Mutations in the colony stimulating factor 1 receptor (CSF1R) gene cause hereditary diffuse leukoencephalopathy with spheroids

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Hereditary diffuse leukoencephalopathy with spheroids (HDLS) is an autosomal-dominant central nervous system white-matter disease with variable clinical presentations, including personality and behavioral changes, dementia, depression, parkinsonism, seizures and other phenotypes1-2. We combined genome-wide linkage analysis with exome sequencing and identified 14 different mutations affecting the tyrosine kinase domain of the colony stimulating factor 1 receptor (encoded by CSF1R) in 14 families with HDLS. In one kindred, we confirmed the de novo occurrence of the mutation. Follow-up sequencing identified an additional CSF1R mutation in an individual diagnosed with corticobasal syndrome. In vitro, CSF-1 stimulation resulted in rapid autophosphorylation of selected tyrosine residues in the kinase domain of wild-type but not mutant CSF1R, suggesting that HDLS may result from partial loss of CSF1R function. As CSF1R is a crucial mediator of microglial proliferation and differentiation in the brain, our findings suggest an important role for microglial dysfunction in HDLS pathogenesis.

HDLS typically presents as an autosomal-dominant disease associated with variable behavioral, cognitive and motor changes1-3. The onset of symptoms is usually in the fourth or fifth decade, progressing to dementia with death within 6 years. In magnetic resonance imaging (MRI), HDLS is characterized by patchy abnormalities in cerebral white matter, often initially asymmetrical but becoming confluent and symmetrical with disease progression4-12. The changes predominantly involve the frontal and parietal white matter, with evolving cortical atrophy affecting these lobes (Fig. 1a,b). As neither the clinical symptoms nor the MRI changes are specific, a definite diagnosis of HDLS relies on pathological examination showing widespread loss of myelin sheaths and axonal destruction, axonal spheroids, gliosis and autofluorescent lipid-laden macrophages (Fig. 1c-i). Occasionally, brain biopsy has been used to confirm the diagnosis5.

To identify the genetic basis of HDLS, we established an international consortium with ethical approval from the Mayo Clinic Institutional Review Board and collected clinical data, MRI studies and blood and brain tissue samples from families with at least one individual with autopsy- or biopsy-proven HDLS. In total, we collected 14 kindreds from the United States, Norway, Germany and Scotland (Fig. 2). We selected family VA for genome-wide linkage studies, and using nonparametric linkage analyses we identified one locus with a lod score >2.5 (chromosome 5; lod = 2.67) and four loci with lod scores >1.0 (Supplementary Fig. 1). Subsequent parametric linkage analysis identified significant linkage on chromosome 5q34 (lod = 3.71, θ = 0 at rs13178296), whereas none of the other

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Figure 1 Neuroimaging and neuropathological findings in HDLS-affected subject FL2-1. Subject FL2-1 developed a mild depression followed by forgetfulness at the age of 50 years. Two years later he had a flat affect, inappropriate behavior, poor concentration, executive dysfunction, restless legs syndrome and insomnia. Examination 3 years after the onset of symptoms showed psychomotor slowing and ideomotor and constructional apraxia. The Mini-Mental State Examination score was 22/30. His gait was slow and shuffling. His postural stability was poor, leading to frequent falls. He had rigidity and bradykinesia in all four extremities symmetrically.

(a,b) Axial T2-weighted magnetic resonance images showed localized hyperintense foci in both frontal and parietal lobes (long arrows), involving the periventricular, deep and subcortical white matter but sparing the subcortical U-fibers. Hyperintense focus in the right forceps minor (small arrow) was seen. In the final stage of his illness, he became mute, reached a vegetative state and died at the age of 55 years. Autopsy was performed. (c) Myelin loss in frontal white matter with a pigmented macrophage and a pale vacuolated axonal spheroid (Luxol fast blue). (d) Spheroids with phosphorylated neurofilament immunohistochemistry. (e) Spheroids with amyloid precursor protein immunohistochemistry. (f) Pigmented macrophages and reactive astrocytes (hematoxylin and eosin). (g) White-matter macrophages with HLA-DR (human leukocyte antigen, DR epitope region) immunohistochemistry. (h) Bizarre white-matter astrocytes. (i) Ballooned cortical neurons with a-β-crystallin immunohistochemistry. Scale bar (c–i), 30 μm.

loci reached significance (Supplementary Fig. 1). Obligate recombinants narrowed the candidate region to 30.3 cM between rs801399 and rs1445716 (Supplementary Fig. 2), corresponding to a ~25-Mb genomic interval containing 233 candidate genes.

To generate a list of potential disease-causing mutations, we performed whole-exome sequencing of two pathologically confirmed affected individuals from family VA (VA-21 and VA-24; Fig. 2). We generated variant profiles for each affected individual and searched for shared heterozygous variants located within the chromosome 5q candidate region. We further predicted that mutations underlying HDLS are likely to be previously unidentified; therefore, we filtered all of the identified base alterations against dbSNP132. This led to the identification of two nonsynonymous mutations: c.80C>T (p.Ser27Leu) in the gene encoding 5-hydroxytryptamine receptor 4 (HTR4) and c.2624T>C (p.Met875Thr) in CSF1R. Both mutations segregated with disease in the extended family VA and were absent in 660 controls. We therefore searched for additional mutations in a cohort of 13 probands from autopsy- or biopsy-proven HDLS families (Fig. 2). Sanger sequencing of the six coding exons of HTR4 and 22 coding exons of CSF1R identified heterozygous CSF1R mutations in all 13 probands, whereas no other mutations in HTR4 were identified (Fig. 3 and Supplementary Table 1). Segregation analyses confirmed transmission of the CSF1R mutations and co-segregation with the disease phenotype in all families for which DNA from multiple affected individuals was available (Fig. 2). We further confirmed the de novo occurrence of one CSF1R mutation in monozygotic twins from family NO without a family history of HDLS (Supplementary Fig. 3). To confirm the rarity of these mutations and provide supporting evidence for pathogenicity, we also sequenced the CSF1R gene in 24 unrelated controls and genotyped the 13 newly discovered mutations in at least 1,436 controls of European ancestry using TaqMan genotyping assays. None of the mutations identified in subjects with HDLS and no other novel CSF1R mutations were found in controls.

The residues affected by the 14 CSF1R mutations identified in families with HDLS are all located in the intracellular tyrosine kinase domain of CSF1R, encoded by exons 12–22. The mutations include ten missense mutations and one single-codon deletion, all affecting residues highly conserved across species and within members of the CSF1-PDGF receptor family of tyrosine kinases (Kit, FLT3 and PDGFRα/β, ref. 13; Fig. 3). We further identified three splice-site mutations, leading to the in-frame deletion of exon 13 (NO) or exon 18 (CA2 and FL2), which removes up to 40 consecutive amino acid residues within the tyrosine kinase domain (Supplementary Fig. 4).

Detailed clinical information was available for 24 individuals with proven CSF1R mutations from 14 HDLS families (Table 1). Mean age at onset was 47.2 ± 14.5 years (range 18–78 years), with mean disease duration of 6.0 ± 3.1 years (range 2–11 years) and a mean age at death of 57.2 ± 13.1 years (range 40–84 years). In some families (FL1, CA1 and VA), age at onset or death differed by more than 25 years among family members, whereas a monozoigtic twin pair (family NO) showed highly similar disease courses with ages at onset and death within 1 year of each other, suggesting that currently unidentified genetic or environmental factors may be important determinants of the age-related disease penetrance. Presenting features and evolving clinical symptoms also varied substantially within and
across families, and ante mortem clinical diagnoses in mutation carriers included frontotemporal dementia, corticobasal syndrome, Alzheimer’s disease, multiple sclerosis, atypical cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and Parkinson’s disease.

As most affected people included in our study were not diagnosed with HDLS, we hypothesized that CSF1R mutation carriers may be present in clinical series of early-onset Alzheimer’s disease, frontotemporal dementia, corticobasal syndrome, multiple sclerosis and Parkinson’s disease, or in individuals affected by ischemic stroke with additional white-matter changes. Sequencing of CSF1R exons 12–22, encoding the protein’s tyrosine kinase domain, in up to 93 Mayo Clinic patients affected with these neurological syndromes led to the identification of an additional CSF1R missense mutation, c.2509G>T (p.Asp837Tyr), in a woman with clinical symptoms resembling cortico-

Table 1 is included above the subject.
cell-surface expression are unlikely to be affected; however, we cannot exclude such effects at this time. These preliminary findings suggest that the kinase activity of mutant CSF1R is disrupted, probably affecting the phosphorylation of downstream targets. We speculate that mutant CSF1R might assemble into nonfunctional homodimers and wild-type–mutant heterodimers, inducing a dominant-negative disease mechanism.

To address whether CSF1R autophosphorylation is also disrupted in individuals with HDLS, we first subjected blood samples from a healthy control and a subject with HDLS, CA1-1, to CSF1R immunoblotting and observed no apparent difference in total or phosphorylated CSF1R levels (Supplementary Fig. 6a,b). However, statistical analysis did not show a significant difference between any of the groups (P > 0.05 for all study groups for each epitope). Although these preliminary in vivo studies do not demonstrate a defect in autophosphorylation, our findings do not necessarily conflict with the data obtained in cultured cells. First, the subjects with HDLS are heterozygous for the CSF1R mutations and, thus, in contrast to our in vitro experiments, the wild-type receptor is still present in these individuals. Second, in our cell-culture experiments, we downregulated CSF1R signaling by serum deprivation to minimize basal receptor activity before stimulation with the CSF1 ligand. CSF1 is a serum trophic factor that the kinase activity of mutant CSF1R is disrupted, probably affecting the phosphorylation of downstream targets. We speculate that mutant CSF1R might assemble into nonfunctional homodimers and wild-type–mutant heterodimers, inducing a dominant-negative disease mechanism.

Unraveling the genetic etiology of HDLS may contribute to the understanding of other adult-onset leukoencephalopathies. De novo mutations in CSF1R could underlie sporadic cases that have been described with clinical and pathological similarities to HDLS24–29. Future CSF1R mutation screening may also determine whether HDLS and pigmentary orthochromatic leukodystrophy are part of a single clinicopathologic entity, as has recently been suggested2. Moreover, the discovery of a mutation in a microglial trophic factor receptor may further elucidate the role of microglia in more common white-matter disorders, particularly those associated with axonal dystrophy such as Binswanger’s disease24,30, multiple sclerosis31 and HIV encephalitis32.

Notably, our findings also shed new light on Nasu-Hakola disease (NHD), a rare condition characterized by systemic bone cysts and dementia with striking similarities to HDLS33–35. NHD is caused by recessive loss-of-function mutations in the DAP12-TREM2 protein complex36,37, which was recently implicated in CSF1R signaling, establishing NHD as a primary microglial disorder38. We speculate that a partial loss of the CSF1R-DAP12 signaling cascade in microglia is responsible for the neurological phenotypes observed in HDLS.

Figure 3 Genomic organization and protein domain structure of CSF1R, with summary of CSF1R mutations. (a) The CSF1R gene extends over 60 kb and contains 22 exons (vertical hatches). (b) Exon structure of the human CSF1R cDNA. Positions of the start codon (ATG) and stop codon (TGA) are indicated. Arrows indicate positions of detected mutations relative to exons and protein domains. (c) Domain structure of the CSF1R protein, showing the immunoglobulin domains (Ig) and the protein tyrosine kinase domain (PTK), interrupted by the kinase insert (shaded). (d) Fifteen heterozygous CSF1R mutations detected in 14 families with autopsy- or biopsy-proven HDLS and in one individual clinically diagnosed with corticobasal syndrome. Family identifiers, cDNA numbering (relative to NM_005211.3) and predicted translational changes are indicated. (e) ClustalW alignment for the parts of the PTK domain where the mutations occur, including multiple CSF1R homologs and all members of the human CSF1/PDGF receptor family. Shown are human CSF1R (NP_002600.1), mouse CSF1R (NP_001032948.2), chicken CSF1R (XP_414597.2) and zebrafish CSF1R (NP_571747.1). Amino acid positions of the mutations are indicated above the alignment.
Table 1  Clinical characteristics of 24 individuals from 14 families with genetically confirmed CSF1R mutations

| Family | Indiv. | Origin | Sex | Onset age | Death age | Initial symptoms | Personality and behavior changes | Dementia | Depression | Parkinsonism | Seizures | Clinical diagnosis | Ref. |
|--------|--------|--------|-----|-----------|-----------|------------------|---------------------------------|----------|------------|-------------|----------|-------------------|------|
| NO     | NO-1   | Norway | F   | 36        | 40        | Dizziness, cognitive impairment | +                  | +         | +          | +          | +       | MS                | New  |
|        | NO-2   |        | F   | 38        | 41        | Depression, cognitive impairment | +                  | +         | +          | +          | +       | MS                | New  |
| MD     | MD-1   | US     | M   | 58        | 61        | Cognitive impairment             | +                  | +         | –          | +          | +       | FTD               | New  |
|        | MD-2   |        | F   | 47        | 58        | Spasticity                      | +                  | –         | +          | +          | +       | Epilepsy           |      |
| FL1    | FL1-1  | US     | F   | 42        | 46        | Speech problems, loss of dexterity | +                  | +         | +          | –          | CBS     |                  |      |
|        | FL1-2  |        | M   | 67        | 74        | Cognitive impairment             | +                  | +         | +          | +          | +       | Parkinsonism       |      |
|        | FL1-3  |        | M   | 78        | 84        | Cognitive impairment             | +                  | +         | +          | +          | +       | AD                |      |
| CA1    | CA1-1  | US     | F   | 18        | –         | Depression                       | +                  | +         | +          | +          | –       | HDLS              | New  |
|        | CA1-7  |        | M   | 43        | –         | Depression                       | +                  | +         | +          | –          | +       | Psychiatric disease |      |
| DE     | DE-1   | Germany| M   | 52        | 63        | Cognitive impairment             | +                  | +         | +          | +          | –       | FTD, atypical CADASIL | New |
|        | FL2    |        | M   | 50        | 55        | Depression                       | +                  | +         | +          | +          | +       | FTD               | New  |
| MI     | MI-1   | US     | M   | 48        | –         | Gait disturbances                | –                  | –         | +          | +          | –       | MS, atypical PD    | New  |
| SC     | SC-1   | US     | F   | 35        | –         | Cognitive impairment             | +                  | +         | +          | –          | –       | Atypical CADASIL  | 9    |
| CA     | CA2-1  |        | M   | 23        | –         | Gait impairments                 | +                  | –         | +          | +          | –       | Leukoencephalopathy with unknown etiology |      |
| IN     | IN-1   | US     | F   | 63        | 67        | Depression, balance issues       | –                  | –         | +          | +          | +       | Atypical dementia  | New  |
| SCT    | SCT-1  | Scotland| F | 46        | 51        | Depression, epilepsy, cognitive impairment | +                  | +         | –          | +          | –       | FTD, cerebrovascular disease | New  |
| MO3    | MO3-1  | US     | F   | 55        | 63        | Depression                       | +                  | +         | +          | +          | –       | FTD               | New  |
| VA     | VA-21  | US     | F   | 77        | –         | NA                              | NA                 | NA        | NA         | NA         | NA      | FTD               | 10   |
|        | VA-24  |        | M   | 58        | 66        | Cognitive impairment             | +                  | +         | +          | +          | +       | HDLS              | b    |
|        | VA-27  |        | M   | 71        | –         | Cognitive impairment             | +                  | +         | –          | –          | –       | HDLS              | b    |
|        | VA-60  |        | M   | 41        | 43        | Cognitive impairment             | +                  | +         | –          | +          | –       | HDLS              | b    |
|        | VA-69  |        | M   | 46        | 49        | Speech problems                  | +                  | +         | –          | +          | –       | FTD, atypical CADASIL | b    |
| MO2    | MO2-1  | US     | F   | 39        | 49        | Cognitive impairment             | +                  | +         | –          | –          | –       | FTD               | New  |
|        | MO2-4  |        | M   | 33        | 43        | Cognitive impairment             | +                  | +         | –          | –          | +       | FTD               | New  |

MS, multiple sclerosis; FTD, frontotemporal dementia; CBS, corticobasal syndrome; AD, Alzheimer’s disease; PD, Parkinson’s disease; NA, not available.

*Individual had an isolated seizure at age 47. Clinical diagnosis of HDLS was made after autopsy confirmation of HDLS in an affected family member. Clinical diagnosis was Pick’s disease.

*Mutations in NOTCH3 were excluded.

and NHD, whereas a complete loss of this signaling cascade in bone marrow-derived macrophages is needed for the formation of bone cysts observed in NHD. In support of this hypothesis, a partial loss-of-function mutation in TREM2 in a family with early-onset dementia without bone cysts has recently been reported. Of note, no bone cysts were reported in any of the subjects with HDLS we studied, and a bone scan in subject CA1-1 did not show bone fractures, hypomineralization or any other bone-structure abnormalities.

In summary, we have shown that mutations affecting the tyrosine kinase domain of CSF1R underlie the white-matter disease HDLS, establishing HDLS as a member of the recently defined class of primary microglial disorders called microgliopathies. Future molecular studies of CSF1R signaling may provide new insights into microglial physiology and the involvement of this cell type in HDLS and neurodegeneration. Moreover, CSF1R mutation screening in patient series with neurodegenerative disease will allow a

Figure 4  CSF-1 induces autophosphorylation of wild-type but not mutant CSF1R.

Autophosphorylation of several tyrosine residues within the kinase domain of CSF1R is crucial for its subsequent signaling, involved in cell survival and proliferation. We studied CSF1R autophosphorylation in HeLa cells, which do not express detectable levels of CSF-1, thereby minimizing endogenous CSF-1–induced signaling. Shown is a representative western blot of lysates from HeLa cells transfected with CSF1RWT or mutant CSF1R M875T and treated with CSF-1 for 5, 15 or 30 min. Lysates from untreated CSF1R-transfected cells are included as a control; GAPDH immunoreactivity was analyzed to ensure equal protein loading. Total CSF1R immunodetection for both DNA constructs was robust. Further, we observed strong phosphorylation of wild-type CSF1R after 5 min of CSF-1 treatment, which was weaker in the 15-min and 30-min treatments, as determined by immunoblotting using CSF1R phosphospecific tyrosine (p-Tyr) antibodies. In contrast, no CSF1R autophosphorylation at any of the selected tyrosine residues was detected after CSF-1 treatment in CSF1R M875T–transfected cells. Experiments were repeated three times with similar outcomes. Comparable results were obtained using CSF1R mutants CSF1R E633K and CSF1R M766T (Supplementary Fig. 5).
more accurate diagnosis of HDLS and could facilitate detection of presymptomatic individuals, which is indispensable for therapy development and early treatment.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

R.R. and Z.K.W. directed the study. R.R., M.B. and A.M.N. wrote the manuscript. M.B. directed the genetic studies and performed the linkage analysis. A.M.N. directed the functional studies. S.L. performed the exome sequencing and variant calling analysis. M.B., N.I.R., A.S.-O., O.A.R. and A.W. performed the gene sequencing and genotyping studies. A.M.N., N.F., A.W., N.K. and P.D. performed the mutations, cell biology and protein biochemistry studies. Z.K.W. directed the international consortium, assisted by I., J.W., C.S., M.D.-H. and J.A.E.A.S., I.A., J.M., S.R., H.A.K., N.I.C., G.R., S.S., A.C.T., I.G., I.A.V.G., B.F.B., D.S.K., R.C.P., D.F.B., J.F.M., R.U., N.G.-R., R.H.S., B.R.M., D.W.D. and Z.K.W. performed clinical evaluations. B.B.M. performed radiological studies. D.W.D. performed neuropathological studies. All authors contributed to manuscript revision.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Subjects. A detailed description of the families with HDLS and control subjects included in this study is provided in the Supplementary Note. The experiments performed in this study were approved by the Mayo Clinic Institutional Review Board, and all biological samples were obtained after informed consent from the individuals or their guardians. Demographic information for the populations of individuals with neurodegenerative disease included in the CSF1R sequencing analyses is summarized in Supplementary Table 2. Demographic information for the affected individuals included in the CSF1R functional analyses is summarized in Supplementary Table 4.

Linkage analyses. We performed a genome-wide linkage scan using 6,090 SNPs in family VA using the Illumina HumanLinkage-12 microarrays (Illumina). Genotypes were determined with BeadStudio (Illumina). MERLIN 1.0 was used to perform a multipoint nonparametric linkage analysis using the Illumina Linkage-12 allele frequencies. Individuals were considered affected in the analyses on the basis of an autopsy confirmation of HDLS, clinical examination (by Z.K.W.) or medical records combined with an autopsy confirmation of HDLS in an affected offspring. The generated Z scores (and their equivalent lod scores) provided evidence for five potentially linked loci on five different chromosomes, each with a corresponding lod score >1.0 (Supplementary Fig. 1a). Parametric analysis was performed on these five loci using an affected-only autosomal-dominant inheritance model. Allele frequencies available from the Illumina Linkage-12 set were used and the disease allele frequency was set at 0.0001.

Exome sequencing. Exome enrichment was conducted largely according to the manufacturer’s protocol for the SureSelect Human All Exon Target Enrichment System (Agilent Technologies, version 2.01, based on the methodology described41). Briefly, 3 µg of genomic DNA was fragmented by sonication using the Covaris S2 device to achieve fragments with a mean size of 300 bp. Sonicated DNA was purified using Agencourt’s AMPure XP Solid Phase Reversible Immobilization paramagnetic bead (SPRI), and we then polished the DNA ends by removing the 3’ overhangs and filling in the 5’ overhangs using T4 DNA polymerase and Klenow fragment (New England Biolabs). After end polishing, a single adenine base was added to the 3’ end of the DNA fragments using Klenow fragment (3’-to-5’ exonuclease minus). The end-repaired DNA was ligated to the Illumina paired-end adaptors in a standard ligation reaction using T4 DNA ligase and 2–4 µM final concentration of adaptoe, depending on the DNA yield after purification after the addition of the adenine. After ligation, samples were purified using SPRI beads, assessed on the Agilent Bioanalyzer and amplified by six PCR cycles. We prepared 500 ng of amplified, purified DNA (DNA library) for hybridization by adding the DNA library to Agilent binding reagents, denaturing at 95 °C and incubating at 65 °C. All subsequent steps were performed at 65 °C. Hybridization buffer was added to the prepared library and the entire mix was added to an aliquot of the Agilent SureSelect Capture Library and incubated at 65 °C for 24 h. After hybridization, streptavidin-coated magnetic beads were used to purify the RNA-DNA hybrids formed during hybridization. RNA capture material was digested via acid hydrolysis after elution from the purification beads. Neutralized captured DNA was purified, desalted and amplified by 12 PCR cycles using Herculase II Fusion DNA polymerase. Libraries were purified after amplification and assessed using the Agilent Bioanalyzer. Final quantification of the library was performed with the Kapa Biosciences real-time PCR assay. Sequencing was performed as described62. After dilution to 10 nM final concentration on the basis of the real-time PCR and bioanalyzer results, the final library stock was used in paired-end cluster generation at a final concentration of 6–8 pM to achieve a cluster density of 600,000/mm² (on the Illumina HiSeq2000 instrument). After cluster generation, 50-nt paired-end sequencing was performed using the standard Illumina protocols. For each subject, we obtained >11 Gb of sequence to achieve a 100× median coverage of the targeted exome.

Read mapping and variant analyses. Raw sequencing data for each individual were mapped to the human reference genome (build hg19) using the Burrows-Wheeler Aligner (BWA version 0.5.8.1536)63. The BWA-aligned sequencing reads were processed by Picard to label PCR duplicates. The Genome Analysis Toolkit (GATK, version 5091) was then used to remove duplicates, perform local realignment and recalibrate map quality scores, producing a ‘cleaned’ BAM file for each individual. SNP calls were made by the Unified Genotyper module in GATK using the cleaned BAM files. The resulting Variant Call Format (VCF, version 4.1)-format files were annotated using the GenomicAnnotator module in GATK to identify and label the called variants that were within the targeted coding regions and that overlapped with known and likely benign SNPs reported in dbSNP version 132. The annotated VCF files were then filtered using the GATK variant filter module with a hard filter setting and a custom setting for initial filtering. Variant calls that did not pass the following filters were eliminated from the call set: (i) MQ0 = 4 & & ((MQ0/ (1.0*DP))-0.1); (ii) QUAL<30.00; (i1)=5.00; (i1)RUM>5;SB<0.00; (i1) cluster size 10; (iv) does not contain dbSNP id; (v) outside the targeted regions.

Sanger sequencing analyses. Coding exons of HTR4 and CSF1R were PCR-amplified using flanking intronic primers tailed with M13 sequences (Supplementary Table 5). PCR products were purified using AMPure (Agencourt Biosciences) and then sequenced in both directions using M13 primers and the Big Dye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems). Sequencing reactions were purified using CleanSEQ (Agencourt Biosciences) and analyzed on an ABI3730 Genetic Analyzer (Applied Biosystems). DNA sequence variants were identified using Sequencer software (Gene Codes).

Genotyping analyses. All newly discovered CSF1R variants were genotyped in control series using custom-designed Taqman SNP genotyping assays on the 7900HT Fast Real Time PCR system. Genotype calls were made using SDS version 2.2 software (Applied Biosystems). The presence of HTR4 c.80C>T was identified in 660 controls by direct bidirectional sequencing of exon 2 using primers HTR4-2F and HTR4-2R for PCR and M13 primers for sequencing (Supplementary Table 5).

cDNA transcript analyses. Total RNA was isolated from brain tissue using the RNeasy Plus kit (Qiagen) or fresh blood using PAXGene Blood RNA kit (PreAnalytix) according to the manufacturer’s instructions. Reverse transcription was performed using the Superscript III system (Invitrogen). cDNA was amplified using primers CSF1Rc17F with CSF1Rc20R (families FL2 and CA2) and CSF1Rc11F with CSF1Rc19R (NO) (Supplementary Table 5). PCR products were purified using the multiscreen system (Millipore) and sequenced using M13 primers sequencing primers and Big Dye chemistry (Applied Biosystems). Sequencing reactions were purified using the Montage system (Millipore) and analyzed on an ABI3730 Genetic Analyzer (Applied Biosystems). Sequence transcripts were analyzed using Sequencer software (Gene Codes).

Mutagenesis of CSF1R cDNA clones. A cDNA construct encoding human CSF1R was purchased from OriGene (RC205288). The p.Glu633Lys, p.Met766Thr, and p.Met875Thr mutations were introduced independently using the QuikChange site-directed mutagenesis kit (Stratagene) and mutagenesis primers (Supplementary Table 6). Mutation-positive clones were identified by sequencing analysis (Supplementary Table 6) and transformed into DH5α-competent Escherichia coli cells, after which DNA was obtained using the Nucleobond Xtra Maxi Plus EF kit (Clontech).

Cell culture transfection and western blotting. HeLa cells were maintained in Eagle’s Minimum Essential Medium supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C and 5% CO₂. One day before transfection, cells were plated at 150,000 cells per well in six-well culture dishes. The next day, cells were transfected with 2 µg of GFP control plasmid DNA (OriGene; PS100010) or CSF1R wild-type (CSF1RWT) or mutant (CSF1R E633K, CSF1R M766T or CSF1R M875T) plasmid DNA using Lipofectamine 2000 transfection reagent (Invitrogen). Two days after transfection, the medium was changed to serum-free medium for 16 h before treatment with 50 ng/ml human recombinant MCSF (R&D Systems). CSF-1 remained in the medium for 5, 15 or 30 min before the cells were harvested in RIPA buffer (Boston BioProducts) for western blotting analysis. HeLa cells without CSF-1 treatment were considered as controls. Cell lysates were diluted in an equivalent volume of...
2× Novex Tris-glycine SDS sample buffer (Invitrogen) for protein denaturation. For **in situ** experiments, frontal-cortex samples from healthy controls and subjects with HDLS, Alzheimer’s disease and amyotrophic lateral sclerosis were homogenized in RIPA buffer, briefly sonicated and centrifuged at 20,817g for 5 min. Remaining supernatant was assessed for total protein content using the BCA Protein Assay (Pierce), and equal amounts of protein were denatured in SDS sample buffer. For cell lysates, equal volumes were run on 10% SDS-polyacrylamide gels (Invitrogen), whereas brain samples were loaded by equal protein. Samples were then transferred to Immobilon membranes (Millipore) and immunoblotted. Total CSF1R levels were detected using the Santa Cruz c-Fms/CSF-1R (C-20) antibody (1:1000). CSF1R autophosphorylation was detected using CSF1R phospho-tyrosine primary antibodies against p-Tyr549 (1:5,000), p-Tyr699 (1:2,000), p-Tyr708 (1:5,000), p-Tyr723 (1:6,000) and p-Tyr809 (1:500) from Cell Signaling Technology. GAPDH primary antibody (1:500,000; Meridian Life Science) was used to ensure equal protein loading. Blots were then incubated with secondary horseradish peroxidase–conjugated antibodies to mouse and rabbit (Promega), and bands were detected by enhanced chemiluminescence using Western Lightning Plus-ECL reagents (Perkin Elmer).

**Blood serum analysis.** Blood was harvested from a healthy control and HDLS-affected subject CA1-1 and allowed to coagulate. Blood was then centrifuged at 1,000g for 15 min, after which albumin and IgG were removed using the ProteoExtract Albumin IgG Removal kit (Calbiochem) per the manufacturer’s instructions. Protein content was measured using the BCA Protein Assay, and equal amounts of protein were denatured in SDS sample buffer and subjected to western blotting as described above.

**URLs.** Picard, http://picard.sourceforge.net/; dbSNP version 132, ftp://ftp.ncbi.nlm.nih.gov/snp/organisms/human_9606/VCF/v4.0/00-All.vcf.gz.

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