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RESEARCH ARTICLE

Clinical and genetic characterization of adult-onset leukoencephalopathy caused by *CSF1R* mutations

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Abstract

Objective: Mutations in the colony-stimulating factor 1 receptor gene (*CSF1R*) were identified as a cause of adult-onset inherited leukoencephalopathy. The present study aims at investigating the frequency, clinical characteristics, and functional effects of *CSF1R* mutations in Taiwanese patients with adult-onset leukoencephalopathy. **Methods**: Mutational analysis of *CSF1R* was performed in 149 unrelated individuals with leukoencephalopathy by a targeted resequencing panel covering the entire coding regions of *CSF1R*. In vitro analysis of the CSF1-induced autophosphorylation activities of mutant CSF1R proteins was conducted to assess the pathogenicity of the *CSF1R* mutations. **Results**: Among the eight *CSF1R* variants identified in this study, five mutations led to a loss of CSF1-induced autophosphorylation of CSF1R proteins. Four mutations (p.K586*, p.G589R, p.R777Q, and p.R782C) located within the tyrosine kinase domain of CSF1R, whereas the p.T79M mutation resided in the immunoglobulin-like domain. The five patients carrying the *CSF1R* mutations developed cognitive decline at age 41, 43, 50, 79, and 86 years, respectively. Psychiatric symptoms and behavior changes were observed in four of the five patients. The executive function and processing speed were severely impaired at an early stage, and their cognitive function deteriorated rapidly within 3–4 years. Diffusion-restricted lesions at the subcortical regions and bilateral corticospinal tracts were found in three patients. **Interpretation**: *CSF1R* mutations account for 3.5% (5/149) of the adult-onset leukoencephalopathy in Taiwan. *CSF1R* mutations outside the tyrosine kinase domain may also disturb the CSF1R function and lead to the clinical phenotype. Molecular functional validation is important to determine the pathogenicity of novel *CSF1R* variants.

Introduction

Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is an inherited white matter disorder encompassing two clinic-pathologically similar entities, namely hereditary diffuse leukoencephalopathy with spheroids (HDLS) and pigmentary orthochromatic leukodystrophy (POLD).[1,2] Patients with ALSP usually develop early-onset dementia, behavior changes, depression, gait ataxia and parkinsonism in the fourth or fifth decade of life, and rapidly deteriorate to bed-ridden status within 3–5 years.[3,4] With the discovery of causative genes,[5,6] these two clinic-pathologically similar conditions (i.e., POLD and HDLS) now can be clearly distinguished.[7] For patients diagnosed with POLD and some literature cases pathologically mislabeled as HDLS, the new nomenclature is "colony-stimulating factor 1 receptor gene (*CSF1R*)-related leukoencephalopathy." On the other hand, the patients diagnosed with HDLS including the original Swedish family reported in 1984 are belonged to "alanyl-transfer tRNA synthetase 2 gene (*AARS2*)-related leukoencephalopathy."[7,8] *CSF1R*-related leukoencephalopathy has become increasingly recognized, and it is estimated to account for 10% of adult-onset leukodystrophy in the Caucasian populations.[9,10]

CSF1R encodes for a tyrosine kinase transmembrane receptor that regulates the survival, proliferation, and differentiation of mononuclear phagocytic cells, including microglia in the brain.[11] Upon stimulation by its ligand colony-stimulating factor 1 (CSF1) or interleukin-34, CSF1R protein would form a homodimer and autophosphorylate the tyrosine residues within its intracellular kinase domain and subsequently activate the downstream signaling pathways.[12,13] The original study identifying *CSF1R* mutations as the cause of ALSP also demonstrated that the pathogenic mutations abolished the CSF1-induced autophosphorylation of CSF1R proteins.[5] The findings that haploinsufficiency of *CSF1R* genetic function is sufficient to cause leukoencephalopathy was again confirmed in the *Csf1r*$^{+/-}$ mouse model.[14] To date, more than 70 different *CSF1R* mutations have been reported worldwide,[3,4,15] and the majority of them are located within exons 12–22 of *CSF1R*, corresponding to the tyrosine kinase domain (TKD) of CSF1R protein. Among these mutations, only a small number of them had been proven to affect the CSF1R function by in vitro studies.[5,16–19] The arbitrariness to claim the pathogenicity without functional verification may raise the uncertainty of some of the *CSF1R* mutations in clinical reports.

To expand the knowledge of molecular spectrum and clinical phenotypes of *CSF1R*-related leukoencephalopathy, the present study investigated the frequency, clinical features, and image characteristics of patients carrying a *CSF1R* mutation in a Taiwanese cohort with adult-onset leukoencephalopathy of unknown causes. In vitro functional analysis of the *CSF1R* mutations was conducted to assess the pathogenicity.

Patients and Methods

Subjects

A consecutive series of 149 unrelated cases with adult-onset leukoencephalopathy were enrolled from the Neurology Department of Taipei Veterans General Hospital between 2001 and 2020. Taipei Veterans General Hospital is a 2974-bed tertiary medical center that serves both veterans and regular citizens in Taiwan. It accepts both self-referred patients and referrals of difficult cases from other hospitals. The inclusion criteria were subjects with (1) marked leukoencephalopathy defined as Fazekas grade 2 or grade 3 on brain magnetic resonance imaging (MRI),[20] and (2) any of the following symptoms: cognitive dysfunction, psychiatric disorder, gait disturbance, or parkinsonism. The exclusion criteria were subjects (1) who ever developed ischemic stroke or intracerebral hemorrhage or (2) carrying pathogenic mutations in *NOTCH3* or *HTRA1*.

All participants were of Han-Chinese descent. Peripheral blood samples were collected after written informed consent was obtained. This study was approved by the Institutional Review Board of Taipei Veterans General Hospital.

Cognitive tests and neuroimaging studies

The demographic information, medical histories, and personal histories of the patients carrying the *CSF1R* mutations were obtained from the patient interview and medical record reviews. Global cognitive performance was assessed using the Mini-Mental Status Examination (MMSE).[21] Tests specific to each cognitive domain were performed, including (1) memory (12-item word recall test),[22] (2) language and executive function (category verbal fluency test),[23] (3) processing speed (triai making test A),[24,25] and (4) attention (forward and backward digit span test from the Wechsler Memory Scale IV).[26] The raw data and percentile score of each cognitive test were shown. The percentile score falling below 1.5 standard deviations (i.e., <16%) of the normative data was defined as significantly abnormal.

Brain MRI, including T_1-weighted images (T_1WI), T_2-weighted images (T_2WI), fluid-attenuated inversion recovery (FLAIR) images, and diffusion-weighted imaging (DWI), as well as brain computed tomography (CT) images were reviewed.

Mutation analysis

Sequence analysis of *CSF1R* was performed by utilizing a high-throughput targeted resequencing panel covering the entire coding regions of *CSF1R* and other 183 genes associated with hereditary leukodystrophy and small vessel diseases (Table S1) on an Illumina HiSeq2500 platform. Alignment of sequenced reads and identification of variants were performed with the reference Human Genome version 38 (hg38/GRCh38) and the reference *CSF1R* coding sequence (NM_005211.3). Sanger sequencing was performed to confirm the identified *CSF1R* variants.

The putative pathogenic *CSF1R* mutations were discriminated by their absence or presence with an extremely rare allele frequency in the genome Aggregation Database (gnomAD version r2.1.1).[27] In silico analysis of pathogenicity of the *CSF1R* mutations was conducted using Combined Annotation Dependent Depletion (CADD),[28] PolyPhen-2,[29] and MutationTaster.[30] Phylogenetic conservation of the mutated amino acid residues were analyzed by aligning the amino acid sequences of multiple CSF1R orthologs utilizing the UniProt website.[31]

Expression plasmids and cell cultures

A human *CSF1R* cDNA clone was purchased from TransOMIC (BC047521; Huntsville, AL). The full-length coding region of human *CSF1R* was cloned into pFLAG-CMV-5a (Sigma-Aldrich, St. Louis, MO) to generate the wild-type CSF1R expression plasmids. Nine *CSF1R* variants were introduced into the wild-type expression plasmids, separately, using the QuikChange Site-Directed Mutagenesis method (Stratagene; Agilent, Santa Clara, CA). These variants were p.T79M (c.236C > T), p.E478K (c.1432G > A), p.T507_H508insP (c.1520_1522dupCGC), p.K586* (c.1754dupT), p.G589R (c.1765G > A), p.R777Q (c.2330G > A), p.R782C (c.2344C > T), p.M875T (c.2624T > C), and p.A914T (c.2740G > A). *CSF1R* p.M875T was a well-known pathogenic mutation[5] and served as a positive control in this study; the remaining eight variants were identified in the present study (Table 1). HeLa cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum in a humidified incubator at 37°C under 5% CO_2.

In vitro analysis of CSF1-induced autophosphorylation of CSF1R

The plasmids expressing wild-type or one of the eight mutant CSF1R proteins were transfected into HeLa cells, respectively, using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA). Forty-eight hours posttransfection, the medium was changed to serum-free medium for 16 h, and the transfected cells were treated with 100 ng/mL human recombinant CSF1 (R&D Systems, Minneapolis, MN) for 10 min. Treated cells were harvested immediately with RIPA buffer and analyzed by Western blotting. Transfected cells without CSF1 treatment were used as controls. Total CSF1R expression levels were

Table 1. The rare *CSF1R* variants identified in this study.

CSF1R variant		Location		Bioinformatics prediction				Population controls	
Nucleotide	Amino acid	Exon	Domain	Mutation Tester	PolyPhen2	CADD Phred score	ClinVar	gnomAD	TaiwanView
c.236C > T	p.T79M	3	IG	Disease causing	Damaging	22.2	Not reported	None	None
c.1432G > A	p.E478K	10	IG	Tolerated	Benign	11.8	Not reported	None	None
c.1520_1522dupCGC	p.T507_H508InsP	11	TM	Tolerated	NA.	NA	Not reported	None	None
c.1754dupT	p. K586*	13	TKD	Disease causing	NA	NA	Not reported	None	None
c.1765G > A	p.G589R	13	TKD	Disease causing	Damaging	29.8	Pathogenic	None	None
c.2330G > A	p.R777Q	18	TKD	Disease causing	Damaging	32	Pathogenic	None	None
c.2344C > T	p.R782C	18	TKD	Disease causing	Damaging	24.1	Not reported	None	None
c.2740G > A	p.A914T	21	C-terminal	Tolerated	Benign	3.4	Not reported	EA: 1/19952	2/1513

ClinVar, a public archive of interpretations of clinically relevant variants (https://www.ncbi.nlm.nih.gov/clinvar/); CADD, Combined Annotation Dependent Depletion; IG, immunoglobulin-like domain; TM, transmembrane domain; TKD, tyrosine kinase domain; C-terminal, carboxyl-terminal; EA, East Asian; gnomAD, genome Aggregation Database (http://gnomad.broadinstitute.org); TaiwanView, 1517 healthy Taiwanese control exomes were publically available in the Taiwan Biobank database (https://taiwanview.twbiobank.org.tw/index); NA, not applicable.

analyzed using the CSF1R antibody (sc-46662; Santa Cruz, Dallas, TX). CSF1R autophosphorylation ability was evaluated using CSF1R phospho-tyrosine primary antibodies against p-Tyr546 (#3083), p-Tyr699 (#12251), p-Tyr723 (#3155), and p-Tyr923 (#3406), respectively (Cell Signaling Technology, Danvers, MA). Actin was used as a loading control to ensure an equal amount of protein loading (MAB1501; Merck Millipore, Burlington, MA).

Results

Identification of *CSF1R* mutations

Among the 149 unrelated patients with leukoencephalopathy, we found eight different *CSF1R* variants (Table 1). Except for *CSF1R* p.A914T presenting with an allele frequency of 0.00005 in the East Asian population in gnomAD, the other seven *CSF1R* variants were absent in the 123,136 exomes and 15,496 genome data of various populations from gnomAD, as well as the 1517 healthy Taiwanese exomes from Taiwan Biobank. Among them, p.G589R and p.R777Q mutations had been reported in patients with ALSP.[4,10,32,33] The p.R782C variant was novel, but *CSF1R* mutations altering the same amino acid residue (e.g., p.R782G and p.R782H) had been identified in patients with leukoencephalopathy.[34,35] The remaining five variants had never been reported before.

Six of the eight *CSF1R* variants were missense mutations, including three located within the TKD (p.G589R, p.R777Q, and p.R782C), two in the immunoglobulin-like domain (p.T79M and p.E478K), and p.A914T at the carboxyl-terminal domain of CSF1R (Table 1, Fig. 1A). The nonsense mutation (p.K586*) caused premature termination of TKD, whereas the p.T507_H508InsP mutation inserted an additional amino acid residue in the transmembrane domain without altering the reading frame. The three mutations located within TKD (p.G589R, p.R777Q, and p.R782C) and p.T79M were

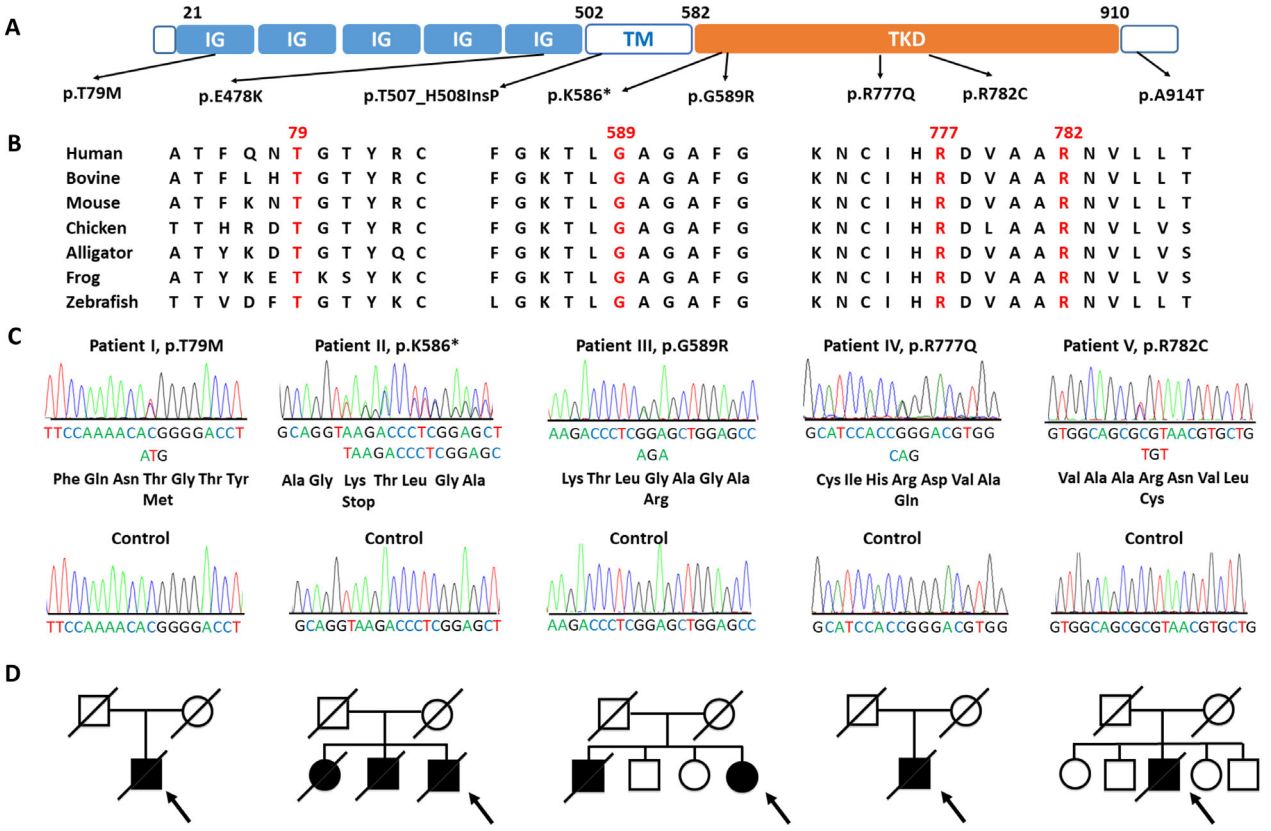

Figure 1. Genetic analysis of the *CSF1R* mutations identified in the Taiwanese patients with leukoencephalopathy. (A) Schematic illustration of the structure of CSF1R protein and position of the rare *CSF1R* variants identified in this study. IG = immunoglobulin-like domain; TM = transmembrane domain; TKD = tyrosine kinase domain. (B) Alignment of multiple CSF1R protein orthologs showing evolutionary conservation of the amino acid residues altered by the putative pathogenic mutations. (C) Sanger sequencing trace demonstrating the five heterozygous *CSF1R* pathogenic mutations and corresponding wide-type control sequences. (D) Pedigrees of the five index patients harboring a *CSF1R* pathogenic mutation. Open symbol: unaffected; filled symbol: affected; symbol with a diagonal line: deceased; arrow: proband; square: male; circle: female.

predicted to be pathogenic by PolyPhen-2 and MutationTaster; the nonsense mutation (p.K586*) was also predicted as disease-causing by MutationTaster (Table 1). The CADD Phred scores of these five mutations ranged from 22.2 to 32, ranking them in the top 6.0/1000 to 6.3/10,000 most deleterious variants in the genome.[28] The amino acids influenced by these four missense mutations (p.T79M, p.G589R, p.R777Q, and p.R782C) were evolutionally conserved across CSF1R orthologues from human to zebrafish (Fig. 1B). The remaining three mutations outside the TKD (p.E478K, p.T507_H508InsP, p.A914T) were predicted to be benign variants by the bioinformatics tools.

In vitro analysis of CSF1-induced autophosphorylation of CSF1R proteins

To assess the functional consequence of *CSF1R* mutations identified in this study, we transfected HeLa cells with wild-type or either one of the mutant CSF1R expression plasmids. The expression level and autophosphorylation ability of mutant and wide-type proteins were compared using Western blotting. The expression of wild-type and eight mutant CSF1R proteins were comparable (Fig. 2). However, the five CSF1R mutants, including T79 M, K586*, G589R, R777Q, and R782C CSF1R, as well as the positive control M875T CSF1R had a total or profound loss of their ability to phosphorylate the Tyr546, Tyr699, Tyr723, and Tyr923 residues upon CSF1 stimulation (Fig. 2). The remaining three CSF1R mutants, including E478K, T507_H508InsP, and A914T CSF1R, had preserved CSF1-induced CSF1R tyrosine kinase activity to phosphorylate the tyrosine residues.

Clinical features of patients harboring a *CSF1R* pathogenic mutation

The pathogenicity of the five *CSF1R* mutations (p.T79M, p.K586*, p.G589R, p.R777Q, and p.R782C) are supported by the absence in population databases, predictions of multiple bioinformatics programs, and well-established in vitro functional analyses (Table 1 and Fig. 2). According to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

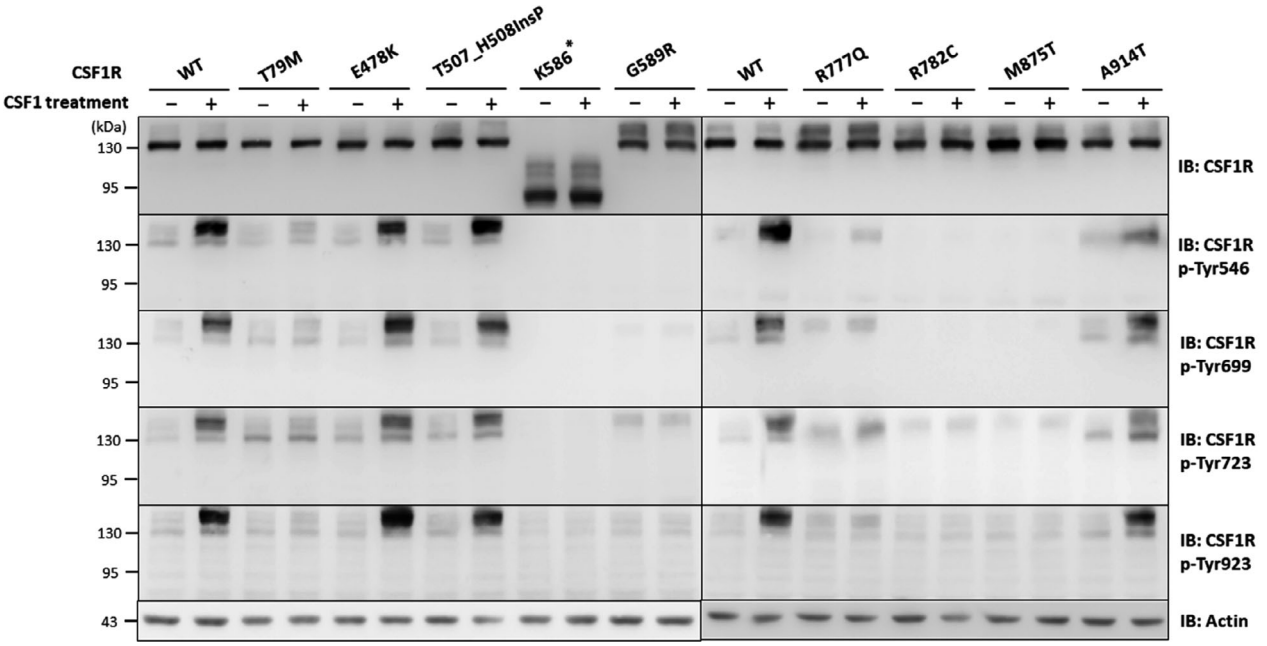

Figure 2. In vitro analysis of CSF1-induced autophosphorylation of CSF1R mutant proteins. Representative western blots of lysates from HeLa cells that were transfected with wide-type (WT) or mutant *CSF1R* expressing constructs with or without colony-stimulating factor 1 (CSF1) treatment. Immunoblotting analysis using anti-CSF1R antibody revealed comparable expression between WT and mutant CSF1R proteins. To evaluate the CSF1-induced autophosphorylation activity of each CSF1R mutant, protein lysates were compared between CSF1-treated and untreated cells expressing either one of the CSF1R mutant proteins. The five CSF1R mutants (T79M, K586*, G589R, R777Q, and R782C CSF1R) and the positive control M875T CSF1R had lost their ability to phosphorylate the Tyr546, Tyr699, Tyr723, and Tyr923 residues within CSF1R upon CSF1 stimulation, whereas the other three CSF1R mutants (E478K, T507_H508InsP, and A914T CSF1R) had preserved function of the CSF1-induced autophosphorylation. Experiments were repeated three times with similar results. The equivalent amount of protein loading is shown using Actin as a loading control.

Table 2. Clinical features and cognitive tests of the patients carrying a *CSF1R* pathogenic mutation.

Patient no Mutation	Patient I p.T79M	Patient II p.K586*	Patient III p.G589R	Patient IV p.R777Q	Patient V p.R782C
Age at onset, sex	86 y, male	79 y, male	43 y, female	41 y, female	50 y, male
Family history of dementia	None	Brother, sister	Brother	None	None
Personal history	HTN, DM, Lipid, CKD, CHF, smoking	None	None	Sjogren's syndrome, Hashimoto thyroiditis	None
Cognitive tests, score (percentile score)					
Age at exam	87 y	82 y	46 y	44 y	54 y
Education	16 y	12 y	9 y	12 y	12 y
Mini-mental status examination	17 (≦1%)	22 (≦1%)	20 (≦1%)	7 (≦1%)	21 (≦1%)
Delayed recalls at 12-item test	1 (<2%)	4 (5%–9%)	5 (10%–30%)	ND	5 (10%–30%)
Category verbal fluency	5 (<3%)	4 (<3%)	6 (<3%)	ND	8 (<11%)
Trial making test A	360 sec (<1%)	190 sec (<2%)	150 sec (<2%)	ND	ND
Forward digit span test	6 (<5%)	5 (<1%)	9 (>19%)	ND	7 (5%–19%)
Backward digit span test	2 (<2%)	3 (4%–16%)	2 (<2%)	ND	2 (<2%)
Behavior changes	None	None	None	Repetition, stereotyping	Compulsive behavior
Psychiatric problems	None	Liable mood, anxiety	Depression	Apathy, social withdraw	Apathy, agitation
Parkinsonism	Postural tremor	Small steps, ataxic gait	Bradykinesia, rigidity, freezing gait	Action and postural tremor	None
Seizure	None	None	None	GTCS	None

HTN, hypertension; DM, Diabetes mellitus; Lipid, hyperlipidemia; CKD, chronic kidney disease; CHF, congestive heart failure; ND, not done; GTCS, generalized tonic clonic seizure; y, years; sec, seconds. The raw score and percentile score of each cognitive test were shown. The percentile score falling below 1.5 standard deviations (i.e. <16%) of the normative data was defined as significantly impaired.

(ACMG/AMP) guideline,[36] the three novel *CSF1R* mutations (p.T79M, p.K586*, and p.R782C) match the PS3, PM2, PP3, and PP4 criteria and are classified as likely pathogenic variants. The other two known *CSF1R* mutations are categorized into pathogenic variants (PS1, PS3, PM2, PP3, and PP4).

Each of the five mutations was identified in one single index patient (Fig. 1C), suggesting that 3.4% (5 of 149) of the patients with adult-onset leukoencephalopathy in Taiwan was attributed to *CSF1R* mutations. Among the five patients, two had a family history of dementia and three were sporadic cases (Fig. 1D).

All the five patients carrying a *CSF1R* mutation suffered from progressive cognitive decline (Table 2). The age at symptom onset ranged from 41 to 86 years, including three patients having symptoms at the fourth to fifth decade of life and two patients developing symptoms in their 80s. Patient IV carrying the *CSF1R* p.R777Q mutation had severe cognitive dysfunction with an MMSE score of 7, and could not perform other cognitive tests. For the remaining four patients, they had impaired cognition (MMSE scores = 17–22) and severe deficits in attention, executive function, and processing speed (Table 2). They performed poorly at the backward digit span test and category verbal fluency with percentile scores below 16% of the normative data. Their processing speed at Trial making test A fell below 2% of the normative data, suggesting severely impaired executive function. More importantly, the cognitive function deteriorated rapidly within 3–4 years. Patient II with *CSF1R* p.K586* mutation had an MMSE score of 22 at age 82 but a score of 5 4 years later; patient III with p.G589R had an MMSE score of 20 at age 46 but a score of 5 two years later.

The majority of the patients (4 out of 5) also suffered from psychiatric symptoms and behavior changes, including apathy, liable mood, depression, agitation, and compulsive and repetitive behaviors (Table 2). The postural tremor was reported in two patients, while gait disturbance was noted in another two. For patient III who carried the p.G589R mutation, she was initially diagnosed with corticobasal degeneration for having bradykinesia, rigidity, and hyperreflexia on the right side of limbs.

Radiologic features of the patients with *CSF1R*-related leukoencephalopathy

Confluent leukoencephalopathy was noted in all the five patients harboring a *CSF1R* pathogenic mutation (Fig. 3,

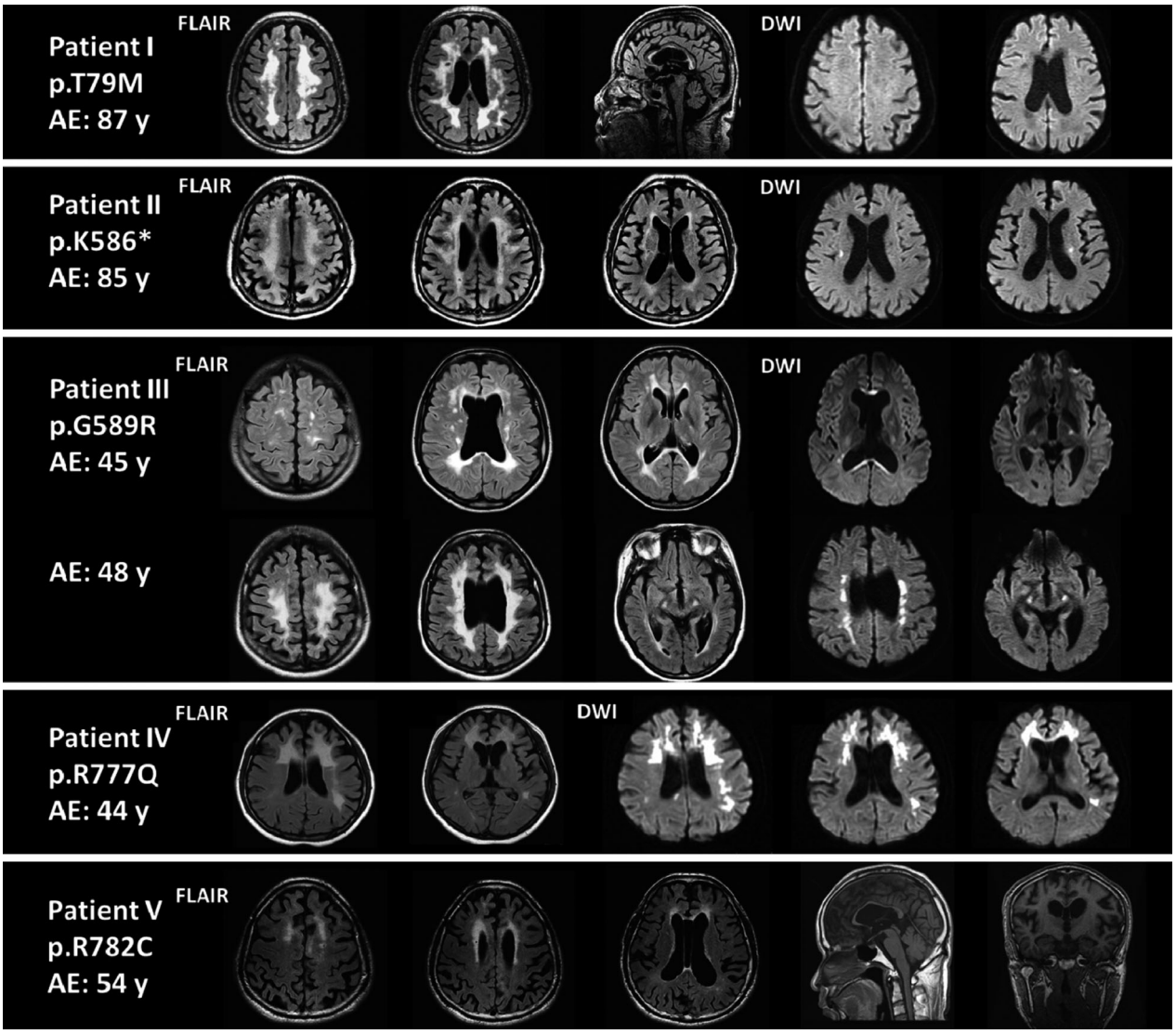

Figure 3. Representative magnetic resonance imaging of the patients with *CSF1R*-related leukoencephalopathy. AE, age at the examination; FLAIR, fluid-attenuated inversion recovery; DWI, diffusion-weighted imaging.

Table 3). White matter hyperintensity on FLAIR images was most prominent at the frontal regions and gradually extended to the parietal lobe. Thinning of the corpus callosum was noted in all five patients, and hyperintensity at the genu or splenium of the corpus callosum was found in patients III and IV. Cavum septum pellucidum was present in three of the five patients (60%), whereas calcification was only detected in one of the three patients (30%) who ever received brain CT.

Diffusion-restricted lesions on DWI were found in three of the four patients (75%). Periventricular lesions were noted in patients III and IV, and a corpus callosum lesion was seen in patient III (Fig. 3). Of note, corticospinal tract involvement on DWI was found in two patients and might be a characteristic feature of *CSF1R*-related leukoencephalopathy. Patient II had bilateral corticospinal tract lesions extending from corona radiate to internal capsule, whereas patient III initially presented with left pyramidal tract lesion at age 45 and then progressed to bilateral pyramidal tract involvement extending from corona radiate, internal capsule to cerebral peduncles at age 48.

Discussion

The present study identified five *CSF1R* pathogenic mutations, including two being reported in literatures and three novel ones, from 149 unrelated Taiwanese

Table 3. Neuroimaging characteristics of the patients with *CSF1R*-related leukoencephalopathy.

Patient no/mutation	Patient I/p.T79M	Patient II/p.K586*	Patient III/p.G589R	Patient IV/p.R777Q	Patient V/p.R782C
T_2-weighted or FLAIR images of MRI					
White matter hyperintensity	Frontoparietal region, anterior temporal pole	Frontoparietal region	Frontoparietal region	Frontal involvement predominant	Frontal involvement predominant
Thinning or hyperintensity of corpus callosum	Present	Present	Present	Present	Present
Cavum septum pellucidum	None	None	Present	Present	Present
DWI (diffusion-weighted imaging) of MRI					
Diffusion-restricted lesions	None	Bilateral corticospinal tracts	Bilateral corticospinal tracts, corpus callosum, periventricular white matter	Frontoparietal periventricular white matter	ND
CT (computed tomography)					
Calcification	None	None	Periventricular, parietal subcortical regions	ND	ND

FLAIR, fluid-attenuated inversion recovery; ND, not done.

patients with molecularly unassigned leukoencephalopathy. All the five mutations compromised the CSF1-induced autophosphorylation function of CSF1R proteins, including the one residing outside the TKD. There are four major findings that have important implications in this study. First, *CSF1R*-related leukoencephalopathy accounts for 3.5% of adult-onset leukoencephalopathy in the Han-Chinese population in Taiwan. The onset age varies from 41 to 86 years in our cases, suggesting that mutation analysis of *CSF1R* is warranted not only in middle-aged patients but also in the elderly with unexplained white matter lesions. Second, our study demonstrated variants outside the TKD of CSF1R still could disturb the kinase activity and cause phenotypes, which highlights the importance of functional validation in newly identified *CSF1R* variants. Third, in our patients, executive function and processing speed were severely impaired, psychiatric symptoms and behavior changes were frequently encountered, and the cognitive function deteriorated rapidly within 3–4 years. These neuropsychiatric features may be characteristic of *CSF1R*-related leukoencephalopathy. Lastly, white matter abnormality with frontoparietal predominance was found in all patients, but such lesions were nonspecific and could be seen in other leukoencephalopathies. Restricted diffusion lesions in the periventricular regions, corpus callosum, and bilateral pyramidal tracts were detected in three cases, and these findings would be more specific to *CSF1R*-related leukoencephalopathy.

More than 30 genes have been implicated in leukodystrophy and genetic leukoencephalopathy,[37,38] but each entity is extremely rare with no single disease having a prevalence >1/20,000.[39] *CSF1R* is recognized as one of the common genetic causes of adult-onset leukoencephalopathy because it accounts for 4%–10.5% of leukoencephalopathy in the United Kingdom and European countries.[9,10,40] In our cohort, 3.4% (5/149) of the Taiwanese patients with leukoencephalopathy carried a *CSF1R* pathogenic mutation. Three of our patients suffered from cognitive decline since the early 40s, which is consistent with the average age of onset at 42 years in the literature.[4,41] Intriguingly, patient I carrying *CSF1R* p.T79M mutation became demented at age 86 years, and patient II with p.K586* mutation developed symptoms at age 79 years. A wide range of onset ages varying from 15 to 78 years had been reported in historical cases,[4,41] but our study further suggested that *CSF1R* mutation carriers might present the first symptom as late as 80s. In other words, screening for *CSF1R* mutations should be considered in patients of all ages with unexplained white matter lesions.

The majority of the *CSF1R* mutations reported in the literature locate within the TKD of CSF1R.[3,4,15] Only a small group of these variants had been investigated in vitro to confirm their deleterious effects on CSF1R function.[2,5,16–19] In the present study and previous ones,[2,5,16,17] missense mutations affecting amino acid residues in the TKD, as well as frameshift or nonsense mutations leading to TKD truncation, would abolish CSF1R kinase activity. Nevertheless, residing within the TKD does not assure the pathogenicity of individual mutation. *CSF1R* p.G747R mutation, located in the TKD, was associated with intact kinase activity of CSF1R.[18] On the contrary, the p.E573L mutation at the transmembrane domain caused impaired CSF1R autophosphorylation.[18] In the present study, the T79M mutant protein had lost

its function to phosphorylate the tyrosine residues, supporting that variants outside the TKD still may play a pathogenic role. The patient with p.E573L mutation and our patient harboring p.T79M mutation both had a late-onset disease with symptom onset at age 70 and 86, respectively. MRI of these two patients both showed diffuse leukoencephalopathy and atrophic corpus callosum, but they had neither calcification on CT nor periventricular lesions on DWI.[18] Our findings strengthen the importance of functional validation of *CSF1R* variants to determine their pathogenicity.

The present study is the first one to demonstrate the cognitive domains primarily affected in the early stage of *CSF1R*-related leukoencephalopathy. Our patients showed severe impairments in processing speed, attention, and executive function. Such features (i.e., subcortical dementia with predominant frontal lobe dysfunction) may reflect the underlying pathogenesis of *CSF1R*-related leukoencephalopathy, in which microglial dysfunction primarily results in axonopathy, followed by demyelination and neuronal degeneration.[3,42] In addition, white matter changes on MRI have been observed 6 years prior to symptom onset,[43] and cortical atrophy over the frontoparietal lobes occurs as disease progression. In addition, patients with *CSF1R* mutations usually have a rapid decline of cognition within 3–4 years and a short survival duration of 6.8 years.[4]

Neuroimaging features can also help diagnose *CSF1R*-related leukoencephalopathy. According to the recently proposed diagnostic criteria,[44] bilateral white matter lesions and thinning of the corpus callosum are the core features of *CSF1R*-related leukoencephalopathy, and spotty calcification also supports the diagnosis. In our five patients, all had the two core features, and one of the three patients who ever received brain CT had calcification. To be noticed, thin-section (1 mm) CT scans and reconstructed sagittal CT images are particularly helpful to detect small calcifications and the characteristic features of "stepping stone appearance in the pericallosal region."[45] Since the brain CT scans were performed at 5-mm section thickness in our three patients carrying the *CSF1R* mutations, small calcification might be overlooked. Previous studies showed that calcifications were detected in 21–67% of the patients with *CSF1R*-related leukoencephalopathy.[46,47] Among these imaging features, thinning of the corpus callosum and diffusion-restricted lesions are better predicting factors of *CSF1R*-related leukoencephalopathy with odds ratios of 42.6 and 10.2, respectively.[46] In addition to the well-known periventricular lesions on DWI, two of our patients had diffusion-restricted lesions at the bilateral pyramidal tracts. The corticospinal tract lesion could be detected by T2WI, FLAIR, or DWI and was found in 63% of the 16 patients in an MRI study of ALSP.[47] The histopathologic analysis of a patient with T_2WI hyperintensity lesions involving bilateral corticospinal tracts from the motor cortex to the midbrain, pons, and medulla revealed vacuolization and reactive gliosis of the corresponding regions.[48]

One limitation of the present study is a lack of pathologic data. However, with the advantage of genetic diagnosis, pathologic examination of brain biopsy tissue is no longer the golden standard to make a definite diagnosis of ALSP. All the five patients harboring *CSF1R* mutations in this study underwent a relentless deterioration in cognitive function and ambulation ability, and three of them had passed away. None of them ever received immunotherapy or other disease-modifying therapy. Although no treatments have been approved for *CSF1R*-related leukoencephalopathy, the latest study confirmed the therapeutic benefits of hematopoietic stem cell transplantation in six of seven patients, in whom the motor and cognitive functions, as well as MRI abnormalities, became stabilized after transplantation.[49]

To conclude, the present study showed that 3.5% of the Taiwanese patients with adult-onset leukoencephalopathy is attributed to *CSF1R*-related leukoencephalopathy. Mutation analysis of *CSF1R* should be considered in patients of all age groups who have cognitive decline and white matter lesions on MRI. Slow processing speed, executive dysfunction, concomitant psychiatric and behavior symptoms, rapid progression of cognitive decline, and corticospinal tract lesions on MRI may be clues to differentiate *CSF1R*-related leukoencephalopathy from other dementia etiologies.

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Conflict of Interest

There is no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Genes in the targeted resequencing panel.

