular white matter lesions were mild and not confluent. These findings suggest that early involvement of the corpus callosum can be a diagnostic clue of HDLS.

A number of neurological diseases involving presenile dementia should be differentiated from HDLS. One of the key points for making the differential diagnosis is the regional distribution of white matter lesions. For example, white matter lesions in X-linked adrenoleukodystrophy are observed predominantly in the parietooccipital lobes (11), whereas those in HDLS are frontal dominant (10). White matter lesions in the temporal tip and external capsules are pathognomonic in CADASIL (12); however, they were not observed in seven HDLS patients, including the present patient (Kinoshita et al. unpublished observation). However, the MRI findings of HDLS closely resemble those of primary progressive MS or VaD, as they commonly show relatively symmetrical white matter lesions with corpus callosum atrophy (8, 13-15). We are now carrying out a detailed examination of the MRI characteristics of HDLS patients in comparison with those of VaD patients (Kinoshita et al. manuscript in preparation).

The patient was sporadic with both parents being healthy until their late sixties or early seventies. The mode of inheritance of HDLS is autosomal-dominant; however, several sporadic patients who met the clinical and neuropathological criteria for HDLS have been reported (5-8). Such patients might be reported under a diagnosis of pigmentary orthochromatic leukodystrophy (POLD), which shows quite similar clinical and neuropathological features to HDLS (4). Based on their clinical and neuropathological similarities, HDLS and POLD have been proposed to belong to a single disease entity (2, 4, 16-18). Therefore, the molecular basis of HDLS and POLD has increasingly attracted much attention since the discovery of *CSF1R* as a causative gene for HDLS.

Our case suggests that the incidence of HDLS may be much higher than previously estimated because sporadic HDLS cases might be overlooked unless postmortem examinations are undertaken. We propose that more careful attention should be paid to HDLS as a possible cause of presenile dementia, regardless of a patient's family history. Now, a premortem diagnosis of HDLS can be established using a molecular analysis of *CSF1R*. Making an early diagnosis is of great importance in furthering the understanding of sequential changes in clinical and neuroradiological findings among patients with HDLS.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Corpus Callosum Atrophy in Patients with Hereditary Diffuse Leukoencephalopathy with Neuroaxonal Spheroids: An MRI-based Study

Michiaki Kinoshita[1], Yasufumi Kondo[1], Kunihiro Yoshida[2], Kazuhiro Fukushima[1], Ken-ichi Hoshi[3], Keisuke Ishizawa[4], Nobuo Araki[4], Ikuru Yazawa[5], Yukihiko Washimi[6], Banyu Saitoh[7], Jun-ichi Kira[7] and Shu-ichi Ikeda[1]

Abstract

Objective Hereditary diffuse leukoencephalopathy with neuroaxonal spheroids (HDLS) is an adult-onset white matter disease that presents clinically with cognitive, mental and motor dysfunction. Several autopsy reports have indicated that the corpus callosum (CC), the largest bundle of white matter, is severely affected in patients with HDLS. The aim of this study was to evaluate corpus callosum atrophy (CCA) quantitatively in HDLS patients.

Methods We assessed CCA in six genetically-proven HDLS patients (HDLS group), in comparison with that observed in 20 patients with vascular dementia (VaD group) and 24 age-matched patients without organic central nervous system (CNS) disease (non-CNS group). Using midsagittal MR images, five measurements of the CC were obtained: the width of the rostrum (aa'), body (bb') and splenium (cc'), the anterior to posterior length (ab) and the maximum height (cd). Next, the corpus callosum index (CCI) was calculated as (aa' + bb' + cc')/ab.

Results All HDLS patients had white matter lesions in the CC and frontoparietal lobes on the initial MRI scans. Compared with that observed in the VaD and age-matched non-CNS groups, the CCI was significantly decreased in the HDLS group (with VaD group, $p<0.01$; with non-CNS group, $p<0.01$).

Conclusion This study showed significant atrophy of the CC in all HDLS patients on the initial MRI scans obtained 6-36 months after onset. We propose that the early appearance of CCA, frequently accompanied by high-intensity in the genu and/or splenium, on T2 images is an important diagnostic clue to HDLS.

Key words: hereditary diffuse leukoencephalopathy with neuroaxonal spheroids (HDLS), colony stimulating factor 1 receptor (CSF1R), white matter lesions (WMLs), corpus callosum atrophy (CCA), corpus callosum index (CCI)

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Introduction

Hereditary diffuse leukoencephalopathy with neuroaxonal spheroids (HDLS) is an adult-onset white matter disease caused by mutations in colony stimulating factor 1 receptor [*CSF1R* (1)]. HDLS is characterized clinically by cognitive, psychiatric and motor dysfunction that is frequently accom-

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Table 1. Clinical Characteristics of Six HDLS Patients

Patient	1	2	3	4	5	6
Sex	F	M	M	M	M	F
Age at disease onset (years)	51	41	53	40	55	27
Age at death (years)	alive	41	56	alive	62	alive
Family history	+	-	+	+	+	-
Neuropsychiatric symptoms						
Cognitive decline	+	+	+	+	+	+
Depression/Anxiety	-	+	+	+	+	?
Behavioral change	+	+	+	+	+	+
Frontal releasing signs	+	+	+	+	+	+
Pyramidal tract signs	+	-	+	-	+	+
Parkinsonism	+	+	+	+	+	?
Apraxia	-	+	+	+	?	+
Epilepsy	+	-	+	-	-	+
CSF1R mutation	R782H	K793T*	R777W	R777W	S759F	I794T*

*: genetically-determined sporadic case carrying a *de novo* mutation, ?: unknown or equivocal because of difficulty to evaluate symptoms

panied by epilepsy (2). The age of onset is quite variable, ranging from 8 to 78 years, and the mean duration of the illness has been reported to be 10 years (2) or 5.8 years (3). Due to its wide variety of neuropsychiatric symptoms and age of onset, HDLS mimics many neurological diseases, including vascular dementia (VaD), cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), frontotemporal dementia (FTD), multiple sclerosis (MS) and multisystem atrophy [MSA (4-7)].

MRI has significant value in evaluating the distribution and degree of white matter lesions (WMLs) and is indispensable for diagnosing HDLS. Recently, Sundal et al. reported the characteristics of 20 MRI scans of 15 patients with pathologically- and genetically-proven HDLS (3). They revealed that WMLs are predominantly frontal in patients with HDLS and that WMLs extending beyond the frontal lobes indicate rapid disease progression.

They also proposed a brain MRI scoring system for evaluating HDLS that appears to be very useful for tracking the natural history of HDLS and predicting the prognosis; however, this system places more emphasis on signal changes in the white matter (total WML score 42) than on atrophy (total atrophy score 13) (3).

Since the discovery of *CSF1R* as the causative gene, it has become possible to make a definitive diagnosis of HDLS in the early stage of the disease (1). We recently experienced a 41-year-old HDLS patient with the K793T mutation in *CSF1R* (patient 2, Table 1) (8). He was independent in daily life [modified Rankin Scale (mRS) 2] at the first presentation. His initial MRI scans (obtained six months after onset) showed minor WMLs within the frontoparietal lobes, corresponding to a total WMLs score of only 9/42 points on the MRI grading scale (3), however, diffuse atrophy of CC was already evident (8). This distinctive finding prompted us to assess early CC involvement in HDLS patients in greater detail.

Corpus callosum atrophy (CCA) has been noted in the postmortem examinations of advanced HDLS patients with diffuse widespread WMLs and severe brain atrophy (9-14).

Sundal et al. reported that CC involvement is frequently observed in HDLS patients on initial MRI scans, with the disease duration ranging from 0.5 to 5.0 years (3); however, in that study, CCA was roughly evaluated according to either its 'presence' or 'absence.' Therefore, in this study, we assessed MRI scans quantitatively to evaluate CCA in HDLS patients, especially in the early stage of disease without the presence of widespread WMLs on MRI.

Materials and Methods

Subjects

We examined 10 brain MRI scans of six HDLS patients (four men and two women; age at MRI scan: 29-57 years; mean age±standard deviation, 47.3±9.3 years) (Table 2). Mutations in *CSF1R* were identified in all patients. Three patients (patients 1, 3 and 4) had a family history consistent with autosomal inheritance (15), and two patients (patients 2 and 6) were confirmed to carry a *de novo CSF1R* mutation. One patient (patient 5) with a *CSF1R* mutation also had an affected brother; however, detailed information on their parents was not available. The detailed clinical information of the six HDLS patients is summarized in Table 1.

When enrolling patients with VaD, we adopted the guidelines of the National Institute for Neurological Disorders and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences [NINDS-AIREN (16)]. We reviewed the medical records at our institution from April 2007 through March 2011. A diagnosis of VaD was made in patients fulfilling both the probable VaD criteria in the NINDS-AIREN and the following additional requirements: 1) an age under 80 years, 2) no family history of neurological or psychiatric diseases, 3) midsagittal MR images available for measurement. We ultimately identified 20 consecutive patients with VaD (11 men and nine women; age at MRI scan: 42-79 years, mean 68.5±7.9 years). The degree of clinical disability at the time of the MRI scan was assessed in the HDLS and VaD groups using the mRS defined by Sulter et al. (17).

The age-matched control group without organic central

Table 2. Summary of the MRI Findings

HDLS (n = 6)	Age at	Age at	Disease	mRS#	HDLS MRI severity score*			Corpus callosum atrophy								Evans
Patient	onset (years)	MRI (years)	duraation until the MRI scan (months)		Total WML score (corpus callosum)	Total atrophy score (corpus callosum)	Total score	ab (L)	aa'	bb'	cc' (B: body)	cd (H: height)	CCI	B/H	B/L	index
1 (1)	51	52	19	2	12 (5)	4 (1)	16	61.0	5.39	5.71	2.53	28.14	0.224	0.090	0.042	0.293
1 (2)		57	72	5	23 (6)	10 (1)	33	64.7	2.62	3.85	2.02	34.90	0.131	0.057	0.031	0.371
2 (1)	41	41	6	2	5 (1)	4 (1)	9	72.9	5.10	6.37	2.95	30.56	0.198	0.097	0.040	0.330
2 (2)		41	11	3	9 (3)	4 (1)	13	73.9	3.72	4.40	2.73	31.33	0.147	0.087	0.037	0.344
3 (1)	53	56	36	3	12 (5)	5 (1)	17	69.0	6.07	6.05	4.18	30.27	0.236	0.138	0.061	0.290
3 (2)		57	48	5	16 (6)	9 (1)	25	NA	NA	NA	NA	NA	NA	NA	NA	0.320
4 (1)	40	41	14	2	15 (6)	9 (1)	24	78.3	8.91	6.08	2.32	34.30	0.221	0.068	0.030	0.360
4 (2)		42	28	3	16 (6)	9 (1)	25	79.1	9.64	4.45	3.13	36.15	0.218	0.087	0.040	0.371
5	55	57	33	4	21 (6)	8 (1)	32	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	27	29	8	4	17 (4)	4 (1)	22	60.7	6.73	9.37	2.33	19.83	0.304	0.119	0.038	0.286
average	44.500	47.300	27.500	3.300			21.600	69.954	6.023	5.785	2.775	30.685	0.210	0.093	0.040	0.331
SD	9.691	9.285	19.679	1.100			7.432	6.818	2.237	1.612	0.628	4.815	0.050	0.024	0.009	0.034
VaD (n = 20)																
average		68.500		2.750				72.620	8.160	7.992	4.689	28.479	0.289	0.169	0.065	0.272
SD		7.940		0.887				5.484	1.566	1.540	0.750	4.151	0.050	0.039	0.011	0.026
non-CNS (n = 24)																
average		49.917						71.141	10.157	10.609	5.635	27.221	0.371	0.208	0.079	0.246
SD		13.789						4.253	2.076	1.808	1.054	2.789	0.056	0.039	0.014	0.022

NA: not analyzed, #: modified Rankin Scale (17), *: proposed by Sundal et al. (3)

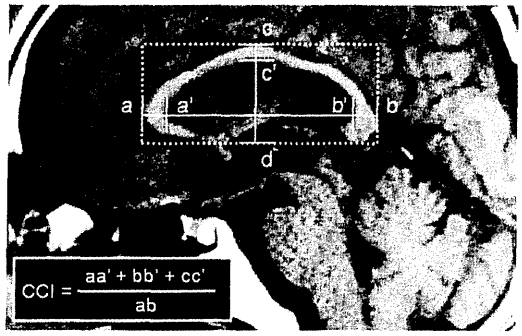

Figure 1. Determination of the corpus callosum index (CCI) using a midsagittal slice on a T1-weighted MR image (HDLS patient 2 in Table 1, 41-year-old man).

nervous system diseases (non-CNS group) included 24 patients (15 men and nine women; age at MRI scan: 29-73 years, mean 49.9±13.8 years). Their diagnoses were as follows: myasthenia gravis (n=6), psychosomatic disorder (n=5), benign paroxysmal positional vertigo (n=3), Guillain-Barré syndrome or Fisher syndrome (n=3), spinal canal stenosis (n=2), spinal cord tumor (n=2; able to exclude multiple sclerosis), brachial plexus neuropathy or neuralgic amyotrophy (n=2) and facioscapulohumeral muscular atrophy (FSH) (n=1). Brain MRI was conducted to rule out intracranial lesions in these patients. None of the patients had WMLs on MRI, except for a small amount of lacunar infarctions.

MRI assessments

The brain MRI scans of the VaD group and non-CNS group were performed in our hospital with a 1.5 Tesla Magnetom Avanto scanner (Siemens AG, Erlangen, Germany). T1-weighted images were obtained using a spin-echo technique (TE=12-26 ms, TR=250-500 ms, slice thickness=3-4 mm). The scans of the HDLS patients were performed in four different hospitals, patients 1-4 being examined using the same scanner as at our hospital (1.5 Tesla Magnetom Avanto, using the same acquisition protocol as the other groups). All of the MRI scans were measured independently once each by one of the authors (YK) and two additional examiners (YT and KT) who were blind to the clinical information, including the diagnoses and ages of the patients. All measurements were obtained using a computerized measurement tool on screen on a picture archiving and communication system (PACS) workstation. We measured the corpus callosum using midsagittal T1-weighted images according to previous reports (18-20). Five measurements of the CC were obtained (Fig. 1): the width of the rostrum (aa'), body (cc', abbreviated as B) and splenium (bb'), the anterior to posterior length (ab, abbreviated as L) and the maximum height (cd, abbreviated as H). The corpus callosum index (CCI), which has been reported to be a marker for brain atrophy in MS patients (18, 19), was calculated as (aa'+bb'+cc')/ab. The ratios B/L and B/H were also calculated. The Evans index (the maximum distance between the two anterior horns/the maximum transverse inner diameter of the skull at the same level) was measured using axial T1-weighted images. A comparison between the three investigators' evaluations of each indicator of the CCA and the Evans index was made with the intraclass correlation coefficiency (*ICC*) and internal consistency using Cronbach's alpha coefficient. In addition, we assessed the severity of WMLs and brain atrophy according to the MRI grading system proposed by Sundal et al. (3).

Statistical analysis

The statistical analysis was carried out using the nonparametric Mann-Whitney U-test to compare the indicators of CCA (aa', bb', cc' (B), ab (L), cd (H), BL, BH and CCI) and the Evans index between the HDLS group and each control group. We considered p values of <0.05 or less to be statistically significant.

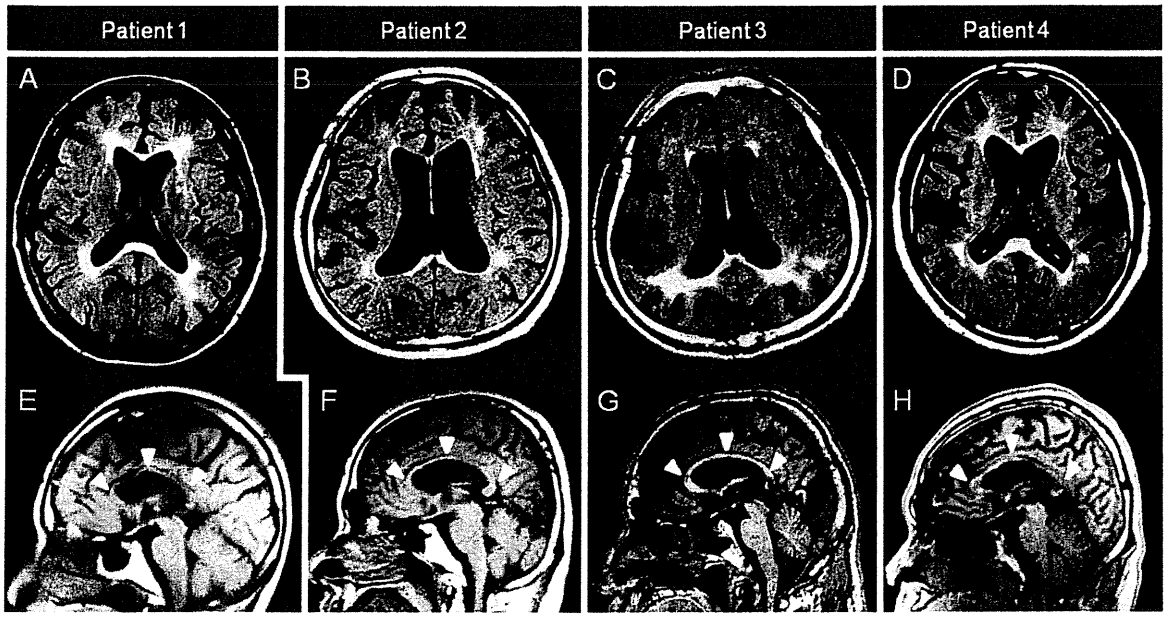

Figure 2. Representative MRI findings of four HDLS patients. A-D: Axial FLAIR images showing patchy or confluent periventricular-dominant white matter lesions and hyperintensity signals in the CC, especially in the splenium. E-H: Midsagittal T1-weighted images showing severe diffuse CC atrophy (arrowheads). (A and E: Patient 1 at age 52 (approximately 19 months after onset); B and F: Patient 2 at age 41 (approximately 11 months after onset); C and G: Patient 3 at age 56 (approximately three years after onset); D and H: Patient 4 at age 42 (approximately 14 months after onset), each patient number corresponding to that in Tables 1 and 2).

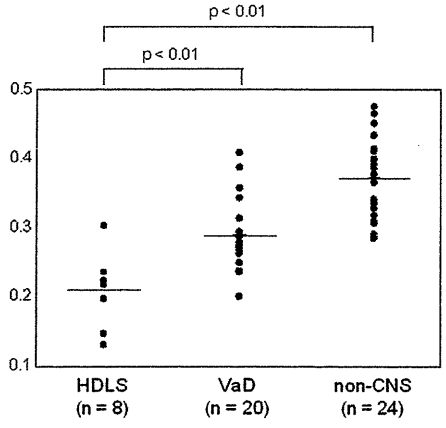

Figure 3. Distribution of the corpus callosum index (CCI) in the patients with HDLS, vascular dementia (VaD) and control individuals without organic central nervous system diseases (non-CNS). The CCI was significantly decreased in the HDLS group (0.210±0.050) compared with that observed in the VaD group (0.289±0.050) ($p<0.01$) and age-matched non-CNS group (0.371±0.056) ($p<0.01$). The horizontal bar indicates the mean of each group.

Results

All patients with HDLS had WMLs in the CC and fronto-parietal lobes (Fig. 2; Table 2). The WMLs extended to the temporal lobes in three patients. The total MRI severity scale for HDLS (3) ranged from 9 to 33 (Table 2). As the disease progressed, volume loss of the cerebral white matter became evident. None of the patients had high-intensity signals in the temporal tips or external capsules. Four patients (seven scans) were screened for cerebral microbleeds using T2*-weighted images; however, no such bleeding was detected, whereas 11 of the 12 patients (91.7%) examined in the VaD group had cerebral microbleeds.

Hyperintense signals and atrophy in the CC were detected on all 10 MRI scans in the six patients (Fig. 2; Table 2). The CCI was significantly decreased in the HDLS group (0.210±0.050) compared with that observed in the VaD group (0.289±0.050) ($p<0.01$) and age-matched non-CNS group (0.371±0.056) ($p<0.01$) (Fig. 3; Table 2). The B/H index (cc'/cd in Fig. 1) was also significantly reduced in the HDLS group (0.093±0.024) compared to that observed in the VaD group (0.169±0.039) ($p<0.01$) and non-CNS group (0.208±0.039) ($p<0.01$). Similarly, the B/L index (cc'/ab in Fig. 1) was significantly reduced in the HDLS group (0.040±0.009) compared to that observed in the VaD group (0.065±0.011) ($p<0.01$) and age-matched non-CNS group (0.079±0.014) ($p<0.01$). The Evans index, an indicator of lateral ventricle dilatation, was significantly larger in the HDLS group (0.331±0.034) than in the VaD group (0.272±0.026) ($p<0.01$) or non-CNS group (0.246±0.022) ($p<0.01$). The inter-rater reliability and internal consistency of the total measurements were almost good (*ICC*=0.755-0.951; Cronbach's alpha=0.907-0.986) (Table 3).

Table 3. Inter-rater Reliability and Internal Consistency of the Indicators of Corpus Callosum Atrophy and the Evans Index

	HDLS group (n = 8)		VaD group (n = 20)		Non-CNS control group (n=24)		Total (n = 52)	
Items	*ICC*	Cronbach's alpha	*ICC*	Cronbach's alpha	*ICC*	Cronbach's alpha	*ICC*	Cronbach's alpha
ab (L: length)	0.992	0.999	0.949	0.987	0.920	0.976	0.951	0.986
aa'	0.899	0.962	0.575	0.840	0.615	0.823	0.755	0.907
bb'	0.820	0.940	0.627	0.840	0.585	0.840	0.792	0.927
cc' (B: body)	0.656	0.883	0.577	0.801	0.736	0.895	0.832	0.940
cd (H: Height)	0.855	0.943	0.892	0.977	0.815	0.953	0.867	0.963
B/H	0.688	0.916	0.724	0.895	0.692	0.889	0.933	0.979
B/L	0.615	0.864	0.598	0.815	0.737	0.895	0.842	0.950
CCI	0.840	0.946	0.738	0.904	0.565	0.876	0.830	0.938
Evans index	0.913	0.970	0.844	0.950	0.853	0.960	0.859	0.952

ICC: Intraclass correlation coefficiency

Discussion

The occurrence of CCA with WMLs has been reported in some demential disorders, such as VaD, including Binswanger's disease, and CADASIL, MS and leukodystrophies, including HDLS. This was a quantitative study conducted to understand CC involvement in HDLS itself, and the aim was not primarily to compare the disease with the occurrence of CCA in VaD for diagnostic purposes to distinguish it from HDLS. We selected a VaD group as a control simply because VaD is the most common and representative disease involving CCA with WMLs.

Due to their clinical and neuroradiological similarities, it is sometimes difficult to differentiate between HDLS and other diseases involving CCA with WMLs, especially in the early stage of the disease. The lack of a family history does not preclude the possibility of HDLS, as several sporadic patients who fulfill the clinical and neuropathological criteria for HDLS have been reported (4, 5, 8, 21). Furthermore, the occurrence of HDLS due to a *de novo CSF1R* mutation has hitherto been confirmed in two families in which the parents of the patient had no mutations of interest (1, 8), and one such family was newly added in this study (patient 6). The most reliable method of discriminating HDLS is currently genetic testing for *CSF1R*. Because genetic testing for *CSF1R* is not presently commercially available, identifying laboratory or neuroradiological hallmarks of HDLS is of value in order to select candidates for further processing to genetic testing.

Recently, Sundal et al. reported that 14 of 15 patients exhibited CC involvement (the disease duration at the initial MRI study ranged from 0.5 to 5.0 years). The CC involvement included atrophy (eight patients), T2 and FLAIR high signal intensity (11 patients) and both atrophy and signal changes in the CC (five patients) (3). These data appear to indicate that hyperintense signals and atrophy in the CC are not correlated with each other. This may be partly because CCA was evaluated using a score of 0 (absence of atrophy) or 1 (presence of atrophy). Therefore, we evaluated CCA quantitatively in this study.

As to data regarding the measurement of CC in healthy Japanese subjects, Takeda et al. reported CC measurements in 205 Japanese individuals without CNS disorders (94 men, mean age: 57.3±20.8 years; range: 6-90 years; and 111 women, 61.2±17.6 years; range: 9-86 years). These values are similar to those of our study, including the widths of the rostrum (aa'): mean ± SD 9.91±1.82 (our non-CNS group, 10.16±2.08), body (bb'): 5.58±1.08 (5.64±1.05), splenium (cc'): 9.94±1.56 (10.61±1.81) and anterior to posterior length (ab): 69.7±4.24 (71.14±4.24), although there was a difference in the range of ages between the two studies. In another study, the CC measurements of 15 Japanese healthy men (mean age: 56.2±6.9) were reported as follows: aa': 10.6±1.4, bb': 5.3±1.1, cc': 10.4±1.4 and ab: 72.7±8.5 (22). These data were also very close to those of our measurements, as well as the ranges of ages, in the non-CNS group. In addition, the CCI was 0.362 when calculated using the mean data for the trial (our non-CNS group: 0.371±0.056). These results support the reliability of the CCI data for our non-CNS group, in spite of the limitation in the number of cases.

In our study, all of the patients had abnormal hyperintensive signals in the CC on their initial MRI scans (disease duration ranging from six to 36 months). Furthermore, the CCI was significantly decreased when compared with that observed in the VaD and age-matched non-CNS groups. In this study, the mean age at MRI in the VaD group (68.5 years) was much higher than that noted in the HDLS group (47.3 years). Takeda et al. showed that the widths of the rostrum (aa'), body (cc', B) and splenium (bb') of the CC decrease with age in normal Japanese individuals (20). Conversely, the maximum height (cd, H) and anterior to posterior length (ab, L) gradually increase with age. These data indicate that the CCI, B/H and B/L decrease with age. Taking the difference in the mean age between the HDLS and VaD groups into consideration, we can say that the decreases in the CCI, B/H and B/L in the HDLS group were conspicuous compared with those observed in the VaD group.

In addition, the CCI observed in the HDLS patients was much less than that at diagnosis in the MS patients (0.345±