ORIGINAL ARTICLE

Clinical and genetic characterization of adult-onset leukoencephalopathy with axonal spheroids and pigmented glia associated with *CSF1R* mutation

T. Konno[a,b], K. Yoshida[c], T. Mizuno[d], T. Kawarai[e], M. Tada[b], H. Nozaki[f], S.-I. Ikeda[g], M. Nishizawa[b], O. Onodera[h], Z. K. Wszolek[a] and T. Ikeuchi[i]

[a]*Department of Neurology, Mayo Clinic, Jacksonville, FL, USA;* [b]*Department of Neurology, Niigata University, Niigata;* [c]*Department of Brain Disease Research, Shinshu University School of Medicine, Matsumoto;* [d]*Department of Neurology, Kyoto Prefectural University of Medicine, Kyoto;* [e]*Department of Clinical Neuroscience, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima;* [f]*Department of Medical Technology, School of Health Sciences Faculty of Medicine, Niigata University, Niigata;* [g]*Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto;* [h]*Department of Molecular Neuroscience, Niigata University, Niigata; and* [i]*Department of Molecular Genetics, Brain Research Institute, Niigata University, Niigata, Japan*

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Background and purpose: The clinical characteristics of *colony stimulating factor 1 receptor* (*CSF1R*) related adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) have been only partially elucidated.
Methods: Clinical data from *CSF1R* mutation carriers who had been seen at our institutions or reported elsewhere were collected and analysed using a specific investigation sheet to standardize the data.
Results: In all, 122 cases from 90 families with *CSF1R* mutations were identified. The mean age of onset was 43 years (range 18–78 years), the mean age at death was 53 years (range 23–84 years) and the mean disease duration was 6.8 years (range 1–29 years). Women had a significantly younger age of onset than men (40 vs. 47 years, $P = 0.0006$, 95% confidence interval 3.158–11.177). There was an age-dependent penetrance that was significantly different between the sexes ($P = 0.0013$). Motor dysfunctions were the most frequent initial symptom in women whose diseases began in their 20s. Thinning of the corpus callosum, abnormal signalling in pyramidal tracts, diffusion-restricted lesions and calcifications in the white matter were characteristic imaging findings of ALSP. The calcifications were more frequently reported in our case series than in the literature (54% vs. 3%). Seventy-nine per cent of the mutations were located in the distal part of the tyrosine kinase domain of *CSF1R* (102 cases). There were no apparent phenotype–genotype correlations.
Conclusions: The characteristics of ALSP were clarified. The phenotype of ALSP caused by *CSF1R* mutations is affected by sex.

Introduction

Hereditary diffuse leukoencephalopathy with spheroids (HDLS) causes dementia, psychiatric symptoms, parkinsonism, seizures and other neurological symptoms and typically begins when patients are in their 40s and 50s [1]. HDLS was first described and pathologically defined in a Swedish family in 1984 [2]. In 2012, *colony stimulating factor 1 receptor* (*CSF1R*) was identified as the causative gene for HDLS [1]. Mutations in *CSF1R* have also been found in families with pigmented orthochromatic leukodystrophy [3], which is another disease affecting the white matter and is clinically and pathologically similar to HDLS. Based on these observations, the name of adult-onset leukoencephalopathy with axonal spheroids and

Correspondence: T. Ikeuchi, Department of Molecular Genetics, Brain Research Institute, Niigata University, 1-757 Asahimachi, Niigata 951-8585, Japan (tel.: +81 25 227 2343; fax: +81 25 227 0793; e-mail: ikeuchi@Bri.Niigata-U.Ac.Jp).

pigmented glia (ALSP) was proposed to encompass both of these *CSF1R*-related diseases [3].

Before discovering the causative gene, only few cases with ALSP had been reported because a neuropathological examination was needed for a definitive diagnosis. Since the clinical presentation of ALSP has not been clearly elucidated, many patients have been clinically misdiagnosed as having Alzheimer's disease [4], frontotemporal dementia [1], corticobasal degeneration [5], multiple sclerosis (MS) [1] or cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [1].

In this study, the aim was to characterize the clinical, radiological and genetic features of ALSP caused by *CSF1R* mutations using data from our case series and a literature review.

Methods

Study subjects and extracting data

In total, 26 Japanese ALSP cases from 24 families carrying *CSF1R* mutations were selected. These cases were identified from suspected ALSP cases referred to our institutions for genetic testing. Genetic analyses were conducted under the approval of the ethics committee of each institution. Whilst a majority of these cases have already been published [4,6–15], seven unpublished cases were included. To extract the detailed clinical phenotypes of ALSP, an investigation sheet was created (Table S1). The sheet covers the following information: sex, current status (living or deceased), age of onset, age of death (if applicable), initial symptoms, the type of mutation, the patient's family history, the mode of inheritance, the family pedigree, the presence of a neuropathological diagnosis, 17 clinical symptoms and 14 brain imaging findings. The sheets were filled out by the attending neurologist using the patient's medical record. The findings observed throughout the disease were collected on the data sheets and analysed.

Next, the literature on ALSP cases with *CSF1R* mutations reported from other institutions since 2012 was reviewed using the search terms 'adult-onset leukoencephalopathy with axonal spheroids and pigmented glia', 'hereditary diffuse leukoencephalopathy with spheroids' and 'CSF1R' in PubMed (searched in December 2015). Ninety-nine *CSF1R* mutation carriers in 66 families were identified, including one obligate carrier [16–39]. After excluding three asymptomatic carriers, the investigation sheets were filled out for 96 symptomatic carriers based on the information described in the literature.

Data analysis

The Student *t* test was used for continuous data and Pearson's chi-squared test and Fisher's exact test (two-tailed) for categorical data. Using the data from cases for whom the disease duration from onset to death could be calculated, a Kaplan–Meier curve of the probability of survival was drawn and the difference between sexes was assessed by a log-rank test. The disease penetrance was estimated using a Kaplan–Meier curve and the difference between sexes was compared using a log-rank test. $P < 0.05$ was considered as statistically significant. All statistical analyses were performed in JMP® Pro 10 (SAS Institute Inc., Cary, NC, USA).

Results

Clinical data

A total of 122 ALSP cases from 90 families carrying *CSF1R* mutations were identified (Table S2). There were 52 men, 66 women and four cases whose sex was not reported. There was no significant difference in the number of cases between men and women ($P = 0.198$). As a whole, the age of onset was 43 ± 11 years (mean ± SD, range 18–78 years), the age of death was 53 ± 12 years (mean ± SD, range 23–84 years) and the disease duration was 6.8 ± 5.4 years (mean ± SD, range 1–29 years). Interestingly, the mean age of onset for women was significantly younger than that for men [40 vs. 47 years, $P = 0.0006$, 95% confidence interval (CI) 3.158–11.177] (Fig. 1a). There was no significant difference in disease duration or the probability of survival between men and women [6.1 ± 3.2 vs. 6.6 ± 5.8 years (mean ± SD); $P = 0.708$, $P = 0.815$, respectively] (Fig. 1b). The disease penetrance of *CSF1R* mutation carriers was age dependent: 10% at age 27 years, 50% at age 43 years and 95% at age 60 years ($n = 117$, a median of 43 years, 95% CI 41–45) (Fig. 1c). There was a significant difference between men and women ($P = 0.0013$): a median of 45 years ($n = 51$, 95% CI 44–51) and 40 years ($n = 62$, 95% CI 38–42), respectively (Fig. 1c).

Initial symptoms were described for 106 cases. The frequencies of the initial symptoms included 59% (63/106) affected by cognitive impairment, 44% (47/106) affected by psychiatric symptoms, 38% (40/106) affected by motor dysfunction, 19% (20/106) affected by speech problems and 8% (9/106) affected by other symptoms, such as stroke-like episodes, sensory disturbance, dizziness, fatigue and epilepsy (Fig. 2a). Reported psychiatric symptoms included anxiety,

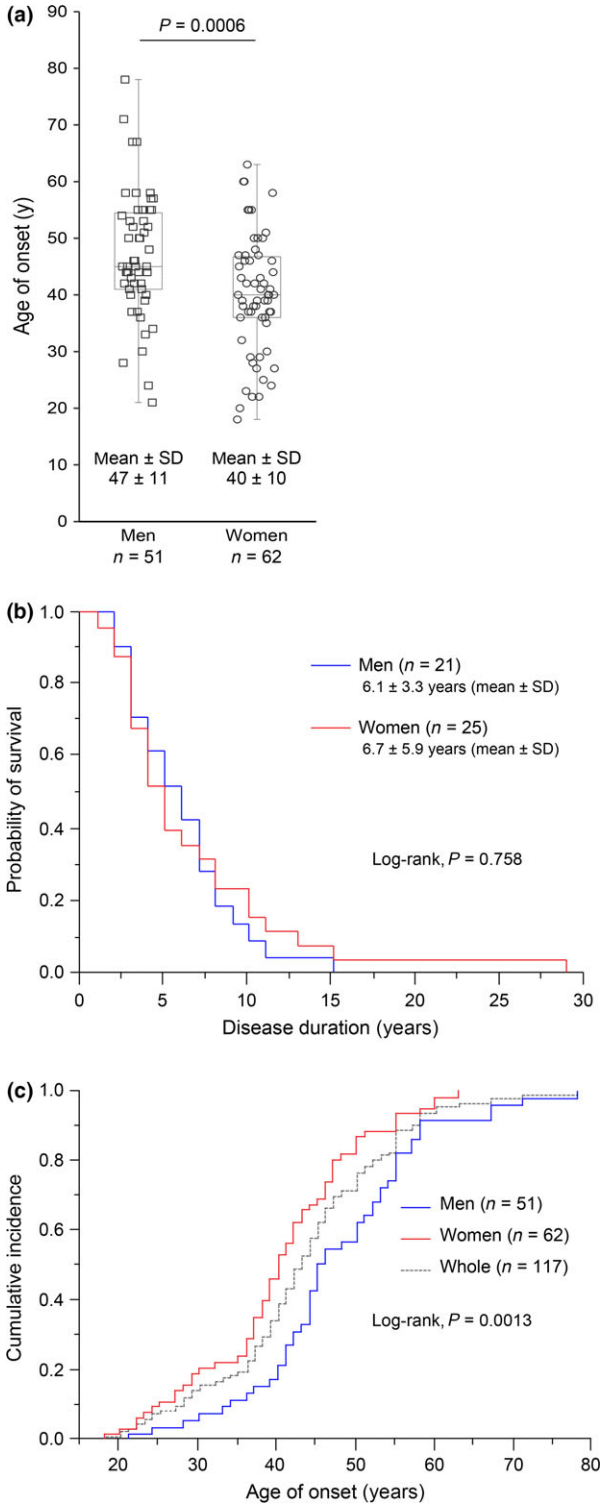

Figure 1 Distribution and disease penetrance of ALSP cases with *CSF1R* mutation. (a) The mean age of onset in women was significantly younger than that of men. (b) Kaplan–Meier curves showing the difference in probability of survival between sexes. There was no significant difference. (c) Kaplan–Meier curves showing the age-dependent penetrance. There was a significant difference between sexes.

depression, apathy, indifference, abulia, irritability, disinhibition, distraction and other behavioural and personality changes. The symptom 'motor dysfunction' referred to parkinsonian symptoms, gait disturbances and spasticity. Speech problems were defined as cases with difficulties speaking or finding words. Half of these cases (55/106) showed two or more symptoms during the early phases of the disease. Cognitive impairment or psychiatric symptoms were observed most frequently in both sexes at every age of onset, but it should be noted that motor dysfunction was found at an exceptionally high rate in women affected in their 20s (Fig. 2b, c).

Based on the detailed clinical data from 26 cases from our cohort, cognitive impairment (96% of subjects), psychiatric symptoms (81%) and pyramidal signs (81%) occurred with the highest frequency throughout the disease (Table 1). Following these were frontal lobe dysfunction (77%), parkinsonism (65%), dysarthria (54%) and dysphagia (50%). Seizure was observed in 31% of the cases. The type of seizures was not always described, but generalized seizure seemed to be the most common. None of these cases showed stroke-like episodes or peripheral neuropathy. Similar results were obtained when our data were combined with those from the literature, but the frequency of each symptom tended to be lower than that of our cohort because of the increased amount of missing data. When the number of men and women with each symptom were compared using the combined data, there was no significant difference (Table 1). Twenty-three cases that had been bedridden were also found and the duration of time from disease onset to incapacitation of 22 of these cases were calculated (one case was excluded because the date when this case had been bedridden could not be obtained). The duration was 3.9 ± 2.7 years (mean $\pm$ SD, range 0–11 years), and there was no significant difference between men and women [3.8 ± 2.4 years ($n = 9$) vs. 3.3 ± 2.2 years ($n = 12$), respectively; $P = 0.666$; the sex of one case was unreported].

Radiological data

Dilation of the lateral ventricles (100%), bilateral white matter lesions (96%), cortical atrophy (92%), thinning of the corpus callosum (88%) and abnormal signalling in the corpus callosum (88%) were the most common magnetic resonance imaging (MRI) findings in our cohort (Table 1). Abnormal signalling in the pyramidal tracts was observed in 58% of cases. Diffusion-weighted imaging revealed diffusion-restricted lesions in 38%. However, gadolinium-enhanced lesions were not detected in any case. Calcifications in the white matter detected by computed tomography (CT) scan were seen in more than half of the cases (54%),

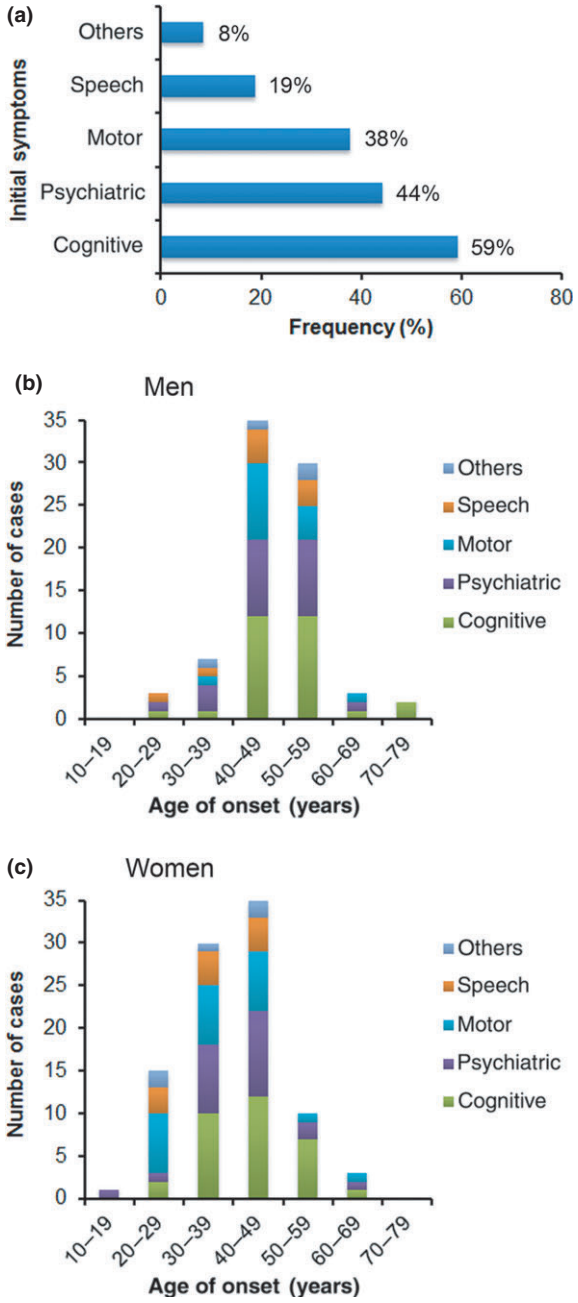

Figure 2 Initial symptoms of ALSP cases with *CSF1R* mutation. (a) Frequency of four major initial symptoms in all cases with a *CSF1R* mutation: cognitive, cognitive impairment; psychiatric, psychiatric symptoms; motor, motor dysfunction; speech, speech problem. Other symptoms include stroke-like episodes, sensory disturbances, dizziness, fatigue and epilepsy. (b), (c) Initial symptoms in both sexes were distributed based on age of onset. Note that motor dysfunction was more often observed in younger women and was observed more frequently than cognitive impairment in affected women in their 20s.

which was strikingly higher than in the literature (3%). There was no significant difference in brain imaging features between the sexes.

Genetic data

Thirty-six cases (40% of all families) had no family history of ALSP. All of the identified mutations are shown in Fig. 3a. There were 58 mutations: 47 missense mutations, seven splice-site mutations, two frameshift mutations, one nonsense mutation and one deletion mutation. All of these mutations were located within the tyrosine kinase domain (TKD) encoded by exons 12–21, but the p.Thr567fsX44 mutation found in exon 12 was outside of the TKD and the p.Ser688GlufsX13 mutation in exon 15 was within the kinase insertion domain, which interrupts the TKD [40]. Mutations were found more frequently in the distal kinase domain encoded by exons 17–21 (46 mutations) than in the proximal kinase domain encoded by exons 12–15 (10 mutations). The most common mutation was a p.Ile794Thr mutation in exon 18 and was found in 14 families around the world, including eight Japanese families, two US families, two German families, one Dutch family and one Taiwanese family. The regional distribution of mutations is shown in Fig. 3b.

Phenotypic correlation

Clinical and radiological findings between cases who had mutations in the proximal kinase domain (exons 12–15) and cases who had mutations in the distal kinase domain (exons 17–21) were also compared because mutations were 4.6 times more likely to be found in the distal kinase domain (Table 2). Two cases with frameshift mutations were excluded in this analysis because frameshift mutations can occur independently of the TKD. Whilst the number of mutation carriers was significantly higher in the distal kinase domain than in the proximal (102 cases vs. 18 cases, $P < 0.0001$), there was no significant difference in the age of onset [43 ± 11 years vs. 44 ± 13 years (mean ± SD), $P = 0.724$], the age at death [52 ± 11 years vs. 55 ± 15 years (mean ± SD), $P = 0.395$] or the disease duration [5.9 ± 4.5 years vs. 7.1 ± 3.8 years (mean ± SD), $P = 0.413$] (Table 2). No difference was found in the clinical and brain imaging features between the two groups, but seizures were observed more frequently in cases who had mutations in the proximal kinase domain ($P = 0.040$) (Table 2).

Discussion

Based on our study, it is considered that the core clinical manifestations of ALSP include the following. (i) Initial/major symptoms: A majority of cases show cognitive impairment as their initial symptom at approximately the age of 40 years. Psychiatric

Table 1 Clinical and brain imaging features of ALSP cases with *CSF1R* mutations

	Our cohort (n = 26)		Total (n = 122)		Men (n = 52)		Women (n = 66)		
	Yes, n (%)	No, n (%)	Yes, n (%)	No, n (%)	Yes, n (%)	No, n (%)	Yes, n (%)	No, n (%)	P value[a]
Clinical features									
Cognitive impairment	25 (96)	0 (0)	115 (94)	1 (1)	50 (96)	0 (0)	61 (92)	1 (2)	1.000
Psychiatric symptoms[b]	21 (81)	3 (12)	92 (75)	3 (2)	43 (83)	0 (0)	47 (71)	3 (5)	0.246
Parkinsonism	17 (65)	7 (27)	74 (61)	10 (8)	34 (65)	3 (6)	38 (58)	7 (11)	0.500
Pyramidal signs[c]	21 (81)	5 (19)	69 (57)	10 (8)	25 (48)	6 (12)	42 (64)	4 (6)	0.189
Seizure	8 (31)	16 (62)	39 (32)	33 (27)	15 (29)	17 (33)	23 (35)	16 (24)	0.347
Frontal lobe dysfunction	20 (77)	2 (8)	38 (31)	2 (2)	15 (29)	1 (2)	20 (30)	1 (2)	1.000
Pathological reflexes	11 (42)	11 (42)	20 (16)	12 (10)	4 (8)	7 (13)	15 (23)	5 (8)	0.056
Aphasia	11 (42)	7 (27)	45 (37)	9 (7)	22 (42)	3 (6)	23 (35)	6 (9)	0.480
Apraxia	6 (23)	8 (31)	42 (34)	9 (7)	19 (37)	4 (8)	20 (30)	5 (8)	1.000
Dysarthria	14 (54)	6 (23)	41 (34)	11 (9)	21 (40)	4 (8)	20 (30)	7 (11)	0.503
Dysphagia	13 (50)	9 (35)	21 (17)	9 (7)	8 (15)	4 (8)	13 (20)	5 (8)	1.000
Ataxia	6 (23)	14 (54)	33 (27)	20 (16)	13 (25)	8 (15)	19 (29)	12 (18)	1.000
Sensory disturbance	2 (8)	16 (62)	12 (10)	22 (18)	6 (12)	10 (19)	6 (9)	12 (18)	1.000
Involuntary movements[d]	3 (12)	16 (62)	26 (21)	23 (19)	11 (21)	12 (23)	14 (21)	10 (15)	0.564
Stroke-like episode	0 (0)	25 (96)	3 (2)	35 (29)	2 (4)	13 (25)	1 (2)	19 (29)	0.565
Peripheral neuropathy	0 (0)	20 (77)	3 (2)	26 (21)	2 (4)	12 (23)	1 (2)	14 (21)	0.598
Bedridden	11 (42)	14 (54)	23 (19)	15 (12)	9 (17)	6 (12)	13 (20)	9 (14)	1.000
Brain imaging features[e]									
Bilateral white matter lesions	25 (96)	0 (0)	84 (69)	0 (0)	36 (69)	0 (0)	45 (68)	0 (0)	
Thinning of the corpus callosum	23 (88)	2 (8)	60 (49)	9 (7)	28 (54)	3 (6)	30 (45)	6 (9)	0.489
Abnormal signal in the corpus callosum	23 (88)	2 (8)	53 (43)	6 (5)	24 (46)	2 (4)	29 (44)	4 (6)	0.685
Abnormal signal in the pyramidal tracts	15 (58)	8 (31)	28 (23)	20 (16)	10 (19)	10 (19)	18 (27)	10 (15)	0.382
Diffusion-restricted lesions	10 (38)	8 (31)	20 (16)	14 (11)	7 (13)	7 (13)	13 (20)	4 (6)	0.154
Gadolinium-enhanced lesions	0 (0)	7 (27)	0 (0)	17 (14)	0 (0)	4 (8)	0 (0)	10 (15)	
Microbleeds on T2*-weighted MRI	1 (4)	9 (35)	1 (1)	9 (7)	1 (2)	4 (8)	0 (0)	5 (8)	1.000
Calcifications in the white matter	14 (54)	6 (23)	17 (14)	8 (7)	6 (12)	2 (4)	11 (17)	6 (9)	1.000
Dilation of the lateral ventricles	26 (100)	0 (0)	69 (57)	4 (3)	30 (58)	2 (4)	36 (55)	2 (3)	1.000
Cortical atrophy	24 (92)	2 (8)	78 (64)	5 (4)	32 (62)	2 (4)	43 (65)	3 (5)	1.000
Brainstem atrophy	4 (15)	20 (77)	5 (4)	41 (34)	1 (2)	19 (37)	4 (6)	22 (33)	0.369
Abnormal signal in brainstem	4 (15)	19 (73)	7 (6)	36 (30)	2 (4)	18 (35)	5 (8)	18 (27)	0.421
Cerebellar atrophy	3 (12)	20 (77)	7 (6)	38 (31)	4 (8)	16 (31)	3 (5)	22 (33)	0.682
Abnormal signal in cerebellum	0 (0)	23 (88)	1 (1)	41 (34)	0 (0)	19 (37)	1 (2)	22 (33)	1.000

MRI, magnetic resonance imaging. Missing data were excluded from these calculations. Therefore the total percentage may not reach 100%.
[a]Comparison of each clinical and brain imaging feature between sexes; [b]including anxiety, depression, apathy, indifference, abulia, irritability, disinhibition, distraction and other behaviour and personality changes; [c]including hyperreflexia, spasticity, increased tone in extremities and pseudobulbar palsy; [d]including tremor, myoclonus, dyskinesia and dystonia; [e]all findings were assessed by MRI scan, but calcifications were detected by computed tomography scan.

symptoms, motor dysfunction and speech problems appear frequently during the disease course and can be an initial symptom. (ii) Rapid disease course: It takes 3.9 years from onset to incapacitation and 6.8 years to death. (iii) MRI findings: Dilation of the lateral ventricles to a size larger than what is acceptable for the patient's age and bilateral white matter lesions without gadolinium enhancement are observed. Thinning of the corpus callosum accompanied by signalling abnormalities is also highly detectable, even in the early phases of the disease [12,13]. Abnormal signalling in the pyramidal tracts and diffusion-restricted lesions in the white matter are supportive of a diagnosis of ALSP. (iv) CT findings: Calcifications are present in the white matter [11,12,22,38,39].

Of note, in addition to the younger age of onset in women, motor dysfunction was the most frequent initial symptom seen in women in their 20s. These findings provide new insights into ALSP because no clinical differences between the sexes have been described previously. These indicate that some young women could present symptoms similar to those seen in non-dementia disorders, such as MS or spastic paraplegia, during the earliest phases of the disease. Indeed, the young women reported to have ALSP in the literature were initially diagnosed as having MS, particularly the primary progressive form, and received immunotherapy without benefit [8,10,14,31]. Thus, clinicians should keep in mind that ALSP may be a differential diagnosis for MS, especially in young women. Therefore, unnecessary

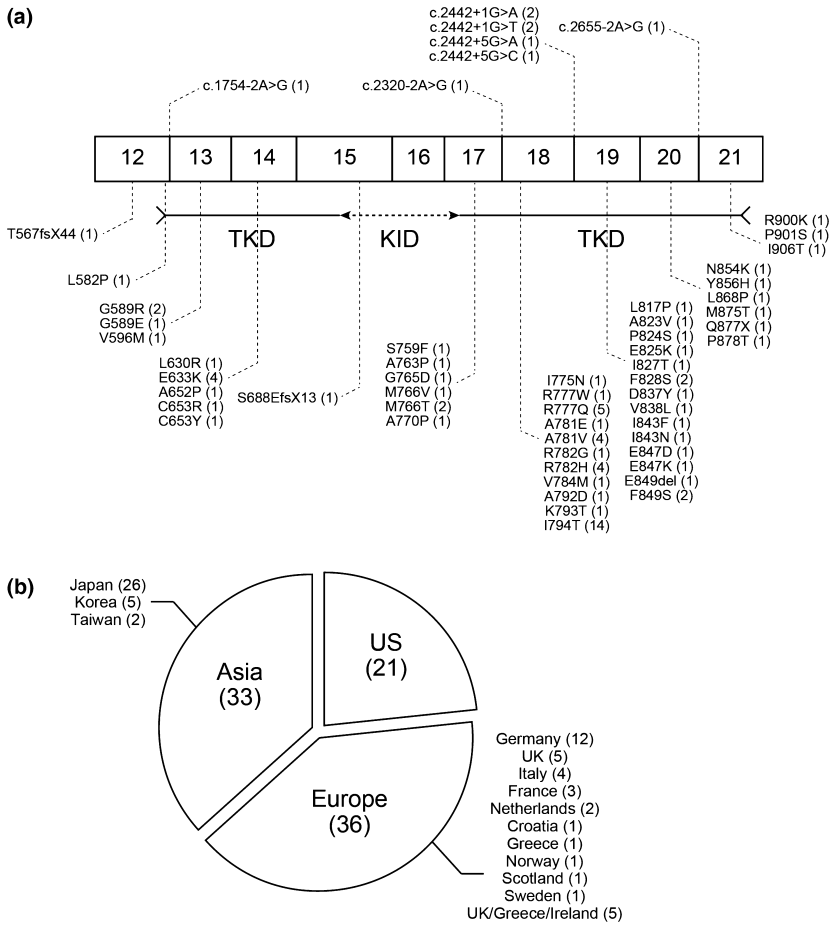

Figure 3 *CSF1R* gene diagram with identified mutations and regional distribution of families carrying *CSF1R* mutations. (a) Exons 12–21 encoding protein tyrosine kinase of CSF1R and the protein tyrosine kinase domain (TKD) are shown based on the information from UniProt (http://www.uniprot.org/uniprot/P07333). TKD is interrupted by the kinase insert domain (KID). Splice-site mutations are put above the diagram, and the other mutations are listed below. All mutations were located within TKD except the T567fsX44 and S688EfsX13 mutations. The numbers in parentheses represent the number of families. (b) Mutation-positive families have been reported in the USA, Europe and Asia. The numbers in parentheses represent the number of families. Multiple locations separated by a slash indicate a mixed cohort from those regions.

immunotherapy treatment should be avoided. The reason why there is a different age of onset and presentation in women is uncertain. In a study of the *Csf1r* haploinsufficient mouse model of ALSP, depression and anxiety-like behaviour were observed only in male mice [41], but this was not seen in our study.

The thinning of the corpus callosum and the presence of brain calcifications are characteristic brain imaging findings of ALSP. These were more frequently observed in our case series than in the literature. Whilst the thinning of the corpus callosum could be seen in other leukoencephalopathies [42], the calcifications seem to be specific to ALSP [12,22]. Not much information about the calcifications could be obtained from the cases that were reviewed in the literature because brain CT findings were rarely described. Therefore, further study is needed to verify this observation.

The number of ALSP cases confirmed by genetic analysis has been increasing in various populations, suggesting that the prevalence of ALSP is higher than previously thought. *CSF1R* mutations have been reported in families from the USA, Europe and Asia, but they have been most frequently observed in Japan; however, there seems to be no obvious relationship between specific mutations, country or race (Table S2). Whilst ALSP is usually inherited in an autosomal dominant fashion, it should be noted that sporadic cases comprise 40% of all families. The reason for the high frequency of sporadic cases is partly explained by the presence of *de novo* mutations [1,7,8,23]. In addition, the age-dependent penetrance plays a role, as shown in Fig. 1c. Indeed, 69- and 79-year-old asymptomatic mutation carriers have been reported [23,27]. Although there was no obvious

Table 2 Clinical and brain imaging features of ALSP cases with *CSF1R* mutations: a comparison between the proximal and distal kinase domains

	Proximal kinase domain (exons 12–15)		Distal kinase domain (exons 17–21)		*P* value
Number of cases	18		102		<0.0001
Men	5 (28%)		47 (46%)		0.195
Age of onset (years) (mean ± SD)	44 ± 13		43 ± 11		0.724
Age of death (years) (mean ± SD)	55 ± 15		52 ± 11		0.395
Disease duration (years) (mean ± SD)	7.1 ± 3.8		5.9 ± 4.5		0.413
	Yes, *n* (%)	No, *n* (%)	Yes, *n* (%)	No, *n* (%)	*P* value
Clinical features					
Cognitive impairment	15 (83)	1 (6)	99 (97)	0 (0)	0.139
Psychiatric symptoms[a]	14 (78)	0 (0)	77 (75)	3 (3)	1.000
Parkinsonism	11 (61)	1 (6)	63 (62)	8 (8)	1.000
Pyramidal signs[b]	13 (72)	1 (6)	54 (53)	9 (9)	0.679
Seizure	12 (67)	3 (17)	27 (26)	29 (28)	0.040[e]
Frontal lobe dysfunction	5 (28)	0 (0)	32 (31)	2 (2)	1.000
Pathological reflexes	2 (11)	2 (11)	18 (18)	10 (10)	0.620
Aphasia	9 (50)	1 (6)	36 (35)	8 (8)	1.000
Apraxia	7 (39)	1 (6)	35 (34)	8 (8)	1.000
Dysarthria	7 (39)	3 (17)	34 (33)	9 (9)	0.677
Dysphagia	2 (11)	1 (6)	18 (18)	8 (8)	1.000
Ataxia	4 (22)	3 (17)	30 (29)	17 (17)	1.000
Sensory disturbance	1 (6)	2 (11)	11 (11)	19 (19)	1.000
Involuntary movements[c]	7 (39)	1 (6)	19 (19)	23 (23)	0.050
Stroke-like episode	1 (6)	5 (28)	2 (2)	29 (28)	0.422
Peripheral neuropathy	0 (0)	4 (22)	2 (2)	22 (22)	1.000
Bedridden	4 (22)	2 (11)	18 (18)	13 (13)	1.000
Brain imaging features[d]					
Bilateral white matter lesions	10 (56)	0 (0)	73 (72)	0 (0)	
Thinning of the corpus callosum	6 (33)	2 (11)	53 (52)	7 (7)	0.285
Abnormal signal in the corpus callosum	6 (33)	2 (11)	46 (45)	4 (4)	0.189
Abnormal signal in the pyramidal tracts	4 (22)	4 (22)	23 (23)	16 (16)	0.707
Diffusion-restricted lesions	3 (17)	1 (6)	17 (17)	13 (13)	0.627
Gadolinium-enhanced lesions	0 (0)	4 (22)	0 (0)	13 (13)	
Microbleeds on T2*-weighted MRI	0 (0)	1 (6)	1 (1)	8 (8)	1.000
Calcifications in the white matter	4 (22)	0 (0)	13 (13)	8 (8)	0.269
Dilation of the lateral ventricles	10 (56)	0 (0)	58 (57)	4 (4)	1.000
Cortical atrophy	12 (67)	0 (0)	65 (64)	5 (5)	1.000
Brainstem atrophy	1 (6)	6 (33)	3 (3)	35 (34)	0.504
Abnormal signal in brainstem	2 (11)	5 (28)	5 (5)	31 (30)	0.318
Cerebellar atrophy	2 (11)	5 (28)	5 (5)	33 (32)	0.296
Abnormal signal in cerebellum	1 (6)	6 (33)	0 (0)	35 (34)	0.167

MRI, magnetic resonance imaging. Missing data were excluded from these calculations. Therefore the total percentage may not reach 100%.
[a]Including anxiety, depression, apathy, indifference, abulia, irritability, disinhibition, distraction and other behaviour and personality changes; [b]including hyperreflexia, spasticity, increased tone in extremities and pseudobulbar palsy; [c]including tremor, myoclonus, dyskinesia and dystonia; [d]all findings were assessed by MRI scan, but calcifications were detected by computed tomography scan; [e]significant difference between the proximal and distal kinase domains.

phenotype–genotype correlation, it should be noted that mutations tend to occur more frequently in the distal part of the TKD than the proximal part. Given that almost all mutations were located within the TKD of *CSF1R*, the loss of tyrosine kinase activity may be necessary for the development ALSP. Functional analysis has already shown that some mutant CSF1R proteins have a loss of function [1,3,12]. Additionally, it has been suggested that the frameshift mutations, which were located outside the TKD, caused ALSP by haploinsufficiency [12]. This implies that frameshift mutations located in more proximal coding regions (exons 2–11) or large deletion mutations that result in loss of TKD could be causative.

This study had several limitations. First, the clinical and radiological data from ALSP cases with *CSF1R* mutations were collected retrospectively. It was possible to extract more detailed information from the 26 cases who were seen and analysed at our institutions, whereas the information from the other cases depended on the description in the literature. This may have led to bias. Our study was also limited by

missing data; there was a tendency to describe only positive symptoms in the literature, and thus the clinical characteristics presented above may more accurately reflect our Japanese cases. Another limitation is that the pathogenesis of identified mutations has not always been proven by functional or segregation analyses. All of the missense mutations were predicted to be 'probably damaging' with score 0.972–1.000 using an *in silico* analysing tool, PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), but one mutation, p.Leu817Pro, was estimated to be 'possibly damaging' with score 0.562 (Table S2).

This study clarified the core features and several characteristics of ALSP and revealed that there was a difference in the clinical presentation between men and women. Considering that ALSP could occur in any population, our findings could be useful to clinical practices and could promote the diagnosis of cases.

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Disclosure of conflicts of interest

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Investigation sheet.
Table S2. List of 122 cases with *CSF1R* mutation included in our study.

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