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Partial Loss of Function of CSF1R in a Patient with White Matter Abnormalities

Takuya Konno, MD, PhD^{1,2}, Takeshi Miura, MD^{2,3}, Andrea M. Harriott, MD, PhD⁴, Naomi Mezaki, MD^{2,3}, Emily S. Edwards, MS, CCRP¹, Rosa Rademakers, PhD⁵, Owen A. Ross, PhD⁵, James F. Meschia, MD¹, Takeshi Ikeuchi, MD, PhD^{3,*}, and Zbigniew K. Wszolek, MD^{1,*}

¹Department of Neurology, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL, 32224, USA

²Department of Neurology, Brain Research Institute, Niigata University, 1-757 Asahimachi, Niigata 952-8585, Japan

³Department of Molecular Genetics, Brain Research Institute, Niigata University, 1-757 Asahimachi, Niigata 952-8585, Japan

⁴Department of Neurology, Massachusetts General Hospital, 55 Fruit St, Boston, Massachusetts, 02114, USA

⁵Department of Neuroscience, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL, 32224, USA

Abstract

Background and purpose—Mutations in *colony stimulating factor 1 receptor (CSF1R)* cause adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP). Patients with ALSP can be misdiagnosed as having acute ischemic stroke due to hyperintensity lesions on diffusion-weighted magnetic resonance imaging. Mutant CSF1R proteins identified in ALSP show a complete loss of autophosphorylation of CSF1R.

Methods—We conducted mutation screening of *CSF1R* in 123 patients with definite acute ischemic cerebrovascular syndrome and positive family history of stroke. The pathogenicity of identified variants was evaluated using functional analyses. The levels of autophosphorylation of CSF1R in response to treatment with ligands of CSF1R were examined in cells transfected with wild-type and mutant CSF1R.

*Corresponding Author: Zbigniew K. Wszolek, MD, Department of Neurology, Mayo Clinic, 4500 San Pablo Road, Jacksonville, Florida, 32224, USA, Phone: 904-953-7229, Fax: 904-953-6036, Wszolek.Zbigniew@mayo.edu; Takeshi Ikeuchi, MD, PhD, Department of Molecular Genetics, Brain Research Institute, Niigata University, 1-757 Asahimachi, Niigata, 951-8585, Japan, Phone: +81-25-227-2343, Fax: +81-25-227-0793, ikeuchi@bri.niigata-u.ac.jp.

PROF. TAKESHI IKEUCHI (Orcid ID: 0000-0001-8828-8085)

PROF. ZBIGNIEW K. WSZOLEK (Orcid ID: 0000-0001-5487-1053)

Disclosure of conflicts of interest

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Results—We identified 8 *CSF1R* variants; 6 were known non-pathogenic polymorphisms, while the other 2 were missense variants inducing substitution of amino acid residues (p.Glu573Lys and p.Gly747Arg). Functional assay showed that the levels of autophosphorylation of p.Gly747Arg were comparable to those of wild-type when treated with ligands. The autophosphorylation of p.Glu573Lys was detectable, but significantly decreased compared with those of wild-type *CSF1R* ($P<0.001$, two-way ANOVA with Bonferroni). The clinical presentation of the patient with p.Glu573Lys was consistent with cerebral embolism. The patient did not have typical clinical findings of ALSP; however, periventricular white matter abnormalities, unrelated to the recent infarct, were evident on brain magnetic resonance imaging.

Conclusions—In contrast to ALSP-associated missense mutations, *CSF1R* p.Glu573Lys variant in a patient with acute ischemic cerebrovascular syndrome showed a partial loss of autophosphorylation of *CSF1R*; its clinical significance warrants further investigation.

Keywords

adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP); colony stimulating factor 1 receptor (*CSF1R*); white matter; Leukodystrophies; Genetic and inherited disorders; Stroke; Leukoencephalopathies

INTRODUCTION

Mutations in *colony stimulating factor 1 receptor (CSF1R)* cause adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP). ALSP is typically characterized by autosomal dominant inheritance, progressive cognitive and neuropsychiatric symptoms, and white matter abnormalities on brain magnetic resonance imaging (MRI) [1, 2]; however, patients who had hemiparesis or stroke-like episodes including transient hemiparesis, amnesia, and aphasia have been occasionally reported [3, 4]. On brain MRI, diffusion-restricted lesions in the white matter are observed [2, 5]. These features of ALSP mimic those of acute ischemic stroke. Indeed, several patients have been diagnosed as having cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy before obtaining genetic confirmation of ALSP [1, 4]. Ligands of *CSF1R*, interleukin-34 (IL-34) and CSF-1, induce autophosphorylation of *CSF1R* [6], which is abolished by ALSP-associated mutations indicating that a loss of *CSF1R* function is relevant to pathogenesis of ALSP [1, 7, 8]. In this study, we screened for *CSF1R* variant in a cohort of patients who had definite acute ischemic cerebrovascular syndrome (AICS) [9] and positive family history of stroke.

METHODS

Patients

Between 2010 and 2013, we consecutively collected 123 patients from the Mayo Clinic Florida Familial Cerebrovascular Diseases Registry. The patients needed to satisfy all of the followings: 1) age over 18 years old, 2) fulfilling the criteria of definite AICS that is defined as acute onset of neurologic dysfunction of any severity consistent with focal brain ischemia and imaging/laboratory confirmation of an acute vascular ischemic pathology [9], and 3) positive family history of cerebrovascular disease. At enrollment, each patient was classified

into one of 5 ischemic stroke subtypes according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification [10] and assessed by stroke scales including the modified Rankin Scale, the Barthel Index, and the National Institutes of Health Stroke Scale [11]. MR brain images were reviewed to grade white matter abnormalities. Hyperintensities on fluid attenuation inversion recovery (FLAIR) sequences were used to score white matter abnormalities as previously described in the Cardiovascular Health Study (CHS) avoiding regions of prior stroke involvement [12]. Briefly, grade 0 is assigned for no abnormalities, grade 1 for a discontinuous periventricular rim of hyperintensity with few punctate subcortical hyperintensities, grade 2 for continuous thin periventricular rim with few patches of subcortical hyperintensities, grade 3 for continuous thicker periventricular rim with few patches of subcortical hyperintensities, grade 4 for thick shaggy periventricular rim and minimal confluent lesions, grade 5 for mild confluent lesions around the frontal and occipital horns, grade 6 for moderate confluent lesions around the frontal and occipital horns, grade 7 for confluent lesions with partial inclusion of the central semiovale, grade 8 for periventricular confluence involving most of the centrum semiovale, grade 9 for the most severe lesions with near complete involvement of subcortical white matter [12]. All patients or their relatives provided informed consent for participating in our genetic sample repository that was used for, but not limited to, genetic analysis done in this study. This study was approved by the institutional review board committees of Mayo Clinic and Niigata University.

Mutation Screening of CSF1R

All of ALSP-associated *CSF1R* mutations have so far been found in exon 12 or later which are responsible for the tyrosine kinase domain (TKD) of *CSF1R* [2]. Therefore, we sequenced *CSF1R* from exons 12 through exon 22, which is the last exon of *CSF1R*, covering whole coding sequences of the TKD and their flanking intronic sequences as previously described [7]. The significance of the identified variants was reviewed by referring to the Ensembl database [13]. The pathogenicity of missense variants was predicted by PolyPhen-2 [14].

Functional Assay

Functional assays of the CSF1R variants were performed as previously described [7]. Briefly, HEK293T cells were transiently transfected with variants CSF1R (p.Glu573Lys, p.Gly747Arg, and p.Ile794Thr) or wild-type CSF1R, and were cultured in the medium containing 10% fetal bovine serum (FBS). Detergent lysates were subject to immunoblot analysis using the antibodies including C-20 (Santa Cruz Biotechnology, Dallas, TX) to detect total CSF1R and phospho-specific antibodies against Tyr546, Tyr708, Tyr723, and Tyr923 (Cell Signaling Technology, Beverly, MA).

In another set of experiments, HEK293T cells transiently transfected were stimulated by IL-34 or CSF-1 in the absence of FBS to induce autophosphorylation of CSF1R. The cells were harvested at 4 time points (0, 10, 20, and 30 minutes) after the stimulation, followed by immunoblot analysis.

Statistical Analysis

The signal intensity of immunoblot was semiquantitatively analyzed with LAS 4000 mini image analyzer (GE Health Science, Piscataway, NJ). Each autophosphorylation signal of CSF1R was normalized by that of total amount of CSF1R. Statistical analysis was performed with one-way ANOVA with Tukey, or two-way ANOVA with Bonferroni using GraphPad Prism 5 (GraphPad Software, La Jolla, CA). Data was presented as mean±SEM.

RESULTS

Demographic characteristics of our cohort were shown in Table 1. Among the 123 patients, we identified 8 heterozygous/homozygous variants in *CSF1R*; 6 were known non-pathogenic polymorphisms or synonymous variants (Supplementary Table 1), while the other 2 were heterozygous missense variants inducing substitution of amino acid residues (p.Glu573Lys and p.Gly747Arg) identified in 1 patient each (Figure 1 and Table 2).

In silico analysis predicted that p.Glu573Lys was probably damaging and p.Gly747Arg was benign (Table 2). Functional analysis showed the levels of phosphorylated CSF1R of p.Gly747Arg in standard media with 10% FBS were comparable to those of wild-type. Autophosphorylation of p.Glu573Lys was detectable but significantly decreased compared with those of wild-type (Figure 2). Upon stimulation of IL-34 a time-dependent increment in autophosphorylation of CSF1R was observed in wild-type CSF1R. The p.Gly747Arg variant showed no difference in the levels of autophosphorylation from those in wild-type; however, ligand-dependent autophosphorylation was significantly decreased in p.Glu573Lys compared with wild-type (Figure 3). Similar results were obtained in the experiment using another ligand, CSF-1 (data not shown).

The patient with p.Glu573Lys was a 70-year-old man who had diabetes mellitus, hypertension, dyslipidemia, and positive family history of stroke in his father and grandfather. Neurologic examination revealed right visual field deficit and aphasia without paresis. Brain MRI showed multifocal acute infarction involving the cerebral cortices and cerebellum, and laminar necrosis in the left parietal cortex (Figure 4A–D). Bilateral white matter abnormalities were depicted with a CHS score of 4 for a shaggy and thick periventricular rim of FLAIR hyperintensity and minimal confluent lesions (Figure 4E, F). The corpus callosum was slightly atrophic but not as much as typically seen in ALSP (Figure 4G). Brain computed tomography scan revealed no calcifications that are common in ALSP (Figure 4H) [15].

DISCUSSION

The aim of this study was not to find the association between the *CSF1R* genetic variants and ischemic stroke but rather to find out if the ALSP patients due to *CSF1R* mutations were diagnosed with ischemic stroke. As a result, we identified 2 rare, nonsynonymous CSF1R variants (p.Glu573Lys and p.Gly747Arg) in 1 patient each from this cohort. Both are heterozygous variants. While p.Gly747Arg showed autophosphorylation comparable to wild-type, p.Glu573Lys had significantly decreased autophosphorylation. In contrast to ALSP-associated mutations causing a complete loss of autophosphorylation (e.g.,

p.Ile794Thr in Figure 2A, 3A) [1, 7], autophosphorylation of the p.Glu573Lys variant was decreased, but still detectable, suggesting a partial loss of autophosphorylation.

While all ALSP-associated missense mutations have been identified within the TKD [2], p.Gly747Arg is within the kinase insert domain which interrupts the TKD (Figure 1). Based on the functional assay, we conclude that this variant is likely benign. As of now, only p.Ser688Glufs*13 variant which causes haploinsufficiency of CSF1R has been identified in the kinase insert domain as a pathogenic variant [7], indicating that any types of variants except for truncating ones may be benign in this domain. p.Glu573Lys is located in the juxtamembrane domain which is adjacent to the N-terminal side of TKD. This domain has a negative regulatory role in the kinase function [16]. There has been reported only one variant, p.Thr567fs*44 in this domain as causative for ALSP [17]. It can be assumed that this variant causes ALSP through haploinsufficiency of CSF1R like the p.Ser688Glufs*13 variant. Although the precise mechanism by which the p.Glu573Lys causes partial loss of CSF1R autophosphorylation remains unclear, its location of the juxtamembrane domain may be attributable.

The clinical presentation of the patient with p.Glu573Lys was consistent with cerebral embolism. Considering that genome-wide association studies have not identified any associations near *CSF1R* gene in ischemic stroke cohorts [18, 19] and any disease associations of p.Glu573Lys variant have not been reported in the Cerebrovascular Disease Knowledge Portal [20], it is unlikely that the p.Glu573Lys variant is directly associated with the patient's stroke. The patient did not have typical clinical findings of ALSP and did not fulfill the diagnostic criteria for ALSP [21], but white matter abnormalities unrelated to the recent infarct were evident. However, the patient had vascular risks. Therefore these abnormalities might represent chronic ischemic changes. It is interesting whether the partial loss-of-function variant is associated with mild clinical phenotype of ALSP such as the white matter abnormalities and very mild atrophy of the corpus callosum seen in this patient; however, it cannot be concluded based on this single observation.

There are several limitations of this study. Firstly, we screened only exons 12–22 of *CSF1R* since no relevant variants have been reported previously in exons 1–11 in patients with ALSP. However, there is the possibility that potentially relevant variants, particularly truncating variants, located in the remaining exons of the gene might be overlooked. Secondly, we could not obtain additional clinical information nor blood samples of other affected family members of the p.Glu573Lys variant carrier. Therefore, we could not confirm a full segregation of this variant in other family members.

CONCLUSIONS

This is the first report of a CSF1R variant causing partial loss of autophosphorylation of CSF1R. The clinical relevance of this partial loss-of-function variant warrants further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. A Schematic Drawing of CSF1R Gene and Protein Domain Structures

Only regions of interest of CSF1R are provided. ex indicates exon; JMD, juxtamembrane domain; KID, kinase insert domain; TKD, tyrosine kinase domain; TM, transmembrane domain; and UTR, untranslated region.

Figure 2. Functional Assay of the CSF1R variants

(A) Immunoblot analyses of total and phosphorylated forms of the variants CSF1R, which were transiently expressed in HEK293T cells cultured in the medium containing 10% FBS. The CSF1R variant of p.Ile794Thr was previously shown to diminish autophosphorylation of CSF1R [7].

(B) Quantification of immunoblot data revealed the levels of autophosphorylation in the p.Glu573Lys variant were significantly decreased at Tyr546, Tyr798, and Tyr923 (n=3). **, $P < 0.01$ versus WT by one-way ANOVA with Tukey.

Figure 3. Functional Assay of the CSF1R Variants in a Condition Treated with IL-34

(A) Immunoblot analyses of ligand-dependent autophosphorylation of CSF1R variants. The CSF1R variant of p.Ile794Thr was previously shown to diminish autophosphorylation of CSF1R [7].

(B) Quantification of immunoblot data revealed that autophosphorylations of CSF1R at p.Tyr546, p.Tyr708, and p.Tyr923 were significantly suppressed in the p.Glu573Lys variant compared with wild-type (n=3). ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$ versus wild-type by two-way ANOVA with Bonferroni.

Figure 4. Brain MRI/CT Images in a Patient with p.Glu573Lys Variant

Axial diffusion-weighted images demonstrate acute infarction in the left cerebellum, the left lateral temporal and parietal cortices, and the right parietal subcortical area (A–C). The lamina necrosis in the left parietal cortex associated with the infarction is evident on T1-weighted image (D). Periventricular white matter hyperintense lesions, which appeared to be greater than expected for the patient's age, are shown on axial fluid-attenuated inversion recovery images (E, F). The corpus callosum is slightly atrophic on a sagittal T1-weighted image but considered to be normal for the patient's age (G). Brain CT scan with 5-mm section thickness shows no apparent calcifications (H).

Table 1

Demographic characteristics of our cohort

Total (n)	123
Sex, %male	57.7
Age (years)	66 ± 16.9
Ethnicity (%)	
Caucasian	87.8
African American	9.8
Asian	1.6
Unknown	0.8
TOAST classification (%)	
Large artery	27.6
Cardioembolic	31.7
Small Vessel	4.9
Others	2.4
Undetermined	32.5
mRS	2 (1–4)
Barthel Index	90 (40–100)
NIHSS	3 (1–7)
CHS scores	3 (2–4)
Comorbidities (%)	
Hypertension	59.3
Diabetes Mellitus	26.8
Atrial Fibrillation	15.4
Migraine	17.9

Data are shown as percentage, mean ± SD or median (inter-quartile range)

CHS, Cardiovascular Health Study; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; TOAST, Trial of Org 10172 in Acute Stroke Treatment

Table 2

Identified Missense Variants of CSF1R

Case	Variant (gene)	Variant (protein)	Location	rs number	Allele frequency		PolyPhen-2
					Allele	1000 Genomes ExAC	
Case 1	c.1717G>A	p.Glu573Lys	Exon 12	rs376280561	A	na 8.236e-06	Probably damaging
Case 2	c.2339G>A	p.Gly747Arg	Exon 17	rs41355444	A	0.001 0.002	Benign

na. not available.