ORIGINAL ARTICLE

Factors predictive of the presence of a *CSF1R* mutation in patients with leukoencephalopathy

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Background and purpose: The purpose was to identify statistically factors that correlate with the presence of a colony-stimulating factor 1 receptor (*CSF1R*) mutation and to reevaluate the accuracy of the current diagnostic criteria for *CSF1R*-related leukoencephalopathy.

Methods: *CSF1R* testing was conducted on 145 consecutive leukoencephalopathy cases who were clinically suspected of having adult-onset leukoencephalopathy with axonal spheroids and pigmented glia. From these, 135 cases whose detailed clinical information was available were enrolled. Forward logistic stepwise regression was performed to generate a probability model to predict a positive *CSF1R* mutation result. The current diagnostic criteria were also applied to our cohort and their sensitivity and specificity were calculated.

Results: Twenty-eight *CSF1R*-mutation-positive cases and 107 *CSF1R*-mutation-negative cases were identified. Our probability model suggested that factors raising the probability of a *CSF1R*-mutation-positive result were younger onset, parkinsonism, thinning of the corpus callosum and diffusion-restricted lesions. It also showed that involuntary movements and brainstem or cerebellar atrophy were negative predictors of a *CSF1R*-mutation-positive result. In our cohort, the sensitivity and specificity for 'probable' or 'possible' *CSF1R*-related leukoencephalopathy were 81% and 14%, respectively.

Conclusions: Clinical and brain imaging features predictive of the presence of a *CSF1R* mutation are proposed. Consideration of these factors will help prioritize patients for *CSF1R* testing.

Introduction

Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) was previously referred to as hereditary diffuse leukoencephalopathy with axonal spheroids or pigmentary orthochromatic leukodystrophy [1,2] due to mutations in the colony-stimulating factor 1 receptor (*CSF1R*) gene [3]. Thereafter, a similar phenotype was reported in individuals with biallelic pathogenic variants in the alanyl-tRNA synthetase 2 (*AARS2*) gene [4,5]. The nomenclature of ALSP has changed with a growing knowledge of its pathology and genetics. Recently, Konno *et al.* have proposed three different leukoencephalopathies: *CSF1R*-related leukoencephalopathy, *AARS2*-related leukoencephalopathy and *CSF1R*/*AARS2*-negative ALSP [6].

Since the discovery of *CSF1R* as the causative gene [3], more than 70 pathogenic mutations have been reported worldwide [6,7]. Recently, the diagnostic criteria for *CSF1R*-related leukoencephalopathy were established by a working group [8]. In these criteria, *CSF1R* testing is not required for the diagnosis of 'probable' and 'possible' cases; however, the diagnosis of such cases is not necessarily easy. For instance, ALSP cases have been misdiagnosed as vascular dementia, cerebral autosomal dominant arteriopathy with subcortical infarcts and

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leukoencephalopathy (CADASIL), multiple sclerosis, frontotemporal degeneration [3], corticobasal degeneration [9] and Alzheimer's disease [10]. As patients with dementia and white matter lesions are seen frequently, it is not realistic to perform *CSF1R* testing on all such cases. It seems clinically useful to predict the probability of a positive *CSF1R* result before genetic testing.

CSF1R genetic testing was done on cases with unidentified leukoencephalopathy in response to requests from clinicians nationwide. The aim of this study was to identify statistically factors that correlate with a positive *CSF1R* result and to reevaluate the accuracy of the current diagnostic criteria for *CSF1R*-related leukoencephalopathy in our cohort.

Methods

Subjects

In all, 145 consecutive leukoencephalopathy cases clinically suspected of having ALSP who were referred to our laboratory for *CSF1R* testing from December 2011 to August 2019 were examined. All the requests of *CSF1R* testing were accepted without any consideration of clinical features. In this study, 135 cases whose clinical information sheet (details shown below) was available were enrolled and 10 cases with the sheet unavailable were excluded. Of 135, 35 overlapped with the previous study [8]. This study was approved by the institutional review board of Shinshu University (no. 643), and written informed consent was obtained from all patients or their caregivers by the chief physician of each institution.

Genetic analysis

Genomic DNA was isolated from peripheral leukocytes from the cases using a Gentra Puregene Blood Kit (QIAGEN, Hilden, Germany). Exons 12–22 of *CSF1R*, covering the mutation 'hot spots' in *CSF1R*-related leukoencephalopathy patients, were amplified by polymerase chain reaction (PCR) according to a previous report [3]. The PCR products were purified and subjected to direct sequencing. Six precomputed deleteriousness prediction scores (SIFT, PolyPhen-2, MutationTaster, CADD, MetaLR and Mutation Assessor) were used to predict the effects of the identified missense variants. Population allele frequencies were taken from the 1000 Genomes Project and the Exome Aggregation Consortium.

Clinical data collection

A clinical information sheet for this study was created to extract the detailed clinical phenotypes of cases with *CSF1R*-related leukoencephalopathy. The sheet covers the following information: age, sex, current status (living or deceased), age at onset, initial symptoms, clinical history, family history, past history, drinking history, modified Rankin Scale (mRS), 12 clinical features (cognitive impairment, confusion, psychiatric symptoms, dysphasia, motor paralysis, cerebellar ataxia, parkinsonism, pyramidal signs, involuntary movements, epilepsy, sensory disturbance and gait disturbance) and nine brain imaging features [cortical atrophy, cerebral white matter lesions, thinning of the corpus callosum, abnormal signal in the corpus callosum, brainstem or cerebellar atrophy, abnormal signal in the brainstem or cerebellum, diffusion-restricted lesions, microbleeds on T2*-weighted magnetic resonance imaging (MRI) and periventricular calcification on computed tomography (CT) scan]. The data were categorized as continuous variables (age at onset, age at testing and mRS), categorical variables not answered with yes or no (sex and current status), categorical variables answered with yes or no (family history, drinking history, clinical features and brain imaging features) and other free descriptions (initial symptoms and clinical and past history). No clear standard for each clinical and brain imaging feature was made, so the judgment of the presence or absence of each feature was left to the attending physician.

Statistical analysis

The findings on the clinical information sheets were assessed using the Mann–Whitney *U* test for continuous variables and Fisher's exact test for categorical variables. Multiple factor analysis was performed using forward logistic stepwise regression. The objective variable was the presence or absence of a *CSF1R* mutation. The explanatory variables were age at onset, family history, drinking history, 12 clinical features and seven brain imaging features ('cerebral white matter lesion' was not analyzed because all cases had white matter lesions and 'periventricular calcification' was not analyzed because there were only a few cases who were investigated for this feature). The cases with missing data were excluded from the logistic regression analysis. The variable standards were as follows: a *P* value less than 0.05 or close to 0.05 was included, whilst a *P* value greater than 0.1 was excluded, and the *CSF1R*-mutation-related factors were screened. A logistic regression model was established, and the generalized determination coefficient (R^2) and the prediction list were used to evaluate the prediction accuracy of the model. A *P* value less than 0.05 was considered to indicate statistical significance. All analyses were performed using SPSS software version 24 (SPSS Inc., Chicago, IL, USA).

Validation of the diagnostic criteria

To calculate the sensitivity and specificity of the diagnostic criteria for *CSF1R*-related leukoencephalopathy established by Konno *et al*. [8], the criteria were applied to our 100 leukoencephalopathy cases who were not included in the original paper [8] based on the clinical information sheets. There was no 'definite' case because the criteria were applied to each case based on the clinical information sheets before *CSF1R* testing. Here, it was assumed that each physician had excluded other diseases before requesting *CSF1R* testing; in other words, that all cases fulfilled the core features 5.

Results

The number of cases in each table or figure is tabulated in Table S1. In the 135 leukoencephalopathy cases, 28 carried a *CSF1R* mutation (Table S2) and 107 were negative for a *CSF1R* mutation. The clinical and brain imaging features of these cases are summarized in Table 1. The mean age at onset and age at testing of the *CSF1R*-mutation-positive group were significantly younger than those of the *CSF1R*-mutation-negative group, but there was no significant difference in disease duration or sex ratio between the two groups. Cases with a positive family history were more commonly found in the *CSF1R*-mutation-positive group (61%) than in the *CSF1R*-mutation-negative group (33%).

The initial symptoms were categorized as cognitive impairment, psychiatric symptoms, motor dysfunction, speech problems and others. Speech problems, including difficulty speaking, aphasia and problems with speech rhythm, were observed more frequently in the *CSF1R*-mutation-positive group than in the *CSF1R*-mutation-negative group.

As for the clinical features, cognitive impairment, psychiatric symptoms and gait disturbance were the most common symptoms overall in both groups. Dysphasia was significantly more frequent in the *CSF1R*-mutation-positive group, whereas involuntary movements were significantly more frequent in the *CSF1R*-mutation-negative group.

As for brain imaging features, thinning of the corpus callosum was prominent in the *CSF1R*-mutation-positive group, whilst an abnormal signal in the brainstem or cerebellum and microbleeds on T2*-weighted MRI were highly suggestive of the *CSF1R*-mutation-negative group. Periventricular calcification on CT scan was also observed more frequently in the *CSF1R*-mutation-positive group (21% in the positive group versus 7% in the negative group).

Logistic analysis for a probability model for predicting the presence of a *CSF1R* mutation

The analysis included 92 cases [*CSF1R*-mutation-positive, 20 cases (13 men, seven women; age at testing 46.4 ± 11.5 years); *CSF1R*-mutation-negative, 72 cases (30 men, 42 women; age at testing 62.1 ± 10.4 years)]; 43 cases were excluded from the analysis because of insufficient data. The results are shown in Fig. 1. Age at onset, parkinsonism, involuntary movements, thinning of the corpus callosum, brainstem or cerebellar atrophy, and diffusion-restricted lesions were selected as independent variables (X_1, X_2, X_3, X_4, X_5 and X_6, respectively). The predictive model for a *CSF1R* mutation was as follows:

$$\mathrm{Logit}(P) = 0.861 + -0.115X_1 + 2.161X_2 + -3.860X_3 + 3.752X_4 + -4.253X_5 + 2.323X_6$$

Logit(P) means log [$(1 - P)/P$], where P is the probability of *CSF1R* mutation positive. The generalized determination coefficients of this regression model were Cox–Snell $R^2 = 0.441$ and Nagelkerke $R^2 = 0.680$; the prediction accuracy rate [the cut-off level of the probability of a *CSF1R* mutation (P) was 0.5; if P was greater than 0.5, it predicted the presence of a *CSF1R* mutation, and if P was less than 0.5, it predicted the absence of a *CSF1R* mutation] was 91.3%, which was considered high.

The probability of a *CSF1R*-mutation-positive result predicted by the model is shown in Fig. 2. It is clear that the probability of a *CSF1R* mutation became lower as the age at onset increased. Furthermore, if a case with unidentified leukoencephalopathy who developed by 60 years had three clinical and brain imaging features, i.e. parkinsonism, thinning of the corpus callosum and diffusion-restricted lesions, the probability that they had a *CSF1R* mutation was greater than 90%. Conversely, cases without these three factors had a probability of a *CSF1R* mutation of less than 20% (Fig. 2a). On the other hand, cases with brainstem or cerebellar atrophy or involuntary movements had a probability of a *CSF1R* mutation of less than 18% even if they showed thinning of the corpus callosum (Fig. 2b).

Accuracy of the current diagnostic criteria for *CSF1R*-related leukoencephalopathy

The number of cases fulfilling each designation is shown in Table 2. One case in the *CSF1R*-mutation-negative group had exclusionary findings such as stroke-like episodes more than twice, except for epilepsy; therefore, this case was included in the 'not

Table 1 Clinical and brain imaging features of *CSF1R*-mutation-positive and *CSF1R*-mutation-negative leukoencephalopathy

	CSF1R mutation				
	Positive (n = 28)		Negative (n = 107)		P value
Age at onset, years, mean ± SD (range)	43.7 ± 11.6 (20–62)		55.6 ± 12.9 (20–80)		<0.001[a]
Age at the test, years, mean ± SD (range)	47.6 ± 12.0 (24–64)		61.7 ± 11.6 (26–86)		<0.001[a]
Disease duration, years, mean ± SD (range)	4.0 ± 4.9 (0–20)		6.1 ± 7.2 (0–36)		0.138
Men, *n* (%)	18 (64)		48 (46)		0.093
Modified Rankin Scale, mean ± SD	3.3 ± 1.3		3.3 ± 1.4		0.679
Initial symptoms, *n* (%)					
Cognitive impairment	14 (50)		51 (48)		0.835
Psychiatric symptoms	4 (14)		11 (10)		0.513
Motor dysfunction	11 (39)		52 (49)		0.404
Speech problems	6 (21)		7 (7)		0.028[a]
Others	5 (18)		23 (21)		0.797
	Yes	No	Yes	No	
Family history, *n* (%)	17 (61)	11 (39)	35 (33)	72 (67)	0.009[a]
History of heavy drinking, *n* (%)	4 (14)	23 (82)	5 (5)	102 (95)	0.08
Clinical features, *n* (%)					
Cognitive impairment	23 (82)	5 (18)	85 (79)	22 (21)	1.000
Confusion	2 (7)	26 (93)	22 (21)	85 (79)	0.162
Psychiatric symptoms	23 (82)	5 (18)	77 (72)	30 (28)	0.339
Dysphasia	21 (75)	7 (25)	46 (43)	60 (56)	0.005[a]
Motor paralysis	10 (36)	18 (64)	41 (38)	66 (62)	0.831
Cerebellar ataxia	4 (14)	22 (79)	32 (30)	74 (69)	0.148
Parkinsonism[b]	13 (46)	15 (54)	35 (33)	72 (67)	0.190
Pyramidal signs[c]	21 (75)	7 (25)	59 (55)	47 (44)	0.083
Involuntary movements	2 (7)	26 (93)	30 (28)	77 (72)	0.024[a]
Epilepsy	8 (29)	20 (71)	16 (15)	91 (85)	0.103
Sensory disturbance	1 (4)	24 (86)	18 (17)	83 (78)	0.119
Gait disturbance	20 (71)	8 (29)	82 (77)	25 (23)	0.623
Brain imaging features, *n* (%)					
Cortical atrophy	23 (82)	5 (18)	78 (73)	25 (23)	0.615
Cerebral white matter lesion	28 (100)	0 (0)	106 (99)	0 (0)	1.000
Thinning of the corpus callosum	25 (89)	3 (11)	60 (56)	44 (41)	0.002[a]
Abnormal signal in the corpus callosum	19 (68)	8 (29)	49 (46)	54 (50)	0.050
Brainstem or cerebellar atrophy	4 (14)	24 (86)	31 (29)	74 (69)	0.147
Abnormal signal in the brainstem or cerebellum	3 (11)	24 (86)	39 (36)	65 (61)	0.010[a]
Diffusion-restricted lesions	18 (64)	8 (29)	47 (44)	53 (50)	0.050
Microbleeds on T2*-weighted MRI	3 (11)	20 (71)	31 (29)	50 (47)	0.025[a]
Periventricular calcification on CT scan	6 (21)	5 (18)	8 (7)	29 (27)	0.058

CSF1R, colony-stimulating factor 1 receptor gene; CT, computed tomography; MRI, magnetic resonance imaging. Missing data were excluded from these calculations; therefore the total percentage may not reach 100%. [a]Significant difference between the *CSF1R*-mutation-positive and *CSF1R*-mutation-negative groups; [b]including rigidity, bradykinesia, parkinsonian gait (small shuffling steps, freezing of gait, and falls), postural instability, masked face, resting tremor and responsiveness to L-dopa; [c]including hyperreflexia and Babinski's sign.

fulfilled' category. When diagnostic sensitivity was calculated as the ratio of the number of cases diagnosed as 'probable' or 'possible' to the total number of *CSF1R*-mutation-positive cases, the sensitivity was 81%. Twelve cases in the *CSF1R*-mutation-negative group were diagnosed as 'not fulfilled'; consequently the specificity was 14%.

Discussion

Twenty-eight cases (21%) carrying a *CSF1R* mutation were identified in 135 adult-onset leukoencephalopathy cases, which corresponded well with the finding that *CSF1R* mutations account for 10%–25% of adult-onset leukodystrophy cases [11]. To identify *CSF1R*-related leukoencephalopathy cases amongst leukoencephalopathy cases of unknown etiology, it is important to identify factors that correlate with the presence of a *CSF1R* mutation before *CSF1R* testing.

Our probability model for predicting the presence of a *CSF1R* mutation suggested that cases with younger onset, parkinsonism, thinning of the corpus callosum and diffusion-restricted lesions were highly probable for the presence of a *CSF1R* mutation. It

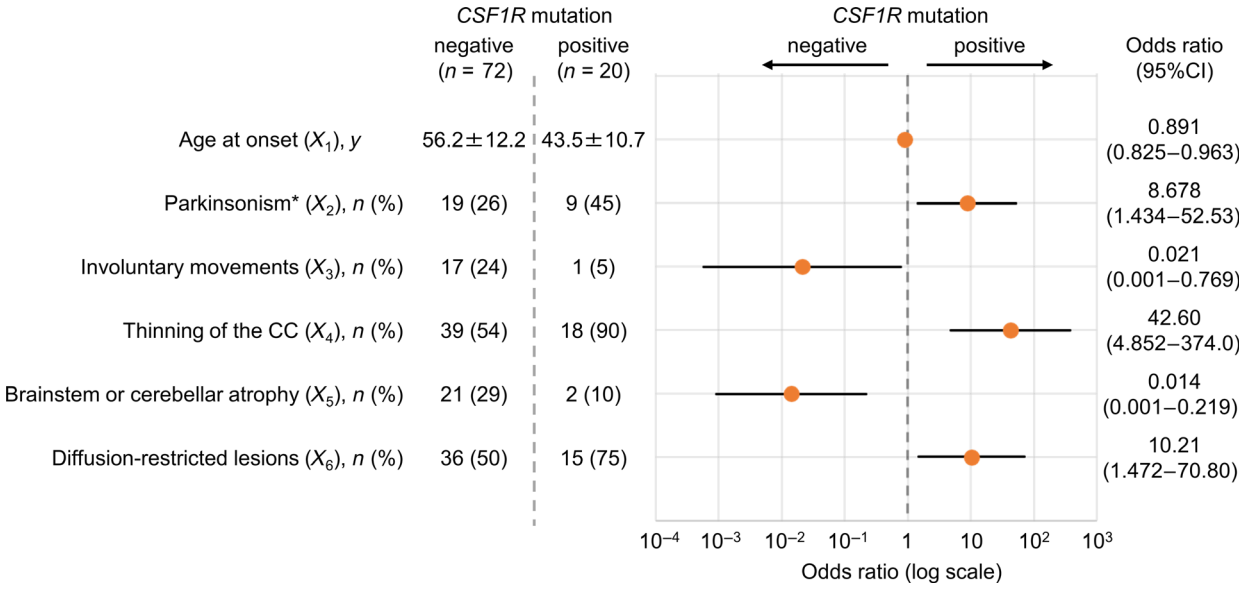

Figure 1 The variables highly related to the presence of a colony-stimulating factor 1 receptor (*CSF1R*) mutation and a logistic regression plot of the odds ratio and 95% confidence interval (CI). Only age at onset was a continuous variable, and the other variables were categorical variables answered with yes or no. CC, corpus callosum; y, years. *Including rigidity, bradykinesia, parkinsonian gait (small shuffling steps, freezing of gait, and falls), postural instability, masked face, resting tremor and responsiveness to L-dopa.

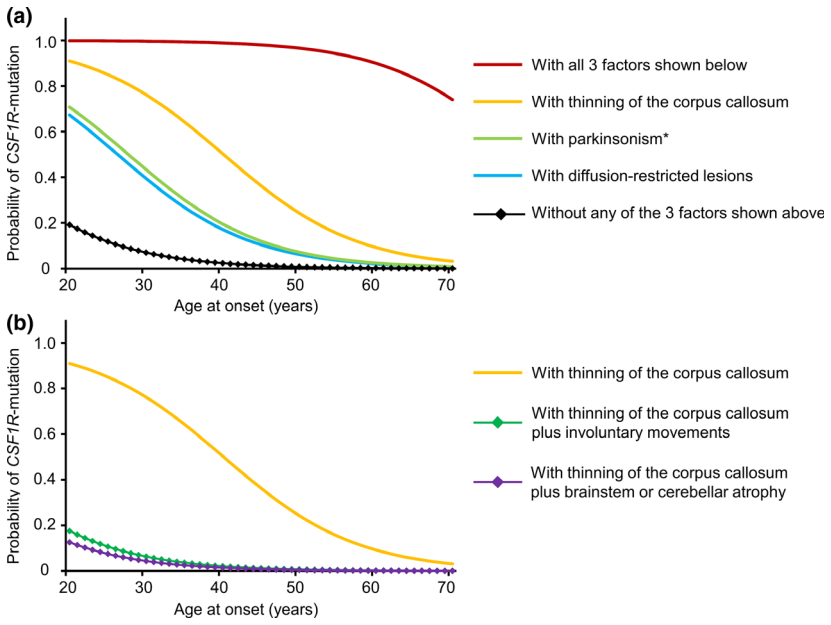

Figure 2 (a) Relationship between the probability of a colony-stimulating factor 1 receptor (*CSF1R*) mutation and three factors (thinning of the corpus callosum, parkinsonism and diffusion-restricted lesions) that increase the probability. *Including rigidity, bradykinesia, parkinsonian gait (small shuffling steps, freezing of gait, and falls), postural instability, masked face, resting tremor and responsiveness to L-dopa. (b) Relationship between the probability of a *CSF1R* mutation and thinning of the corpus callosum and two factors (brainstem or cerebellar atrophy and involuntary movements) that lower the probability.

also showed that involuntary movements and brainstem or cerebellar atrophy were negative predictors for a *CSF1R* mutation. This model could judge whether our unidentified leukoencephalopathy cases had a *CSF1R* mutation with an accuracy of more than 90%. Actually, the first model for predicting the presence of a *CSF1R* mutation by forward logistic stepwise regression included parkinsonism and gait disturbance; however, they had the highest polychoric correlation value of 0.712 (Table S3). Therefore, forward logistic stepwise regression analysis was performed again after excluding gait disturbance. To the best of our knowledge, this is the first study in which logistic regression has been used to identify factors for predicting the presence of a *CSF1R* mutation.

Table 2 Accuracy of the current diagnostic criteria for *CSF1R*-related leukoencephalopathy

	No. of cases	Probable (*n*, %)	Possible (*n*, %)	Not fulfilled (*n*, %)		
CSF1R-mutation-positive leukoencephalopathy	16	9 (56)	4 (25)	3 (19)	Sensitivity[a]	81%
					Specificity	14%
CSF1R-mutation-negative leukoencephalopathy	84	18 (21)	54 (64)	12 (14)		

CSF1R, colony-stimulating factor 1 receptor gene. [a]Sensitivity was calculated as the ratio of the number of cases who were diagnosed as 'probable' or 'possible' to the total number of *CSF1R*-mutation-positive leukoencephalopathy cases.

The current diagnostic criteria define the age at onset of *CSF1R*-related leukoencephalopathy as less than 60 years and exclude cases aged less than 10 years [8]. Our model proved that, the younger the age at onset, the higher the probability of having a *CSF1R* mutation (the lower limit for the age at onset was set at 20 years because the youngest onset age in our study was 20 years). Cognitive impairment or psychiatric symptoms (core feature 2a in the criteria) and bilateral cerebral white matter lesions (core feature 4a in the criteria) were observed frequently in leukoencephalopathy cases of various etiologies; therefore, these clinical and brain imaging features did not contribute to a positive result for a *CSF1R* mutation. The only clinical feature that raised the probability of a *CSF1R* mutation was parkinsonism (core feature 2c in the criteria). Sundal *et al*. reported that subjects with *CSF1R*-related leukoencephalopathy frequently exhibited parkinsonism features such as gait disturbance (100%), postural instability (94%) and rigidity (44%) [12]. Thinning of the corpus callosum (core feature 4b in the criteria) was identified as one of the major contributing factors to a positive result for a *CSF1R* mutation. An abnormality of the corpus callosum is not specific to *CSF1R*-related leukoencephalopathy; however, it is suggestive of *CSF1R*-related leukoencephalopathy when it is seen from the early stages of the disease [13]. It is very likely that leukoencephalopathy cases with a prolonged disease duration will fulfill most of the items in the criteria, despite the etiology.

In our cohort, the specificity of the current diagnostic criteria was significantly lower than that described in the original report [8] (exact binomial 95% confidence interval 8%–24% vs. 28%–56%; the latter was calculated by us with the data presented in the report). This is because, in our *CSF1R*-mutation-negative group, many cases with cognitive impairment or psychiatric symptoms fulfilled the 'probable' or 'positive' designation and only one case was diagnosed as 'not fulfilled' by stroke episodes more than twice. Basically, the current diagnostic criteria were designed to avoid missing atypical cases of *CSF1R*-related leukoencephalopathy; therefore, the minimum clinical and neuroimaging features were used to define the 'possible' designation. As a result, it is inevitable that 'possible' will include a certain number of cases with other white matter diseases. It is further emphasized that *CSF1R*-related leukoencephalopathy cases rarely present with involuntary movements or brainstem or cerebellar atrophy on MRI.

There are several limitations of this study that merit consideration. First, there was no involvement in the selection of patients for *CSF1R* testing, which was completely dependent on each clinician who requested *CSF1R* testing. Secondly, the clinical data for analysis were based on the clinical information sheet that each clinician was asked to fill up. Therefore, the judgment of the presence or absence of each feature was left to the clinician. If all the cases could be evaluated based on clear standards, the results might be different. Thirdly, there were 35 cases who were included in the previous study [8]. These cases were excluded to validate the diagnostic criteria, but a total of 135 cases including them were first analyzed by logistic regression to identify factors for predicting the presence of a *CSF1R* mutation.

In conclusion, clinical and brain imaging features predictive of a positive *CSF1R* mutation result by logistic regression analysis are proposed. Factors that raise the probability of a *CSF1R* mutation are younger onset, parkinsonism, thinning of the corpus callosum and diffusion-restricted lesions. Conversely, factors that lower the probability of a *CSF1R* mutation are involuntary movements and brainstem or cerebellar atrophy. Consideration of these factors will help to prioritize patients for *CSF1R* testing.

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Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

Supporting Information

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

Table S1. The number of cases in each table or figure.
Table S2. Identified mutations, prediction scores and allele frequency in the databases.
Table S3. Polychoric correlation coefficients of variables for forward logistic stepwise regression.

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