Pathologic basis of the preferential thinning of the corpus callosum in adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP)

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Abstract

Background: Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is an early onset dementia characterized by axonal loss in the cerebral white matter with swollen axons (spheroids). It had been reported that the preferential thinning and “focal lesions” of the corpus callosum were observed on T2-weighted MRI in ALSP patients. The present study aimed to reveal the pathologic basis of them in relation to brain lesion staging (I ~ IV: Oyanagi et al. 2017).

Methods: Seven autopsied brains of ALSP and five controls were neuropathologically examined.

Results: Even at Stage I, corpus callosum body showed evident atrophy, and the atrophy advanced with stage progression. Spheroid size and density were maximal at Stage II in both centrum semiovale and corpus callosum body, but spheroids were larger in corpus callosum body than in centrum semiovale. Microglia in the body at Stage II had a larger cytoplasm than those in centrum semiovale. But spheroids and microglia in the “focal lesions” were identical with those of centrum semiovale.

Conclusion: Preferential thinning of corpus callosum was considered to be formed in relation to peculiar morphological alteration of microglia there in ALSP. Instead, “focal lesions” were formed in connection with the lesions in centrum semiovale.

1. Introduction

Among the hereditary leukoencephalopathies causing early onset dementia, adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP)/hereditary diffuse leukoencephalopathy with axonal spheroids (HDLS) is an autosomal-dominant cerebral white matter disease characterized by axonal loss with swollen axons (spheroids) and marked alteration of microglia, astrocytes and oligodendroglia in the lesions [1–15]. The colony stimulating factor 1 receptor gene (CSF1R) [8,16–18] and the alanyl-transfer (t)RNA synthetase 2 (AARS2)
gene [19–22] have been implicated as causative genes of ALSP. CSF1R is expressed mainly in microglia [23], which are thought to play an important role in the pathogenesis of white matter degeneration in ALSP [1,8,11,17,18,24,25]. AARS2 encodes a mitochondrial enzyme, and its mutation has recently been reported to cause a leukoencephalopathy showing clinical and pathological features identical to those of ALSP attributable to CSF1R mutation [19–22].

Brain imaging has demonstrated marked atrophy of the corpus callosum even at the early stage of ALSP [4,12,26–30]. Using quantitative determination of corpus callosum thickness (corpus callosum index: CCI) demonstrated by midsagittal MRI [31,32], the present authors have shown that this corpus callosum atrophy is significant in ALSP and occurs early in the disease course [26]. However, the pathological features of the corpus callosum in ALSP, particularly those of “focal corpus callosum lesions” that are frequently demonstrable by high-intensity T2-weighted MRI [18,27,29,30,33–35], have not yet been reported in detail and the mechanism has not been elucidated.

Many autopsy reports have macroscopically pointed a marked thinning of the corpus callosum in patients with ALSP, and only two papers reported the histological findings of the corpus callosum [25,36], however, chronological alteration of the histopathological findings of the corpus callosum along with the progression of the brain lesion staging and the pathologic basis of those findings of the corpus callosum have not been reported and the mechanism of the thinning is still unknown. The present study aimed to clarify the detailed neuropathological features of the corpus callosum in ALSP and the mechanism, by focusing especially on 1) the features in microglial alteration compared with those in centrum semiovale, 2) the “focal corpus callosum lesions” revealed by MRI, and 3) pathologic basis of the preferential thinning of the corpus callosum.

2. Materials and methods

2.1. Examined cases

All of the patients examined in the present study, except for #3 [37], were the same as those documented in our recent previous investigation [1]. We examined seven autopsied brains of ALSP patients from five families (four males and three females; ages at autopsy 41–58 years; mean age ± standard deviation 51.86 ± 6.67 years) [8,10,24,26,27,36–38]. All of the patients were Japanese, and mutations in CSF1R were identified in six of them. The remaining patient was diagnosed as having ALSP on the basis of clinical and neuropathological findings [37]. AARS2 genes status was not examined in any of the patients. Table 1 shows the characteristics of the seven ALSP patients, including their family lines, gene mutations, sex, age at onset, age at death, clinical symptoms and the modified Rankin scale (mRS) [39] at death, brain weight and thickness of the corpus callosum.

The brain lesions of ALSP were staged according to Oyanagi et al. (2017) as follows [1]. Stage I, patchy axon loss in the cerebral white matter without brain atrophy; Stage II, large patchy areas of axon loss with slight atrophy of the cerebral white matter and slight dilatation of the lateral ventricles; Stage III, extensive axon loss in the cerebral white matter and dilatation of the lateral and third ventricles without marked axon loss in the brainstem and cerebellum; Stage IV, cerebral white matter destruction with marked dilatation of the ventricles and axon loss in the brainstem and/or cerebellum.

Patient #1 was at Stage I, patients #2 and #3 were at Stage II, patients #4, #5 and #6 were at Stage III, and patient #7 was at Stage IV. Five controls (three males and two females; ages at autopsy 62–76 years; mean age ± standard deviation 69.4 ± 5.03 years), who had also been used in our previous study [1], were included for comparison of the neuropathological features.

2.2. Neuropathological procedures

Formalin-fixed paraffin-embedded 6-μm-thick coronal sections of the corpus callosum and centrum semiovale from these seven autopsied brains were subjected to neuropathological examination with hematoxylin and eosin (HE) and Klüver-Barrera (K-B) staining, as well as immunohistochemistry for phosphorylated (p)-neurofilament (NF), Iba-1 (ionized calcium binding molecule-1) which has been reported to be a...
Corpus callosum thickness

The thickness of the corpus callosum measured at the smallest parts of the body level was 3.475 ± 0.456 (mean ± SD) mm in the controls. As for the ALSP cases, the thickness of the corpus callosum at the body level was reduced even at Stage I, and thinning advanced with stage progression (Table 1, Fig. 1). “Focal lesion” at the corpus callosum splenium (Fig. 1C, arrowhead) connected with the lesions in the centrum semiovale (Fig. 1C).

Axon loss and spherically swollen axons (spheroids)

Axonal or myelinated fiber density in the lesions of the centrum semiovale was slightly and patchily decreased at Stage I (Figs. 2A, 3A), reduced in the lesions at Stage II (Figs. 2C, 3C), and extensive destruction was evident at Stages III (Figs. 2G, 3G) and IV (Figs. 2I, 3I). However, the density in the corpus callosum body appeared to be preserved due to compaction at Stage I (Figs. 2B, 3B), and decreased with disease progression from Stage II, although better preservation was evident relative to the centrum semiovale (Figs. 2C, D, G, H, I, J, 3C, D, G, H, I, J). The ventral half of the corpus callosum splenium showed moderate loss of axons in Patient #2 at Stage II (Figs. 2F, 3F), and the dorsal half of the splenium, corresponding to the “focal lesions” evident on MRI, showed loss of axons (Figs. 2E, 3E) (Table 3).

Spheroids with a maximal diameter of 20 μm were scattered in the lesions in the centrum semiovale (Fig. 3A) and the body of the corpus callosum (Fig. 3B) at Stage I, and those with a maximal diameter of 40 μm were observed extensively in the lesions in the centrum semiovale (Fig. 3C), and in the body (Fig. 3D) and dorsal part of the splenium (Fig. 3E) of the corpus callosum in Patient #2 at Stage II, although spheroids in the ventral part of the splenium were less than 20 μm in diameter at this stage (Fig. 3F). A few spheroids with a maximum diameter of 15 μm were present in the centrum semiovale (Fig. 3G) and body of the corpus callosum (Fig. 3H) at Stage III, and in the centrum semiovale (Fig. 3I) at Stage IV, but scarcely observed in the body of the corpus callosum (Fig. 3J) at Stage IV (Table 3).

No calcium deposition was observed in the portions examined.

Iba-1-immunopositive microglial cells

Many ramified cells with long and thin processes, whose cytoplasmic diameters were less than 10 μm, were seen in the centrum semiovale (Fig. 4A) and body of the corpus callosum (Fig. 4B) at Stage I (Table 4). On the other hand, rounded cells with a maximal cytoplasmic diameter of 25 μm and short, thick processes were abundant in the centrum semiovale (Fig. 4C) and dorsal part of the corpus callosum splenium (Fig. 4E) in Patient #2 at Stage II, corresponding to the “focal lesions” revealed by MRI. Round ameboid cells with maximal diameter of 25 μm without remarkable cell processes were present in the body of the corpus callosum (Fig. 4D), whereas cells with thick processes were evident in the ventral part of the splenium (Fig. 4F) in Patient #2 at Stage II (Table 4). Oval ameboid cells without evident cell processes, whose cytoplasmic diameters were maximally 15 μm, were seen in the centrum semiovale (Fig. 4G), and ameboid cells with a maximal cytoplasmic diameter of 20 μm were observed in the body of the corpus callosum (Fig. 4H) at Stage III (Table 4). Scattered small ramified cells with a cytoplasmic diameter less than 10 μm were seen in both the corpus callosum body (Fig. 4I) and centrum semiovale (Fig. 4J) at Stage IV (Table 4).

Table 2 Antibodies used.

| Detection                        | Antibody type     | Dilution | Increase of antigenicity | Source                        |
|----------------------------------|-------------------|----------|--------------------------|-------------------------------|
| Phosphorylated neurofilament     | Mouse/ monoclonal | 1:2000   | Boiling                  | COVANCE, Princeton, USA       |
| IBA-1                            | Rabbit/ polyclonal| 1:4000   | Autoclaving              | Wako Pure Chemical Industries, Osaka, Japan |
| CD163                            | Mouse/ monoclonal | 1:200    | Autoclaving              | Leica Biosystems, Newcastle Upon Tyne, UK |
| CD68                             | Mouse/ monoclonal | 1:800    | Boiling                  | Dako, Glostrup, Denmark       |

Antigenicity was increased by autoclaving (121 °C, 20 min) in 0.01 mol/L citrate-buffered solution (pH 6.0) for Iba-1 and CD163. Boiling in a microwave oven (500 W, 7 min) in 0.01 mol/L citrate-buffered solution (pH 7.6) for phosphorylated neurofilament and CD68.
Fig. 1. Coronal sections of the corpus callosum at each ALSP lesion stage. A: patient #1 at Stage I, B; patient #2 and D; Patient #3 at Stage II, E; patient #4, F; patient #5 and G; patient #6 at Stage III, and H; patient #7 at Stage IV. No remarkable paleness was observed in the corpus callosum or centrum semiovale in the control brains (data not shown). Paleness of myelin staining and atrophy was exacerbated with disease progression in the body of the corpus callosum of patients with ALSP. Thickness of the corpus callosum at the body level was measured at the smallest parts square with the corpus callosum between the arrows in each patient (A - H). Thickness of the corpus callosum is shown in Table 1. Red arrowhead shows the lesion in the dorsal part of the splenium in patient #2 at Stage II (C), which corresponds to the “focal corpus callosum lesion” revealed by MRI, connecting with the centrum semiovale lesions. Klüver-Barrera (K-B) preparation. Bars; 5 mm at Stages I and II (A-D); 10 mm at Stages III and IV (E-H). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Loss of myelinated fibers in the centrum semiovale and corpus callosum. Paleness of myelin staining was exacerbated with disease progression in the body of the corpus callosum (B, D, H, J) and centrum semiovale (A, C, G, I). The dorsal part of the corpus callosum in Patient #2 at Stage II, corresponding to the “focal lesions” revealed by MRI, showed severe paleness (E) as compared with the ventral part (F). Klüver-Barrera (K-B) preparation. Bars; 200 μm.
Fig. 3. Axonal changes.

No significant axon loss or spheroids were observed in the corpus callosum and centrum semiovale in the control brains (data not shown). Axonal density was slightly and patchily sparse in the centrum semiovale at Stage I (A), markedly reduced at Stage II (C), and extensive destruction was evident at Stages III (G) and IV (I). Axonal density in the corpus callosum body decreased slightly at Stage I (B), and reduced severely at Stages II (D), III (H) and IV (J). But the severity was slighter than that of centrum semiovale due to compaction of axons in the corpus callosum. The dorsal half of the splenium in Patient #2 at Stage II, corresponding to the “focal lesions” evident on MRI, showed marked loss of axons (E), whereas axons in the ventral half were relatively preserved (F).

Spheroids with a maximal diameter of 20 μm were scattered in the centrum semiovale (A) and the body of the corpus callosum (B) at Stage I, and those with a maximal diameter of 40 μm were abundant in the centrum semiovale (C), and the body (D) and dorsal part of the splenium (E) of the corpus callosum in Patient #2 at Stage II. But the size of the spheroids in the corpus callosum body and in the dorsal part of the splenium is relatively larger than that in the centrum semiovale at Stage II and largest as compared with that in other stages. Spheroids in the ventral part of the splenium were less than 20 μm in diameter at Stage II (F). A few spheroids with a maximum diameter of 15 μm were present in the centrum semiovale (G) and body of the corpus callosum (H) at Stage III, and in the centrum semiovale at Stage IV (I), but were scarcely evident in the corpus callosum body at Stage IV (J).

No calcium deposition was observed in any of the portions examined. Immunohistochemistry for phosphorylated (p-) neurofilament (NF) (SMI-31) (C, D, and G-J). Double staining using periodic acid-Schiff (PAS) and immunohistochemistry for p-NF (A, B, E and F). Bars; 10 μm.

Table 3
Axonal loss and swollen axons.

| Patient | #1   | #2   | #3   | #4   | #5   | #6   | #7   |
|---------|------|------|------|------|------|------|------|
| Stage (Oyanagi et al. 2017) [1] | I    | II   | II   | III  | III  | III  | IV   |
| Axonal loss | patchy diffuse diffuse extensive extensive extensive extensive extensive extensive |
| Corpus Callosum Body | diffuse diffuse extensive extensive extensive extensive extensive extensive |
| Splenium <dorsal> | extensive extensive extensive extensive extensive extensive extensive extensive |
| Splenium <ventral> | extensive extensive extensive extensive extensive extensive extensive extensive |
| Swollen axons | 20 μm maximal, scattered 40 μm maximal, many 15 μm maximal, a few 15 μm maximal, a few 15 μm maximal, a few |
| Centrum Semiovale | 20 μm maximal, scattered 40 μm maximal, many 15 μm maximal, a few 15 μm maximal, a few 15 μm maximal, a few |
| Corpus Callosum Body | 20 μm maximal, scattered 40 μm maximal, many 15 μm maximal, a few 15 μm maximal, a few 15 μm maximal, a few |
| Splenium <dorsal> | 20 μm maximal, scattered 40 μm maximal, many 10 μm maximal, few 10 μm maximal, few 10 μm maximal, few |
| Splenium <ventral> | 20 μm maximal, scattered 40 μm maximal, many 10 μm maximal, few 10 μm maximal, few 10 μm maximal, few |

Semiquantitative evaluation of the findings of immunohistochemistry for phosphorylated (p-) neurofilament (NF) (SMI-31) of all the patients. The numbers indicate diameters of swollen axons (spheroids).
3.4. CD163-immunopositive microglial cells

Amoeboid cells with a maximal cytoplasmic diameter of 15 μm and a few short processes were scattered in both the centrum semiovale (A) and corpus callosum body (B) at Stage I (Table 4). On the other hand, round cells with maximal cytoplasmic diameter of 25 μm with short and thick processes were abundant in the centrum semiovale (C) and dorsal part of the splenium (E) in Patient #2 at Stage II, corresponding to the “focal lesions” revealed by MRI, whereas round cells without remarkable cell processes were present in the body of the corpus callosum (D), and cells sizing maximal diameter of 20 μm with thick processes were evident at the ventral part of the splenium (F) in Patient #2 at Stage II. Oval amoeboid cells without remarkable cell processes were present in the centrum semiovale (G) and corpus callosum body (H) with maximal cytoplasmic diameters of 15 μm and 20 μm at Stage III, respectively. Scattered small ramified cells less than 10 μm were seen in both the corpus callosum (I) and centrum semiovale (J) at Stage IV. Iba-1 immunohistochemistry (brown) (D, I and J). Double staining using periodic acid-Schiff (PAS) and immunohistochemistry for Iba-1 (brown) (A - C, and E - H). Bars; 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. CD68-immunopositive microglial cells

Oval-shaped amoeboid cells with a maximal cytoplasmic diameter of 20 μm were scattered in both the centrum semiovale (Fig. 5A) and corpus callosum body (Fig. 5B) at Stage I (Table 4). Polygonal cells with a maximal cytoplasmic diameter of 20 μm, possessing several thick short processes, were abundant in the centrum semiovale (blue in Fig. 5C) and dorsal (blue in Fig. 5E) and ventral (blue in Fig. 5F) parts of the splenium in Patient #2, whereas oval amoeboid cells with a maximal diameter of 20 μm were scattered in the body of the corpus callosum at Stage II (Fig. 5D) (Table 4). A few or less small oval or polygonal cells with a diameter of less than 10 μm and sparse processes were present in the centrum semiovale and body of the corpus callosum at Stages III and IV (Figs. 5G - 5J) (Table 4).

4. Discussion

Microglia have been classified morphologically into ramified and amoeboid types. Ramified microglia have long, thin and branched processes and are regarded as a “resting type” [44], whereas round or oval-shaped amoeboid microglia have short and thick processes and are considered to be an “activating type” [45]. Activating microglia are thought to be induced by certain cytokines, such as interleukin-34 (IL-34), which act via CFS1R after being secreted from damaged neurons in the brain [46-48].

Several reports have been published pointing the specificity of the microglia and of the pathological peculiarity of the corpus callosum. Cuprizone induced demyelination and increase of microglia specifically in the corpus callosum [49], one-year-old Csf1r+/− mice revealed thinning of the corpus callosum [50], and an individual human with a homozygous splice mutation (c.1754_1G > C) in CSF1R demonstrated...
Microglia with immunopositive for Iba-1 and CD163 in the corpus callosum body at Stages II and III differed from those in the centrum semiovale, since the dorsal part of the splenium connects the ventral half in patient #2 at Stage II, and the ventral half in patient #2 at Stage II, differing a difference in the features and intensity of the spheroids may be formed in relation to the activation of the microglia. Thus the density of microglia in the dorsal lesion and centrum semiovale indicates a difference in the features and intensity of the spheroids were maximal at Stage II in both the centrum semiovale and corpus callosum body; and spheroids were larger in the corpus callosum body than in the centrum semiovale at this stage, corresponding to the degree of activation of microglia in these regions. Histological and semiquantitative evaluation of Iba-1, CD163 or CD 68 immunopositive microglia of all the patients. The numbers indicate diameters of the cytoplasm of microglia.

Table 4
Evaluation of microglial alteration.

| Patient | #1 | #2 | #3 | #4 | #5 | #6 | #7 |
|---------|----|----|----|----|----|----|----|
| Stage (Oyanagi et al. 2017) [1] | I | II | II | III | III | III | IV |
| Centrum Semiovale | ramified, less than 10 μm, many | large round with short processes, 25 μm maximal, many | large round with short processes, 25 μm maximal, many | small ramified, 15 μm maximal, scattered | small ramified, 15 μm maximal, scattered | small ramified, 15 μm maximal, scattered | small ramified, less than 10 μm, scattered |
| Corpus Callosum Body | ramified, less than 10 μm, many | large amoeboid, 25 μm maximal, many | amoeboid, 20 μm maximal, scattered | amoeboid, 20 μm maximal, scattered | small amoeboid, less than 10 μm, scattered | small ramified, less than 10 μm, scattered |
| Splenium <dorsal> | large round with short processes, 25 μm maximal, many | | | | | |
| Splenium <ventral> | | | | | | |
| CD163 | Centrum Semiovale | small amoeboid, 15 μm maximal, scattered | polygonal, 20 μm maximal, many | polygonal, 20 μm maximal, many | small amoeboid, less than 10 μm, a few | small amoeboid, less than 10 μm, a few | small amoeboid, less than 10 μm, few |
| | Corpus Callosum Body | small amoeboid, 20 μm maximal, scattered | amoeboid, 20 μm maximal, scattered | | small amoeboid, less than 10 μm, a few | small amoeboid, less than 10 μm, a few | small amoeboid, less than 10 μm, few |
| | Splenium <dorsal> | | | | | |
| | Splenium <ventral> | | | | |
| CD68 | Centrum Semiovale | amoeboid, 20 μm maximal, scattered | large amoeboid, 20 μm maximal, many | large amoeboid, 20 μm maximal, many | amoeboid, less than 10 μm, a few | amoeboid, less than 10 μm, a few | small amoeboid, less than 10 μm, a few |
| | Corpus Callosum Body | amoeboid, 20 μm maximal, scattered | large amoeboid, 20 μm maximal, many | large amoeboid, 20 μm maximal, many | amoeboid, less than 10 μm, a few | amoeboid, less than 10 μm, a few | small amoeboid, less than 10 μm, a few |
| | Splenium <dorsal> | | | | | |
| | Splenium <ventral> | | | | |

Histological and semiquantitative evaluation of Iba-1, CD163 or CD 68 immunopositive microglia of all the patients. The numbers indicate diameters of the cytoplasm of microglia.

Agenesis of corpus callosum [51].

In the present investigation, the morphology of microglia had changed even at Stage I, such as the size of microglia being larger, in the corpus callosum body than in the centrum semiovale at this stage. Morphological alteration of microglia was maximal at Stage II, corresponding to the severe degeneration and/or regeneration of axons and other cell components in the lesions at this point in the brains of ALS patients. Microglia with immunopositive for Iba-1 and CD163 in the corpus callosum body at Stages II and III differed from those in the centrum semiovale. Microglia in the centrum semiovale had larger cytoplasm but evident cell processes were absent. These findings suggested that the features and intensity of the microglial reaction in the corpus callosum differed from those in the centrum semiovale, and that the process of axonal degeneration/regeneration also differed between the two. Earlier and more evident atrophy of the corpus callosum as compared with that of the centrum semiovale was considered to be caused by different reaction of microglia at the corpus callosum from that of the centrum semiovale and by decrease of commissural fibers and compaction of the remaining fibers in the present study. In addition, the size and density of spheroids were maximal at Stage II in both the centrum semiovale and the body of corpus callosum; and spheroids were larger in the corpus callosum body than in the centrum semiovale at this stage, corresponding to the degree of activation of microglia in these regions. Thus spheroids may be formed in relation to the activation of the microglia.

On the other hand, the morphology and density of microglia in the dorsal half of the splenium, representing the “focal lesions” evident on MRI [27,30,33–35], and the ventral half in patient #2 at Stage II, differed, suggesting a difference in the features and intensity of the microglial reaction in these areas. The similarity in the morphology and density of microglia in the dorsal lesion and centrum semiovale indicates that the “focal lesions” revealed by MRI developed from those in the centrum semiovale, since the dorsal part of the splenium connects the corpus callosum to the centrum semiovale via commissural fibers [52,53]. The difference in the location of “focal lesions” revealed by MRI...
among patients with ALSP reported may be due to different localization of involved areas in the centrum semiovale.

Microglial activation appeared to subside through Stages III and IV, whereas depletion of axons and disappearance of spheroids advanced in the corpus callosum, together with hollowing of the centrum semiovale. The pathological mechanism responsible for this remains unknown, and the mechanism responsible for sparing of U-fibers around the hollows at the Stages III and IV is also unclear. The forefront of the lesions extended to the brainstem, cerebellum, optic nerve and spinal cord at Stages III and IV.

Specific responses and alteration of the microglia occurring in the corpus callosum in various pathologic conditions [49–51] may indicate a possibility of particular alteration of microglia and preferential thinning of the corpus callosum in ALSP. However, there have been no reports on the expression of CSF1R, nor on the structure and amount of the CSF1R protein for each site and each cell type. Further study should be performed on these points in relation to the particular response of the microglia and the preferential thinning of the corpus callosum.

In conclusion, the present study confirmed that axonal loss advances in the body of the corpus callosum with lesion staging in patients with ALSP, as in the centrum semiovale, but that the microglial reaction in the former differs from that in latter, especially at Stages II and III. Spheroids in the body of the corpus callosum are larger than those in the centrum semiovale at Stage II. The preferential thinning of corpus callosum was considered to be formed in relation to peculiar morphological alteration of microglia there in ALSP, instead, the “focal corpus callosum lesions” revealed by MRI in patients with ALSP are formed in connection with the lesions in the centrum semiovale.

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Ethical approval

This study was approved by the Ethics Committee of Shinshu University, Japan (No. 4415).
Informed consent

Informed consent was obtained from all individual patients and control subjects included in the present study.

Credit author statement

M Kinoshita, K Oyanagi; planning and organizing the research, K Ishizawa, K Ishihara, M Yoshida, T Inoue, Y Mitsuyama, Y Sekijima, S Ikeda, Y Kondo; providing cases examined and analysis of the clinical data, K Yoshida; genetic analysis, M Yamada; immunohistochemistry. The corresponding author is responsible for ensuring that the descriptions are accurate and agreed by all authors.

Declaration of Competing Interest

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

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Fig. 6. CD 68-immunopositive microglial cells. Oval-shaped amoeboid cells with a maximal diameter of 20 μm were scattered in both the centrum semiovale (A) and corpus callosum body (B) at Stage I. Many were present in the centrum semiovale (C), the body level (D) and dorsal part of the splenium of the corpus callosum in Patient #2 corresponding to the “focal lesions” revealed by MRI (E), but few were evident in the ventral part of the splenium (F) at Stage II. However, a few cells less than 10 μm in diameter were seen in the centrum semiovale and body of the corpus callosum at Stages III and IV (G–J). CD68 immunohistochemistry (brown). Bars: 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
