

CSF1R mutations link POLD and HDLS as a single disease entity

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ABSTRACT

Objective: Pigmented orthochromatic leukodystrophy (POLD) and hereditary diffuse leukoencephalopathy with axonal spheroids (HDLS) are rare neurodegenerative disorders characterized by cerebral white matter abnormalities, myelin loss, and axonal swellings. The striking overlap of clinical and pathologic features of these disorders suggested a common pathogenesis; however, no genetic or mechanistic link between POLD and HDLS has been established. Recently, we reported that mutations in the colony-stimulating factor 1 receptor (*CSF1R*) gene cause HDLS. In this study, we determined whether *CSF1R* mutations are also a cause of POLD.

Methods: We performed sequencing of *CSF1R* in 2 pathologically confirmed POLD families. For the largest family (FTD368), a detailed case report was provided and brain samples from 2 affected family members previously diagnosed with POLD were re-evaluated to determine whether they had HDLS features. In vitro functional characterization of wild-type and mutant *CSF1R* was also performed.

Results: We identified *CSF1R* mutations in both POLD families: in family 5901, we found c.2297T>C (p.M766T), previously reported by us in HDLS family CA1, and in family FTD368, we identified c.2345G>A (p.R782H), recently reported in a biopsy-proven HDLS case. Immunohistochemical examination in family FTD368 showed the typical neuronal and glial findings of HDLS. Functional analyses of *CSF1R* mutant p.R782H (identified in this study) and p.M875T (previously observed in HDLS), showed a similar loss of *CSF1R* autophosphorylation of selected tyrosine residues in the kinase domain for both mutations when compared with wild-type *CSF1R*.

Conclusions: We provide the first genetic and mechanistic evidence that POLD and HDLS are a single clinicopathologic entity. *Neurology*® 2013;80:1033-1040

GLOSSARY

ALSP = adult-onset leukoencephalopathy with axonal spheroids and pigmented glia; **bvFTD** = behavioral variant frontotemporal dementia; **CSF1R** = colony-stimulating factor 1 receptor; **CSF1R_{WT}** = wild-type colony-stimulating factor 1 receptor; **FTD** = frontotemporal dementia; **H&E** = hematoxylin & eosin; **HDLS** = hereditary diffuse leukoencephalopathy with axonal spheroids; **POLD** = pigmented orthochromatic leukodystrophy; **STMS** = Short Test of Mental Status.

Familial pigmented orthochromatic leukodystrophy (POLD) and hereditary diffuse leukoencephalopathy with axonal spheroids (HDLS) are white matter disorders. Thus far considered distinct diseases, clinical and pathologic features suggest that POLD and HDLS are of the same disease spectrum. The pigmented macrophages identified in HDLS families are similar to those in POLD,¹⁻⁵ whereas axonal dilations found in POLD families coincide with axonal spheroids described in HDLS.^{3,5-7} Clinically, most patients with familial POLD and HDLS present with psychiatric symptoms, which progress into dementia, often of a frontotemporal phenotype.^{3,5,8-11} MRIs are consistent with this clinical presentation, because both POLD and HDLS patients portray frontal-predominant atrophy.¹²

Although clinical and pathologic data are strikingly similar between POLD and HDLS, a common genetic cause would further solidify these diseases as a single entity.

Supplemental data at
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Recently, we reported mutations in the colony-stimulating factor 1 receptor (*CSF1R*) gene as the first known cause of HDLS.¹³ We identified a unique pathogenic mutation in the *CSF1R* kinase domain in each autopsy- or biopsy-confirmed HDLS family included in the study, and in 1 clinically diagnosed patient with corticobasal syndrome. The antemortem diagnoses among patients in these HDLS families varied significantly, including frontotemporal dementia (FTD), corticobasal syndrome, Alzheimer disease, multiple sclerosis, atypical cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, and Parkinson disease,¹³ suggesting that HDLS may masquerade as other neurologic conditions leading to underdiagnosis. The objective of this study was to determine whether *CSF1R* mutations also cause familial POLD.

METHODS Standard protocol approvals, registrations, and patient consents. We studied 2 neuropathologically confirmed POLD families (families FTD368 and 5901) and 1,089 neurologically normal, control individuals ascertained at Mayo Clinic Jacksonville. This study was approved by the Mayo Clinic Institutional Review Board and all subjects and/or their proxies gave informed consent to take part in this study.

Neuropathology. Paraffin-embedded fixed tissue sections (5–7 μ m thick, mounted on glass slides) were available from cerebral cortex and basal ganglia on patients FTD368 II-1 and II-2. Tissue sections were stained with hematoxylin & eosin (H&E), and with Luxol fast blue/periodic Schiff. Unstained sections were processed for autofluorescence. Sections were also immunostained with a DAKO Autostainer (DAKO, Carpinteria, CA) using Envision reagents and 3,3'-diaminobenzidine as the chromogen, with the following antibodies: amyloid precursor protein (mouse monoclonal [22C11], 1:10,000; Chemicon, Temecula, CA); phosphorylated neurofilament (mouse monoclonal [SMI-31], 1:40,000; Sternberger Monoclonals, Lutherville, MD); ubiquitin (mouse monoclonal [MAB1510], 1:60,000; Chemicon, Burlingame, CA); α B-crystallin (rabbit polyclonal, 1:10,000; gift from Dr. Jack Liang, Harvard University); human leukocyte antigen-DR for microglia (mouse monoclonal [LN-3], 1:100; eBioscience, San Diego, CA); and glial fibrillary acidic protein (rabbit polyclonal, 1:5,000; Biogenex, Fremont, CA).

DNA extraction, sequencing, and haplotype analyses. DNA was extracted from blood (FTD368 III-1; 5901 III-2), frontal cortex (FTD368 II-2), and formalin-fixed, paraffin-embedded tissue (FTD368 II-1) using Gentra Puregene kit and QIAamp FFPE methods (Qiagen, Valencia, CA). In the probands (FTD368 III-1 and 5901 III-2), *CSF1R* exons 12–22 were PCR-amplified using M13-tailed primers,¹³ and segregation analyses of the c.2345G>A (p.R782H) mutation in patients FTD368 II-1 and II-2 were performed using custom primers (5'-CACGACGTTGTAACGACTGTTCACTCCAGCAGGGAC-3' and 5'-GGATAACAATTTACACAGGCCAATCTTGGCCACATGAC-3'). PCR products were purified using the AMPure system (Beckman Coulter, Brea, CA) and sequenced in both directions using M13 primers and Big Dye Cycle Sequencing chemistry (Applied Biosystems, Foster City, CA). Sequence

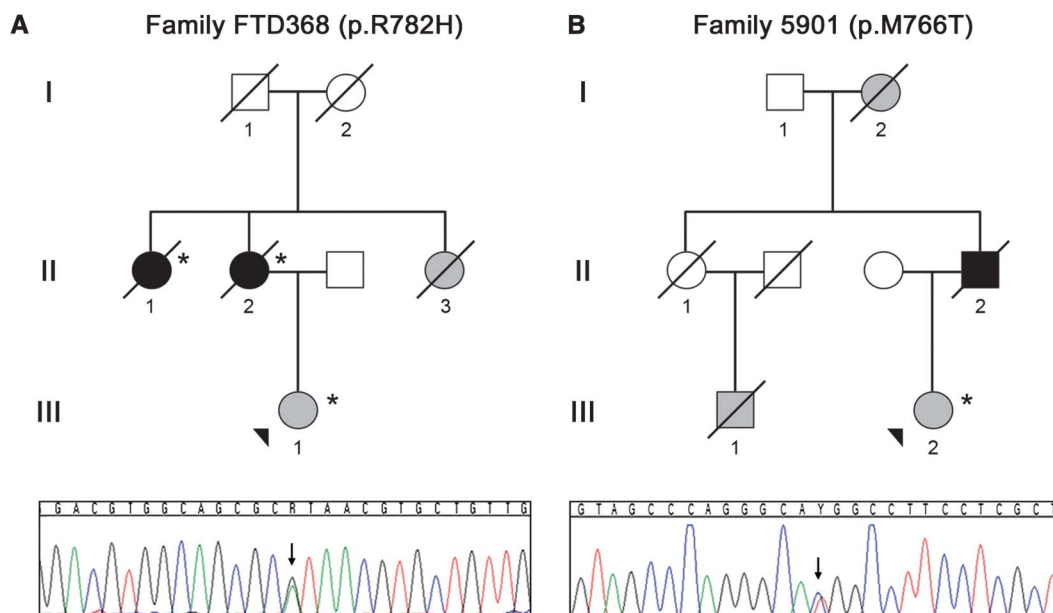
reactions were purified with CleanSEQ (Beckman), sequenced on an ABI3730xl Genetic Analyzer, and analyzed using Sequencher (Gene Codes, Ann Arbor, MI). A custom TaqMan single nucleotide polymorphism genotyping assay was designed for c.2345G>A (p.R782H), and controls were genotyped on the 7900HT Fast Real Time PCR system. Genotype calls were made using SDS version 2.2 software (Applied Biosystems). For haplotype sharing studies, 10 STR markers flanking *CSF1R* were PCR-amplified with 1 fluorescently labeled primer, analyzed on an ABI3730xl analyzer, and scored using GENOTYPER software.

***CSF1R* cDNA clone mutagenesis.** A cDNA construct encoding the human *CSF1R* gene was purchased from OriGene (Rockville, MD; RC205288). Mutations p.M875T and p.R782H were introduced independently using the QuickChange site-directed mutagenesis kit (Stratagene, Santa Clara, CA) and mutagenesis primers (M875T: 5'-GAAGGATGGATACCAACCGCC-CAGCCTGCATTG-3' and 5'-CAAATGCAGGCTGGGCCGTTTGGTATCCATCCTTC-3'; R782H: 5'-GACGTGGCAGCGCATAACGTGCTGTTG-3' and 5'-CAACAGCACGTTATGCGCTGCCACGTC-3'). Mutation-positive clones were identified by sequencing and transformed into DH5 α *Escherichia coli* cells, after which DNA was obtained using the Nucleobond Xtra Maxi Plus EF kit (Clontech, Mountain View, CA).

Cell culture and immunoblotting. HeLa cells were maintained in Eagle minimum essential medium with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C, 5% CO₂. Cells were transfected with 2 μ g of tGFP control plasmid DNA (OriGene) or *CSF1R* plasmid DNA (wild-type or mutant) using Lipofectamine 2000 (Life Technologies, Grand Island, NY). Two days posttransfection, the media was changed to serum-free media for 16 hours before treatment with 50 ng/mL human recombinant CSF-1 protein (R&D Systems, Minneapolis, MN). CSF-1 treatment continued for 5, 15, or 30 minutes before harvesting. HeLa cells without CSF-1 treatment were collected as controls. Sample protein was denatured in 2 \times Novex Tris-glycine sodium dodecyl sulfate sample buffer (Life Technologies), and equal volumes were run on 10% sodium dodecyl sulfate–polyacrylamide gels (Life Technologies), transferred to Immobilon membranes (Millipore, Billerica, MA), and immunoblotted. Total *CSF1R* levels were detected using the rabbit polyclonal c-Fms/CSF-1R (C-20) antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA). *CSF1R* autophosphorylation was detected using rabbit polyclonal *CSF1R* phospho-tyrosine primary antibodies against p-Y⁵⁴⁹ (1:5,000), p-Y⁶⁹⁹ (1:2,000), p-Y⁷⁰⁸ (1:5,000), p-Y⁷²³ (1:6,000), and p-Y⁸⁰⁹ (1:500) purchased from Cell Signaling Technology (Danvers, MA). A GAPDH primary antibody (1:500,000) (Meridian Life Science, Memphis, TN) was used to ensure equal protein loading. Blots were then incubated with anti-mouse or anti-rabbit secondary horseradish peroxidase–conjugated antibodies (Promega, Madison, WI) and bands were detected by enhanced chemiluminescence using Western Lightning Plus-ECL reagents (Perkin Elmer, Waltham, MA).

RESULTS FTD368 III-1, clinical case report. The proband of family FTD368 (FTD368 III-1; figure 1) was a white, non-Hispanic female who at the age of 51 years began to have difficulties at work. She missed a meeting, showed up for another meeting several days early, and failed a work-related examination. She experienced forgetfulness and repeated stories, but remembered conversations. She began to struggle with arithmetic. Her husband noted a large discrepancy in her checking account, potentially from impulsive buying. She became more irritable toward her husband and retired after the

Figure 1 Families with POLD and *CSF1R* mutations



Shown are abbreviated pedigrees of 2 families, family FTD368 (A) and family 5901 (B) affected by pigmented orthochromatic leukodystrophy (POLD) included in this study. Gray-filled symbols represent clinically affected individuals, whereas black-filled symbols represent individuals with pathologically confirmed POLD. The proband of each family is indicated with an arrowhead. Cases for which DNA was available for genetic studies are noted with an asterisk. DNA sequence traces observed in a sample from the proband from each family are shown below each pedigree. The single base substitution in each trace is indicated with an arrow.

forgetfulness at work became apparent. After retiring, she initially functioned well on her own. She had no other medical, neurologic, or psychiatric history. On the Short Test of Mental Status (STMS), she lost 2 points on recall, 2 points on drawing, and scored 34 of 38. The remainder of her neurologic examination, including balance, coordination, alternating motion, muscle tone, and gait, was normal. An MRI scan showed prominent frontal brain atrophy, and extensive white matter hyperintensity changes throughout the anterior portion of the frontal lobes, as well as surrounding the ventricular system in the temporal, parietal, and occipital lobes.

One year later, she was having greater difficulty multitasking. Her memory had worsened, she repeated herself frequently, and had some evidence of disinhibition and a tendency to say socially inappropriate things. She was still doing some cooking and driving locally, but had become slow to react to changes in situations and events. Her STMS score decreased 8 points to 26 of 38, but her motor neurologic examination was normal.

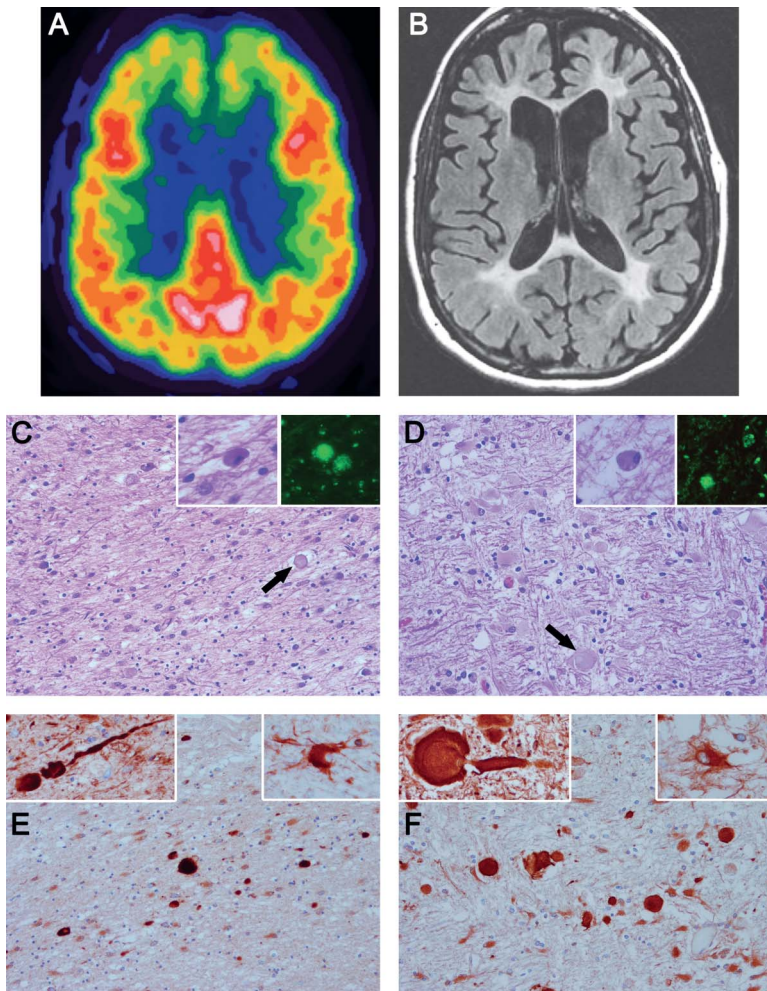
Two years after first being seen, she had worsened substantially. She spent her days sitting, had ceased all household chores, and needed assistance with dressing and bathing. She tended to put layers of clothes on, but often put them on backward. Her weight was stable. Her STMS score had decreased to 17 of 38. Her walking was slower, but balance was satisfactory. The MRI findings were not dramatically different compared with 2 years prior. At the time of the initial visit, the clinical

diagnosis was behavioral variant FTD (bvFTD). PET using 18-fluorodeoxyglucose tracer showed hypometabolism when first seen in 2006 (figure 2A). The CT brain scan at that time showed enlargement of the frontal horns of the lateral ventricles. A year later, the MRI scan showed frontal lobe atrophy, frontal lobe white matter hyperintensities, and similar changes in the parietooccipital white matter, with enlargement of the frontal horns of the lateral ventricles, and milder diffuse cerebral atrophy (figure 2B). An MRI scan performed 2 years later showed progression of white matter hyperintensities and slight increase in atrophy.

The proband's mother and 2 maternal aunts (FTD368 II-1, II-2, and II-3; figure 1) showed similar symptoms and were clinically diagnosed with dementia between ages 40 and 70. Her grandparents did not have dementia at the time of death. The grandfather died in his 40s of cancer. The grandmother died at age 65 years of a ruptured aortic aneurysm. Neuropathologic examination of the proband's aunt (FTD368 II-1) and mother (FTD368 II-2) led to the rare diagnosis of POLD in both patients.¹⁴

Neuropathology re-evaluation. Archival tissue from FTD368 II-1 and II-2, reported previously,¹⁴ was re-evaluated with histologic and immunohistochemical methods as in previous reports.^{2,5,8,15} The findings in II-1 and II-2 were essentially the same. For comparison, FTD368 II-2 (figure 2, C and E) was compared with an HDLS case with a documented *CSF1R*

Figure 2 Neuroimaging and neuropathologic findings in pigmented orthochromatic leukodystrophy



(A) 18-Fluorodeoxyglucose PET scan performed in 2006 from FTD368 II-1 shows hypometabolism in both frontal lobes. (B) MRI scan performed in 2007 shows atrophy of the frontal lobes, white matter hyperintensities in the white matter of the frontal lobes and similar changes in the parieto-occipital white matter, enlargement of the frontal horns of the lateral ventricles, and milder diffuse cerebral atrophy. (C–F) Microscopic images of white matter from FTD368 II-2 (C and E) compared with typical hereditary diffuse leukoencephalopathy with axonal spheroids (HDLS) (D and F). On hematoxylin & eosin stains, the white matter has pallor because of myelinated fiber loss and gliosis, with axonal spheroids (arrows in C and D). Within the affected white matter are macrophages with basophilic to brown fine granules (left insets in C and D) that have autofluorescent pigment (right insets in C and D) when unstained sections are viewed with a fluorescent microscope. Immunohistochemistry for amyloid precursor protein (panels E and F) shows many axonal spheroids in affected white matter, which are confirmed to be axonal in origin by immunoreactivity with phosphorylated neurofilament (left insets in E and F). A characteristic feature of HDLS is the presence of bizarre hypertrophic astrocytes demonstrated with immunohistochemistry for α B-crystallin (right insets in E and F). C–F: original magnification $\times 200$; all insets: original magnification $\times 400$.

mutation (figure 2, D and F). Cerebral white matter in both POLD and HDLS had staining pallor on H&E because of myelinated fiber loss. Axonal spheroid number varied (arrows in figure 2, C and D) but was most numerous in areas with early white matter pathology and less abundant in burnt-out white matter areas that had few axons and merely dense astrocytic gliosis. Immunohistochemistry for amyloid precursor protein most readily detected axonal spheroids (figure 2, E and F), as well as phosphorylated

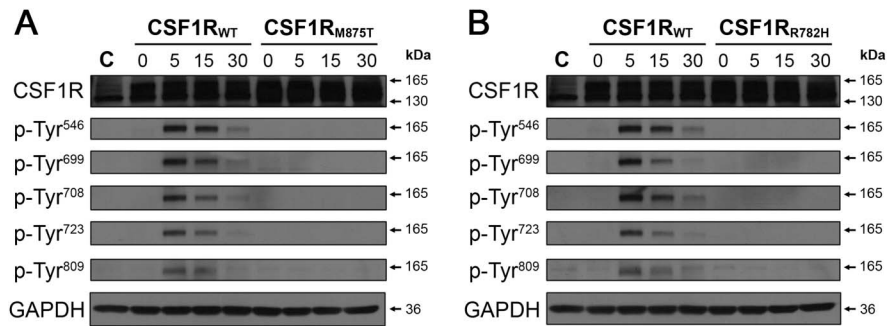
neurofilament (insets, figure 2, E and F). There was variable ubiquitin immunoreactivity in the spheroids (not shown). In both conditions, there were sparse to moderate pigment-laden macrophages visible on H&E (insets, figure 2, C and D) and the pigment was autofluorescent, as originally reported¹⁴ (insets, figure 2, C and D). The small heat shock protein, α B-crystallin, showed bizarre hypertrophic astrocytes in affected white matter (insets, figure 2, E and F), and scattered ballooned neurons were observed in the overlying cortex, as noted previously in HDLS.^{2,8}

Genetic screening. *CSF1R* exons 12–22 sequencing analyses in FTD368 III-1 showed a mutation in exon 18, c.2345G>A, introducing a p.R782H missense mutation in the *CSF1R* protein. By direct sequencing, the mutation was confirmed in the proband's mother (FTD368 II-2) and maternal aunt (FTD368 II-1) using primers designed to generate a short amplicon. The mutation was excluded in 1,089 white, non-Hispanic control subjects using a TaqMan single nucleotide polymorphism genotyping assay.

p.R782H *CSF1R* autophosphorylation is abrogated. In the brain, *CSF1R* is a tyrosine kinase receptor highly expressed in microglia and, to a lesser extent, in neurons. Upon binding to the CSF-1 ligand, *CSF1R* forms homodimers before autophosphorylation of its kinase domain. This autophosphorylation precedes *CSF1R*-dependent phosphorylation of downstream targets, rendering this process critical for CSF-1 signaling. In our previous study, we performed functional analyses of HDLS-causing *CSF1R* mutations p.M875T, p.M766T, and p.E633K and found that *CSF1R* autophosphorylation is abrogated in mutant *CSF1R*-transfected cells.¹³ To determine whether the p.R782H mutation identified in POLD family FTD368 in this study similarly affects CSF-1-induced *CSF1R* autophosphorylation, we transfected wild-type *CSF1R* (*CSF1R*_{WT}), a kinase-deficient *CSF1R* (*CSF1R*_{M875T}),¹³ or *CSF1R*_{R782H} in HeLa cells. Immunoblotting revealed that wild-type and mutant *CSF1R* overexpression levels were similar. Upon stimulation with CSF-1, autophosphorylation on multiple *CSF1R* tyrosine residues was observed for *CSF1R*_{WT} after 5 minutes of CSF-1 exposure that gradually decreased over 30 minutes. Conversely, neither the *CSF1R*_{M875T} nor the *CSF1R*_{R782H} mutants showed detectable levels of autophosphorylation at any time post-CSF-1 treatment (figure 3).

POLD family 5901. In a recently identified unpublished family with a history of pathologically confirmed POLD, family 5901, we also analyzed the clinically affected proband's DNA (5901 III-2) for mutations in *CSF1R* exons 12–22. Sequencing analyses revealed the previously published and functionally characterized c.2297T>C mutation in exon 17,

Figure 3 Functional effects of *CSFR1* mutations in cell culture



CSF-1 treatment leads to autophosphorylation of wild-type but not mutant *CSF1R*. HeLa cells were transfected with either a tGFP control plasmid, or a plasmid containing wild-type (*CSF1R*_{WT}), the previously characterized mutant (*CSF1R*_{M875T}) (A), or uncharacterized mutant (*CSF1R*_{R782H}) (B) *CSF1R*. Cells were treated with CSF-1 for 5, 15, or 30 minutes. Each blot was probed with antibodies against total *CSF1R* and with antibodies that detect *CSF1R* autophosphorylation at several tyrosine residues (p-Tyr). Note that CSF-1 treatment only causes *CSF1R* autophosphorylation in cells expressing *CSF1R*_{WT}. GAPDH (glyceraldehyde 3-phosphate dehydrogenase) was used as a loading control.

predicted to cause the amino acid change p.M766T.¹³ This mutation was previously excluded in 1,449 white, non-Hispanic controls. Genetic marker analyses did not suggest a common founder for POLD family 5901 and HDLS family CA1 (figure e-1 on the *Neurology*[®] Web site at www.neurology.org).

DISCUSSION To date, there have been several reports with compelling evidence that the rare adult-onset neurodegenerative white matter diseases, POLD and HDLS, are 1 clinicopathologic entity. Despite the striking overlap in clinical and neuropathologic features of these diseases, a genetic or mechanistic link has not been established. One seemingly discrepant feature between these disorders had been that the HDLS inheritance pattern was usually autosomal dominant, whereas some individual POLD cases arose in families in which no parent displayed neurologic dysfunction, suggesting autosomal recessive inheritance or incomplete penetrance,^{7,16} and questioning the involvement of a single disease gene in both disorders.

Using a combination of genome-wide linkage analyses and exome sequencing, we recently identified mutations affecting the kinase domain of *CSF1R* as the genetic cause in 14 autopsy- or biopsy-proven HDLS families.¹³ A different *CSF1R* mutation was identified in each HDLS family, and the de novo occurrence of at least 1 mutation was confirmed. In the present study, we obtained DNA from one of the largest reported POLD families (FTD368) and 1 smaller unpublished pathologically confirmed POLD family (5901) to test the hypothesis that *CSF1R* mutations could also cause POLD. Similar to the HDLS families, sequencing analyses identified mutations in the *CSF1R* kinase domain in both POLD families. In family 5901, we identified the known p.M766T mutation, previously found in HDLS family CA1, whereas the mutation

identified in family FTD368, p.R782H, had not been identified in our previous study. While this work was underway, the p.R782H mutation was reported by an independent group as a causative mutation in a biopsy-proven HDLS family,¹⁷ further strengthening the evidence of homogeneity between these diseases.

CSF1R is a tyrosine kinase receptor expressed on the surface of microglia, and to a lesser extent in neurons.^{18,19} This protein consists of 3 domains critical to its function: an extracellular ligand-binding domain, a single-pass transmembrane domain, and an intracellular kinase domain for downstream signaling.²⁰ In a nondiseased state, *CSF1R* stimulation by its CSF-1 ligand induces receptor homodimerization, after which *CSF1R* autophosphorylates various kinase domain tyrosine residues to allow subsequent phosphorylation of its cellular targets. The *CSF1R* mutations recently found in HDLS families are all located within the *CSF1R* kinase domain and were shown to abrogate autophosphorylation on several critical tyrosine residues.¹³ Importantly, both *CSF1R* mutations identified in POLD families in this study were also located within the kinase domain. Functional analyses of these mutations (p.R782H as part of this study and p.M766T as part of our previous work) showed similar deficits in autophosphorylation in cultured cells, suggesting that familial POLD and HDLS share a common disease mechanism.

Patients with POLD and HDLS often present with varying neuropsychiatric symptoms, including depression, behavioral changes, and dementia leading to variable clinical diagnoses including FTD, Alzheimer disease, Parkinson disease, and multiple sclerosis.^{7,8,13–17,21–25} The clinical presentation in POLD family FTD368 included symptoms of depression, abnormal behavior, and an inability to function, progressing to dementia in the 3 siblings from the second generation. The proband

(FTD368 II-1), first described in this report, was seen in the Mayo Clinic Alzheimer's Disease Research Center and presented with behavioral changes apparent at work and memory deficits that worsened over time, leading to a clinical diagnosis of bvFTD. Both the frontal and temporal lobes were affected on MRI scans, although extensive white matter hyperintensity changes were also

observed, not typical of bvFTD.^{22,26,27} Family FTD368 illustrates the difficulties associated with clinically diagnosing POLD and HDLS, and explains why in the absence of pathologic examination, misdiagnosis is common. Based on our current findings, we argue that genetic screening for *CSF1R* mutations should, therefore, be considered in patients presenting with a range of neurodegenerative syndromes, including bvFTD, to provide a more accurate diagnosis.

In both kindreds described in this report, the POLD diagnosis was based on neuropathologic evaluations of autopsy or biopsy tissue.¹⁴ The brains of familial POLD cases generally show extensive myelin and axonal damage, as well as the distinctive feature of pigmented macrophages and glia accompanied by cytoplasmic autofluorescent lipofuscin granules.^{4,7,16,24,28} The white matter atrophy and myelin loss is similar in patients with pathologically confirmed HDLS; however, axonal spheroids are prominent in affected white matter in addition to myelinated fiber loss, gliosis, and pigment-laden glial cells.^{3,8,9,23,25,29} However, they can be sparse in areas of white matter with burnt-out pathology and few residual axons, and must be sought for in areas where the white matter pathology is ongoing. In a study of the originally described POLD family in 1936, it was reported that axonal dilations consistent with axonal spheroids were present in the affected brain tissue.¹⁶ Additional case reports have since described such a phenomenon in other POLD families.⁵

In the current HDLS and POLD classification, neither acronym adequately reflects the complete set of molecular genetic, clinical, and pathologic features observed in these 2 closely overlapping disorders. In particular, use of the term leukodystrophy in POLD implies a defect in production or catabolism of myelin, which is not the case. The discovery of defects in *CSF1R*, a trophic factor for microglia, suggests that the axonal spheroids in HDLS are a secondary disease process and that HDLS and POLD might be considered primary microgliopathies that lead to secondary axonal and myelin pathology. While the presence of morphologically abnormal (i.e., pigment-laden) microglia in POLD is more closely linked to the genetic defect, the specificity of autofluorescent pigment in microglia is not. Recently, we proposed the term *adult-onset leukoencephalopathy with axonal spheroids and pigmented glia* (ALSP) to encompass HDLS and POLD. Given that *CSF1R* mutations are now known to cause both disorders, we propose the continued use of ALSP to encompass the microgliopathies previously described as HDLS and POLD. Furthermore, we have included a table (table) with the clinical, imaging, and pathologic features of this *CSF1R*-related disease to assist in the future diagnosis of patients.

Taken together, we identified mutations in *CSF1R*, the gene known to cause familial HDLS, in

Table Clinical, imaging, and pathologic features found in patients with the white matter diseases HDLS and POLD, now collectively referred to as ALSP

Demographics	
Age at onset, y, mean (range)	40 (8-78)
Duration, y, mean (range)	8 (2-34)
Symptoms and signs	
Depression/anxiety ^a	++
Behavioral changes ^a	++
Dementia ^a	++
Parkinsonism	++
Pyramidal signs	++
Epilepsy	++
Ataxia	++
Clinical look-alikes	
MS ^b	Young patients
Multiple subcortical infarcts (including CADASIL)	Any patients
Dementia (vascular, Alzheimer, FTD)	Older patients
Vascular parkinsonism	Any patients
Various leukodystrophies	Any patients
Imaging findings	
White matter lesions	+++
Frontal predominance (PV, SC)	+++
Corpus callosum	++
Pyramidal tract (IC, BS)	+
Cerebellum	–
Initially patchy/focal, later confluent	++
Enhancement	–
Atrophy (cortical and subcortical)	+++
Frontal predominance	+++
Corpus callosum	+
Lateral ventricles enlargement	++
Pathology	
Myelin/axon destruction	+++
Axonal spheroids	+++
Pigmented macrophages	+++

Abbreviations: ALSP = adult-onset leukoencephalopathy with axonal spheroids and pigmented glia; BS = brainstem; CADASIL = cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; FTD = frontotemporal dementia; HDLS = hereditary diffuse leukoencephalopathy with axonal spheroids; IC = internal capsule; MS = multiple sclerosis; POLD = pigmented orthochromatic leukodystrophy; PV = periventricular; SC = subcortical; – = absent; + = occasional; ++ = common; +++ = universal.

^aOften mood and behavioral changes early on, with dementia in later stages.

^bHowever, patients with ALSP display neither optic nerve involvement nor oligoclonal band in CSF.

2 families with autosomal dominant inheritance of pathologically confirmed POLD. This finding confirms the hypothesis that POLD and HDLS are a single disease entity.

AUTHOR CONTRIBUTIONS

Dr. Nicholson: drafting/revising the manuscript for content; analysis or interpretation of data; acquisition of data. Matt C. Baker, NiCole A. Finch, Nicola J. Rutherford: analysis or interpretation of data; acquisition of data. Drs. Wider, Graff-Radford, Nelson, Clark, Wszolek, Dickson, and Knopman: drafting/revising the manuscript for content, including medical writing for content; contribution of vital reagents/tools/patents; obtaining funding. Dr. Rademakers: drafting/revising the manuscript for content, including medical writing for content; study concept and design; analysis or interpretation of data; study supervision; obtaining funding.

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DISCLOSURE

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Editor's Note to Authors and Readers: Levels of Evidence in *Neurology*[®]

Effective January 15, 2009, authors submitting Articles or Clinical/Scientific Notes to *Neurology*[®] that report on clinical therapeutic studies must state the study type, the primary research question(s), and the classification of level of evidence assigned to each question based on the classification scheme requirements shown below (left). While the authors will initially assign a level of evidence, the final level will be adjudicated by an independent team prior to publication. Ultimately, these levels can be translated into classes of recommendations for clinical care, as shown below (right). For more information, please access the articles and the editorial on the use of classification of levels of evidence published in *Neurology*.¹⁻³

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Classification scheme requirements for therapeutic questions

Class I. A randomized, controlled clinical trial of the intervention of interest with masked or objective outcome assessment, in a representative population. Relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences.

Class II. A randomized, controlled clinical trial of the intervention of interest in a representative population with masked or objective outcome assessment that lacks one criterion a-e in Class I or a prospective matched cohort study with masked or objective outcome assessment in a representative population that meets b-e in Class I. Relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences.

Class III. All other controlled trials (including well-defined natural history controls or patients serving as their own controls) in a representative population where outcome is independently assessed or independently derived by objective outcome measurements.

Class IV. Studies not meeting Class I, II, or III criteria including consensus or expert opinion.

AAN classification of recommendations

A = Established as effective, ineffective, or harmful (or established as useful/predictive or not useful/predictive) for the given condition in the specific population. (Level A rating requires at least two consistent Class I studies.)

B = Probably effective, ineffective, or harmful (or probably useful/predictive or not useful/predictive) for the given condition in the specific population. (Level B rating requires at least one Class I study or two consistent Class II studies.)

C = Possibly effective, ineffective, or harmful (or possibly useful/predictive or not useful/predictive) for the given condition in the specific population. (Level C rating requires at least one Class II study or two consistent Class III studies.)

U = Data inadequate or conflicting; given current knowledge, treatment (test, predictor) is unproven.