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【 CASE REPORT 】

CSF1R Mutation p.G589R and the Distribution Pattern of Brain Calcification

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Abstract:
We herein report the case of a 47-year-old female with the colony-stimulating factor 1 receptor (*CSF1R*) mutation p.G589R, which is related to hereditary leukoencephalopathy with axonal spheroid (HDLS). The patient presented with an early-onset cognitive decline and progressive aphasia. Brain magnetic resonance imaging revealed HDLS-related alterations. In addition, brain computed tomography revealed interspersed spotty calcifications in the frontal and parietal subcortical white matter, while a characteristic "stepping stone" appearance was observed in the frontal pericallosal regions. Our findings emphasize the importance of calcification appearances in establishing an HDLS diagnosis and in screening for *CSF1R* mutations.

Key words: CSF1R, hereditary diffuse leukoencephalopathy with spheroids, cognitive decline, calcification

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Introduction

Hereditary diffuse leukoencephalopathy with spheroids (HDLS) is an autosomal dominant disease characterized by progressive cognitive and behavioral dysfunctions. Typically, HDLS is characterized by the following neuropathological features: (i) microscopic axonal swellings or "spheroids," (ii) pigmented macrophages in the axons and myelin in areas of white matter loss, and (iii) diffuse degenerative changes in the cerebral white matter and the corpus callosum. It is well established that HDLS is caused by mutations in the colony-stimulating factor 1 receptor (*CSF1R*) gene (OMIM# 164770) located at 5q32 (1-3). The disease onset typically occurs in the fourth or fifth decade of life and is followed within a few years by a progressive cognitive decline, behavioral changes, and/or Parkinsonism (4).

Many reports have described Japanese patients with cognitive decline related to *CSF1R* mutations (5-12). The detection of brain calcification with a "stepping stone" appearance in the frontal pericallosal regions using computer tomography (CT) has been recently reported as a useful approach for an HDLS diagnosis (13). Calcification in the white matter is commonly observed in patients with *CSF1R* mutations (4). We experienced a case of a 47-year-old woman harboring a mutation in *CSF1R*, namely, the c.1765G>Ap.589R mutation. The patient presented with progressive aphasia and distinctive brain magnetic resonance imaging (MRI) manifestations. Given these findings, we advocate the importance of neuroimaging in validating the decision to perform genetic tests in patients with an early onset of cognitive decline.

Case Report

The patient was a 47-year-old Japanese woman. At 44 years of age, her family noticed that she had difficulties in verbally expressing herself. At 46 years of age, the patient frequently had difficulties in performing daily activities and displayed gait disturbances, small steps, and a stooped posture. Her body weight decreased by approximately 10 kg in 1 year. In that same year, at 46 years of age, she was admitted to Juntendo University Hospital. The patient did not have any remarkable history of medical conditions. Furthermore, there was no family history related to a cognitive decline. The patient manifested non-fluent aphasia, categorized as transcortical motor aphasia, limb-kinetic apraxia in her left hand, and dressing apraxia. Furthermore, she scored 19/

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30 on the Mini-Mental State Examination and 19/30 on the revised Hasegawa's Dementia Scale. Her speech was non-fluent, unclear, and sometimes presented with a rushed quality. The number of spoken words was extremely low, although her comprehension of words was intact. There were no remarkable findings on blood or cerebrospinal fluid examinations.

Brain MRI revealed hyperintensities in the deep white mater, severe cortical atrophy with frontal predominance, and progressive thinness in the corpus callosum (Figure a and b). Brain CT revealed diffuse spotty calcification in subcortical areas and a "stepping stone" appearance on the left side of the frontal pericallosal regions (Figure c and d). Single-photon emission computerized tomography (SPECT) with N-isopropyl-p-[^{123}I] iodoamphetamine indicated bilateral hypoperfusion in the frontal and parietal lobes, dominantly on the right side, and the cingulate gyrus. We therefore clinically diagnosed the patient with HDLS and subsequently performed a genetic screening test for *CSF 1R* mutations. Prior to the gene analysis, we obtained written informed consent from the patient and her family members.

The DNA was extracted from the peripheral blood and sequenced in accordance with the Sanger method using the BigDye Terminator v1.1 Cycle Sequencing Kit and a 3130 Genetic Analyzer (Life Technologies, Foster City, USA). All coding exons and exon-intron boundaries (exons 1 to 22) in the *CSF1R* gene were screened. Sequences and polymerase chain reaction (PCR) conditions have been described previously (2). The genotyping results indicated a heterozygous mutation, c.1765G>A, p.G589R, in exon 13 of *CSF1R* (Figure e). This result matched the reference gene in GenBank (NM_005211) and has been recently reported as a pathogenic mutation in HDLS (4). Based on these results, we confirmed our diagnosis of HDLS.

A gene database analysis using Mutation Taster (http://www.mutationtaster.org/) indicated this mutation as "disease-causing." Polymorphism Phenotyping v2 (PolyPhen-2; http://genetics.bwh.harvard.edu/pph2/) and a Sorting Intolerant From Tolerant (SIFT; http://sift.jcvi.org/) algorithm further confirmed the mutation as "damaging" (HumDiv, 1.000 and HumVar, 1.000). SIFT indicated p.G589R to be deleterious. It is worth noting that the p.G589R mutation was not listed in the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org/). In addition, p.G589 is evolutionarily conserved, as indicated by the National Center for Biotechnology Information HomoloGene (www.ncbi.nlm.nih.gov/pubmed) (Figure f). We therefore concluded that p.G589R was a pathogenic mutation.

Discussion

We herein report the case of a patient who initially presented with motor aphasia combined with specific findings of diffuse and spotty calcification on brain computed tomography (CT) scans and characteristic brain MRI findings indicative of HDLS. In this case, the interspersed spotty calcification was a critical key to establishing the differential diagnosis. Indeed, HDLS may easily be misdiagnosed as early-onset Alzheimer's disease, frontotemporal dementia, or atypical parkinsonism.

Spotty calcification on the brain CT scans has been suggested as a characteristic finding in patients with HDLS (4, 13). Table 1 summarizes previous reports describing patients confirmed with *CSF1R* mutations and brain calcification detected on CT scans (5, 6, 12-15). Spotty calcification was primarily observed in the frontal subcortical white matter in all summarized patients (100%, 17/17) and secondarily in the parietal subcortical white matter (41.2%, 7/17). However, calcification was not commonly detected in the basal ganglia or cerebellum. Although it has not been referred to in many previous reports, a "stepping stone" appearance in the frontal pericallosal regions was additionally observed in our patient. Further studies are needed to verify the prevalence and specificity of this "stepping stone" appearance in the diagnosis of HDLS with *CSF1R* mutation. Konno et al. (4) previously reported cognitive decline in all of their patients with *CSF1R* mutations (100%, 17/17), combined with prevalent personality and behavioral changes (64.7%), Parkinsonism (52.9%), and pyramidal signs (70.6%). In contrast, depression and seizure were rarer symptoms.

The neuroimaging results in our patient are similar to those reported in patients with Nasu-Hakola disease, which is characterized by systemic bone cysts and early-onset progressive cognitive decline with leukoencephalopathy. Nasu-Hakola disease is caused by loss-of-function mutations in the DNAX-activating protein of the 12-kDa triggering receptor expressed on myeloid cells (DAP12-TREM2) protein complex (16). Brain CT findings in patients with Nasu-Hakola disease also show multiple calcifications in the basal ganglia (17). Furthermore, multiple cystic bone lesions are caused by osteoclast cytoskeletal reorganization due to the aberrant DAP12 cascade, which is related to CSF1R signaling (18). Nasu-Hakola disease and HDLS may share common pathomechanisms related to brain calcification, at least in part.

In our patient, progressive non-fluent aphasia was the initial symptom. However, HDLS leads to various clinical phenotypes and prognoses (19). Personality and behavioral changes are usually the common initial symptoms in HDLS. To our knowledge, only three cases with *CSF1R* mutations have been reported to exhibit difficulties in expressing words or impaired verbal fluency as the initial symptom [(20-22); (Table 2)]. In all HDLS patients, aphasia has been categorized as motor aphasia. Lee et al. (21) described a case with progressive non-fluent aphasia and hypometabolism in the bilateral putamen and cortical areas as indicated by 2-deoxy-2-[fluorine-18]-fluoro-D-glucose positron emission tomography (^{18}F-FDG-PET) integrated with CT (21). In our case, SPECT indicated hypoperfusion dominantly on the right side of the frontal and parietal lobes. Previous studies

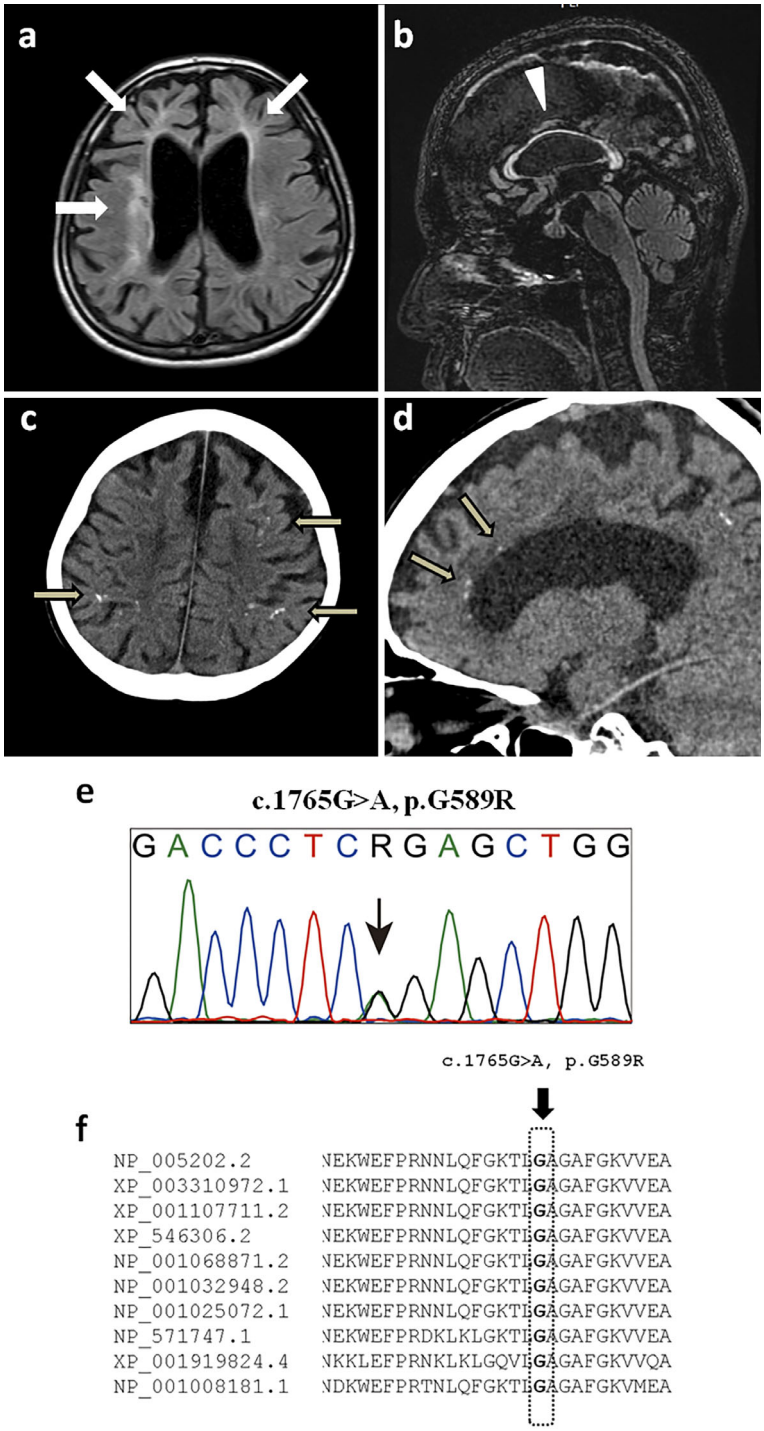

Figure. **Result of direct sequencing of *CSF1R*, neuroimaging findings, and conservation of the mutation p.G589R. (a) Brain MRI axial-view, fluid-attenuated inversion recovery weighted shows white matter lesions in the bilateral subcortex and diffuse atrophic changes in the cortex with predominant frontal lobe (white arrows). (b) Brain MRI sagittal-view shows progressive thinning of the corpus callosum (white arrowhead). (c) Brain CT axial-view shows interspersed spotty calcification in the region of the frontal and parietal subcortical white matter (gray arrows). (d) Brain CT 1-mm-thick sagittal-view shows a "stepping stone" appearance in the frontal pericallosal region on the left side (gray arrows). (e) Direct sequencing reveals a heterozygous mutation, c.1765G>A, p.G589R in exon 13 of *CSF1R*. (f) Conservation of c.1765G>A, p.G589R. Protein homologues were aligned using NCBI homolo gene (http://www.ncbi.nlm.nih.gov/pubmed/). GeneBank accession numbers: *Homo sapiens*, NP_05202.2; *P. troglodytes*, XP_003310972.1; *M. mulatta*, XP_001107711.2; *C. lupus familiaris*, XP_546306.2; *B. taurus*, NP_001068871.2; *M. musculus*, NP_001032948.2; *R. norvegicus*, NP_ 001025072.1; *D. rerio*, NP_ 571747.1; *X. tropicalis*, NP_ 001008181.1.**

Table 1. **Clinical Overview of Patients with *CSF1R* Mutations and the Regions of Calcification on Brain CT.**

Reference	Mutations in *CSF1R*	Gender	Age at onset	Clinical findings							Regions of calcification			
				Initial symptom	Cognitive decline	Personality and behavioral changes	Depression	Parkinsonism	Pyramidal sign	Seizure	Frontal subcortical whitematter	Parietal sub-cortical whitematter	Basal ganglia	Stepping stone appearance in the frontal prricallosal regions
14	p.G589E	F	47	dysarthria, loss of balance, falls, hand tremor	+	+	-	-	+	+	+	+	-	NA
12	p.A823V	F	50	cognitive impairment	+	-	-	-	-	-	+	-	-	NA
5	p.G765D	F	37	cognitive impairment/ personality and behavior change	+	+	-	+	+	-	+	-	-	NA
	p.A781E	F	36	cognitive impairment/ personality and behavior change	+	+	-	-	+	-	+	-	-	NA
	p.I794T	M	40	cognitive impairment	+	+	-	-	+	+	+	+	-	NA
	c.2442+1 G>T	M	53	cognitive imapairment	+	+	-	+	+	-	+	-	-	NA
	p.P824S	F	45	cognitive imapairment/ depression	+	+	+	+	+	-	+	-	-	NA
6	p.A792D	M	41	cognitive impairment	+	+	-	-	-	-	+	-	+	+
15	p.E847V	F	32	gait disturbance and cognitive impairment	+	+	-	+	+	+	+	+	-	NA
13	p.G589R	F	37	Gait disturbance	+	-	-	+	-	-	+	-	-	+
	p.A652P	F	30	Gait disturbance	+	+	-	-	+	-	+	+	-	NA
	c.2442+ 5G>A	F	27	Gait disturbance	+	-	-	+	+	-	+	-	-	+
	c.2442+ 5G>A	M	58	Cognitive decline	+	+	-	-	+	-	+	+	-	NA
	c.2442+ 5G>C	F	23	Cognitive decline	+	-	-	-	+	-	+	+	-	NA
	p.M766T	F	18	Cognitive decline	+	-	+	+	-	-	+	-	-	+
	p.G589E	M	58	Cognitive decline	+	-	+	+	+	+	+	-	-	NA
Our case	p.G589R	F	44	Cognitve decline/aphasia	+	+	-	+	-	-	+	+	-	+
Average		M:F= 5:12	39.8 ± 11.9		100% (17/17)	64.7% (11/17)	17.6% (3/17)	52.9% (9/17)	70.6% (12/17)	23.5% (4/17)	100% (17/17)	41.2% (7/17)	5.9% (1/17)	

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Table 2. Cases with *CSF1R* Mutations Presenting with Progressive Verbal Non-fluency as an Initial Symptom.

Reference	20	21	22	Our case
Gender	Male	Female	Male	Female
Gene analyisis of *CSF1R*	p.R782G	c.2442+1 G>T	p.E664K	p.G589R
Age at onset	57	47	56	47
Initial symptom	Slurred speech and difficulty finishing sentences	Imaired verbal fluency	Word finding difficulty	Difficulty of words expression
Type of aphasia at onset	Motor aphasia	Transcortical motor aphasia	Motor aphasia	Transcortical motor aphasia
Apraxia	+	-	-	+
Cognitive decline	+	+	+	+
Leukoencepahlopathy in the dep white matter on brain MRI	+	+	+	+
Hypoperfision regions in brain PET/SPECT	NA	Hypoperfusion in thalamus and diffuse cortical area	NA	Bilaterally hypoperfusion in the frontal and parietal lobes.

have reported the presence of diffuse hypometabolism in the frontal and parietal areas using PET, or frontotemporal or frontoparietal hypoperfusion using 99-Tc-ethyl cysteinate dimer (99mTc-ECD) single-photon emission CT (3, 23, 24). Patients with *CSF1R* mutations may, therefore, predominantly demonstrate functional decline in the frontal lobe, which is in line with a previous finding (4). Further studies are needed to assess the relationship between verbal symptoms and frontal lobe dysfunction in patients with *CSF1R* mutations.

In conclusion, we herein described a patient with early-onset cognitive decline and a *CSF1R* mutation who initially presented with aphasia. Interspersed calcification in the frontal subcortical white matter is a decisive finding in making a diagnosis for HDLS and subjecting patients to genetic testing for CSF1R mutations.

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

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