

Published in final edited form as:

Nat Genet. ; 44(2): 200–205. doi:10.1038/ng.1027.

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

Rosa Rademakers^{1,*}, Matt Baker¹, Alexandra M. Nicholson¹, Nicola J. Rutherford¹, NiCole Finch¹, Alexandra Soto-Ortolaza¹, Jennifer Lash², Christian Wider^{1,3}, Aleksandra Wojtas¹, Mariely DeJesus-Hernandez¹, Jennifer Adamson¹, Naomi Kouri¹, Christina Sundal¹, Elizabeth A. Shuster², Jan Aasly⁴, James MacKenzie⁵, Sigrun Roeber⁶, Hans A. Kretzschmar⁶, Bradley F. Boeve⁷, David S. Knopman⁷, Ronald C. Petersen⁷, Nigel J. Cairns⁸, Bernardino Ghetti⁹, Salvatore Spina⁹, James Garbern¹⁰, Alexandros C. Tselis¹¹, Ryan Uitti², Pritam Das¹, Jay A. Van Gerpen², James F. Meschia², Shawn Levy¹², Daniel F. Broderick¹³, Neill Graff-Radford², Owen A. Ross¹, Bradley B. Miller¹⁴, Russell H. Swerdlow¹⁵, Dennis W. Dickson¹, and Zbigniew K. Wszolek²

¹Department of Neuroscience, Mayo Clinic Florida, Jacksonville, Florida, USA ²Department of Neurology, Mayo Clinic Florida, Jacksonville, Florida, USA ³Department of Clinical Neurosciences, Centre Hospitalier Universitaire Vaudois (CHUV-UNIL), Lausanne, Switzerland ⁴Department of Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway ⁵Department of Pathology, University of Aberdeen, Aberdeen, Scotland ⁶Center for Neuropathology and Prion Research, Ludwig-Maximilians University Munich, Munich, Germany ⁷Department of Neurology, Mayo Clinic Minnesota, Rochester, Minnesota, USA ⁸Alzheimer's Disease Research Center, Department of Neurology, Washington University School of Medicine, St Louis, Missouri, USA ⁹Department of Pathology and Laboratory Medicine and Indiana Alzheimer Disease Center, Indiana University School of Medicine, Indianapolis, USA ¹⁰Department of Neurology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA ¹¹Department of Neurology, Wayne State University School of Medicine, Detroit, USA ¹²HudsonAlpha Institute for Biotechnology, Huntsville, Alabama, USA ¹³Department of Radiology, Mayo Clinic Florida, Jacksonville, Florida, USA ¹⁴Department of Pathology, Texas Tech University Health Sciences Center, Lubbock, Texas, USA ¹⁵Department of Neurology, University of Kansas School of Medicine, Kansas City, USA

Abstract

Hereditary diffuse leukoencephalopathy with spheroids (HDLS) is an autosomal dominantly inherited central nervous system white matter disease with variable clinical presentations including personality and behavioral changes, dementia, depression, parkinsonism, seizures, and others^{1,2}. 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

*Corresponding author's contact information Department of Neuroscience, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, FL 32224, Tel: (904)-953-6279, Fax: (904)-953-7370 rademakers.rosa@mayo.edu.

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.J.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 mutagenesis, cell biology and protein biochemistry studies. Z.K.W. directed the international consortium, assisted by J.L., C.W., C.S., M.D-H and J.A. and E.A.S., J.A., J.M., S.R., H.A.K., N.J.C., B.G., S.S., A.C.T., J.G., J.A.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.B.M., D.W.D. and Z.K.W. performed clinical evaluations, B.B.M. radiological and D.W.D neuropathological studies. All authors contributed to manuscript revision.

COMPETING FINANCIAL INTERESTS The authors declare no competing financial interests.

(encoded by *CSF1R*) in 14 families affected by HDLS. In one kindred, the *de novo* occurrence of the mutation was confirmed. Follow-up sequencing analyses identified an additional *CSF1R* mutation in a patient clinically diagnosed with corticobasal syndrome (CBS). *In vitro*, CSF-1 stimulation resulted in the rapid autophosphorylation of selected tyrosine-residues in the kinase domain of wild-type but not mutant CSF1R, suggesting that HDLS may result from a partial loss of CSF1R function. Since CSF1R is a critical 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 six years. On magnetic resonance imaging (MRI), HDLS is characterized by patchy cerebral white matter abnormalities, 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). Since 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)^{1,4-8,10-12}. Occasionally, brain biopsy has been used to confirm the diagnosis⁹.

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, blood and brain tissue samples from families with at least one patient with autopsy- or biopsy-proven HDLS. In total, we collected 14 kindreds from the United States, Norway, Germany and Scotland (Fig. 2). Family VA was selected for genome-wide linkage studies, and non-parametric linkage analyses 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, $\theta = 0$ at rs13178296), while none of the other loci reached significance (Supplementary Fig. 1). Obligate recombinants narrowed the candidate region to 30.3cM between rs801399 and rs1445716 (Supplementary Fig. 2), corresponding to a ~25Mb genomic interval containing 233 candidate genes.

To generate a list of potential disease-causing mutations, we performed whole-exome sequencing of two pathologically confirmed patients from family VA (VA-21 and VA-24, Fig. 2). We generated variant profiles for each patient 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 non-synonymous mutations: c.80C>T (p.S27L) in the gene encoding the 5-hydroxytryptamine receptor 4 (*HTR4*) and c.2624T>C (p.M875T) in the macrophage colony-stimulating factor 1 receptor gene (*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 6 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; Supplementary Table 1). Segregation analyses confirmed transmission of the *CSF1R* mutations and co-segregation with the disease phenotype in all families where DNA from multiple affecteds 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 to provide supporting evidence for pathogenicity, we also sequenced the *CSF1R* gene in 24 unrelated controls and genotyped the 13 novel mutations in at least 1436 Caucasian controls using Taqman genotyping assays. None of the mutations identified in HDLS patients and no other novel *CSF1R* mutations were found in controls.

The 14 *CSF1R* mutations identified in HDLS families are all located in the intracellular tyrosine-kinase domain of *CSF1R* encoded by exons 12-22. The mutations include 10 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-protein kinases (*Kit*, *FLT3* and *PDGFR α/β*)¹³ (Fig. 3). We further identified three splice-site mutations, leading to the in-frame deletion of exon 13 (NO) or exon 18 (CA2/FL2), deleting up to 40 consecutive amino acids within the tyrosine kinase domain (Supplementary Fig. 4).

Detailed clinical information was available for 24 patients 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/VA), age at onset or death differed by more than 25 years among family members, whereas a monozygotic twin pair (family NO) showed highly similar disease course with ages at onset and death within one year from 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 significantly within and across families, and *ante mortem* clinical diagnoses in mutation carriers included frontotemporal dementia (FTD), CBS, Alzheimer disease (AD), multiple sclerosis (MS), atypical cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), and Parkinson disease (PD).

Since most patients included in our study were not diagnosed with HDLS, we hypothesized that *CSF1R* mutation carriers may be present in clinical series of early-onset AD, FTD, CBS, MS and PD, or ischemic stroke patients with additional white matter changes. Sequencing analyses of *CSF1R* exons 12-22 encoding the protein tyrosine kinase domain in up to 93 Mayo Clinic patients affected with each of these neurological syndromes led to the identification of an additional *CSF1R* missense mutation c.2509G>T (p.D837Y) in a woman with clinical symptoms resembling CBS (Supplementary Tables 2-3). The identification of a *CSF1R* mutation in this limited patient series underscores that HDLS may be an under-diagnosed disease.

CSF1R is a cell-surface receptor primarily for the cytokine CSF-1, which regulates the survival, proliferation, differentiation and function of mononuclear phagocytic cells, including microglia of the central nervous system¹⁴. *CSF1R* is composed of a highly glycosylated extracellular ligand-binding domain, a trans-membrane domain and an intracellular tyrosine-kinase domain¹⁵. Binding of CSF-1 to *CSF1R* results in the formation of receptor homodimers and subsequent auto-phosphorylation of several tyrosine residues in the cytoplasmic domain¹⁶. *CSF1R* autophosphorylation precedes *CSF1R*-dependent phosphorylation of several proteins, including the phosphatase SHP-1 and the kinases Src, PLC-g, PI(3)K, Akt and Erk¹⁶⁻¹⁸. In the brain, *CSF1R* is predominantly expressed in microglial cells, although low levels of *CSF1R* have been reported in cultured neurons¹⁹⁻²¹. An increase in *CSF1R* copy number and point mutations leading to constitutive activation of the *CSF1R* receptor have been associated with tumor development, including hematological malignancies and renal cell carcinomas^{22,23}.

To assess the functional importance of the *CSF1R* mutations identified in this study, we first studied the effect of the mutations on *CSF1R in vitro*. We transiently expressed DDK-

tagged wild-type (CSF1R_{WT}) and mutant (CSF1R_{E633K}, CSF1R_{M766T} and CSF1R_{M875T}) CSF1R in cultured cells. Upon stimulation with CSF-1, autophosphorylation on multiple CSF1R tyrosine-residues was observed for CSF1R_{WT}, while none of the mutants showed detectable levels of autophosphorylation (Fig. 4 and Supplementary Fig. 5). Since all mutations are in the CSF1R kinase domain, dimerization and/or cell surface expression are unlikely to be affected; however, we cannot exclude this at this time. These preliminary findings suggest that mutant CSF1R kinase activity is abrogated, likely affecting the phosphorylation of downstream targets. We speculate that mutant CSF1R might assemble into non-functional homodimers and wild-type/mutant heterodimers inducing a dominant-negative disease mechanism.

To address whether CSF1R autophosphorylation is also disrupted in HDLS patient samples, we first subjected blood samples from a healthy control and HDLS patient CA1-1 to CSF1R immunoblotting, which revealed no apparent difference in CSF1R total or phosphorylation levels (Supplementary Fig. 6a). Further, CSF1R immunoblotting was performed in frontal cortex brain tissue of healthy controls as well as patients with HDLS. Brain samples from AD and ALS patients were included as neurodegenerative disease controls. Our data showed varied levels of total and phosphorylated CSF1R in these brain samples (Supplementary Fig. 6b); however, statistical analysis did not reveal a significant difference between any of the groups. Although these preliminary *in vivo* studies do not reveal a defect in autophosphorylation, these findings do not necessarily conflict with the data obtained in cultured cells. First, HDLS patients are heterozygous for the *CSF1R* mutations and therefore, in contrast to our *in-vitro* experiments, wild-type receptor is still present in these patients. In our cell culture experiments, CSF1R signaling was down regulated by serum deprivation to minimize basal signaling through this receptor before stimulation with the CSF-1 ligand. CSF-1 is a serum protein, so without this deprivation *in vivo*, immediate changes in CSF-1-induced CSF1R autophosphorylation may not be apparent as we cannot disregard wild-type receptors at the cell surface that have already been activated. Unfortunately, without access to an immortalized cell line derived from an HDLS patient, we are currently unable to accurately assess acute receptor activation *in vivo*. Finally, the post-mortem brain samples from HDLS patients included in these studies exhibit extensive degeneration, leaving the possibility that cells with greater disruption of CSF1R signaling are underrepresented in the tissue sample.

Unraveling the genetic etiology of HDLS may significantly contribute to the understanding of other adult-onset leukoencephalopathies. *De novo* mutations in *CSF1R* could explain the disease in sporadic patients 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 (POLD) are part of a single clinicopathologic entity, as was recently 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 disease^{24,30}, multiple sclerosis³¹ and HIV encephalitis³².

Interestingly, 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 complex^{36,37}, 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 and NHD, whereas a complete loss of this signaling cascade in bone marrow-derived macrophages is needed for the bone-cysts formation 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 was recently reported³⁹. Also, no bone-cysts were reported in any of our HDLS patients and a bone scan in a patient 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 of HDLS, establishing HDLS as an important novel member of the recently defined class of primary microglial disorders, called 'microgliopathies'⁴⁰. Future molecular studies of CSF1R signaling might offer novel insights into microglial physiology and the involvement of this cell type in HDLS and neurodegeneration. Moreover, *CSF1R* mutation screening in neurodegenerative disease patient series will now allow an accurate diagnosis of HDLS and could facilitate detection of presymptomatic individuals, which is indispensable for therapy development and early treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to all family members who participated in this study. We further would like to thank Ms. Dale Gamble for her help in coordinating sample and data collection for the ischemic stroke cases and Dr. Ra'id Ossi for review of brain MRI scans for the ischemic stroke cases. This work is funded by a Mayo Benefactor and the Mayo Foundation. Mayo Clinic Jacksonville is a Morris K. Udall Parkinson's Disease Research Center of Excellence supported by the NINDS (P50 NS072187). ZKW is partially funded by the NIH R01 NS057567 and 1RC2NS070276], and by Mayo Clinic Florida CR programs (MCF 90052018 and MCF 90052030). ZKW is further supported by the family of Carl Edward Bolch, Jr., and Susan Bass Bolch and Dystonia Medical Research Foundation. RR is funded by NIH grants R01 NS065782, R01 AG26251 and P50 AG16574 and by the Peebler PSP Research Foundation. OAR is supported by the American Heart Association, James & Esther King Biomedical Research Program, the Florida Department of Health and the Myron and Jane Hanley Award in Stroke research. CW is supported by the Leenaards Foundation and the Swiss Parkinson Foundation. The Mayo Clinic Florida Cerebrovascular Diseases Registry (IRB No. 08-003878; JFM, Principal Investigator) is supported by the Mayo Foundation for Medical Education and Research. CS is supported by grants from Sven and Dagmar Saléns, Stiftelse, Sweden, and the Swedish Society of Medicine Gothenburg (GLS). This work is further funded by NIH PHS P30 AG 10133 (Indiana Alzheimer Disease Center, to BG) and NIH U24 AG 21886-01S1 (National Cell Repository for Alzheimer's disease, to TF).

REFERENCES

1. Axelsson R, Roytta M, Sourander P, Akesson HO, Andersen O. Hereditary diffuse leucoencephalopathy with spheroids. *Acta Psychiatr Scand Suppl.* 1984; 314:1–65. [PubMed: 6595937]
2. Wider C, et al. Leukoencephalopathy with spheroids (HDLS) and pigmentary leukodystrophy (POLD): a single entity? *Neurology.* 2009; 72:1953–9. [PubMed: 19487654]
3. Schiffmann R, van der Knaap MS. Invited article: an MRI-based approach to the diagnosis of white matter disorders. *Neurology.* 2009; 72:750–9. [PubMed: 19237705]
4. Baba Y, et al. Hereditary diffuse leucoencephalopathy with spheroids: clinical, pathologic and genetic studies of a new kindred. *Acta Neuropathol.* 2006; 111:300–11. [PubMed: 16523341]
5. Freeman SH, et al. Adult onset leukodystrophy with neuroaxonal spheroids: clinical, neuroimaging and neuropathologic observations. *Brain Pathol.* 2009; 19:39–47. [PubMed: 18422757]
6. Hancock N, Poon M, Taylor B, McLean C. Hereditary diffuse leucoencephalopathy with spheroids. *J Neurol Neurosurg Psychiatry.* 2003; 74:1345–7. [PubMed: 12933955]
7. Itoh K, et al. Autosomal dominant leukodystrophy with axonal spheroids and pigmented glia: clinical and neuropathological characteristics. *Acta Neuropathol.* 2006; 111:39–45. [PubMed: 16328511]

8. Marotti JD, Tobias S, Fratkin JD, Powers JM, Rhodes CH. Adult onset leukodystrophy with neuroaxonal spheroids and pigmented glia: report of a family, historical perspective, and review of the literature. *Acta Neuropathol.* 2004; 107:481–8. [PubMed: 15067553]
9. Mateen FJ, et al. Sporadic leucodystrophy with neuroaxonal spheroids: persistence of DWI changes and neurocognitive profiles: a case study. *J Neurol Neurosurg Psychiatry.* 2010; 81:619–22. [PubMed: 20176606]
10. Swerdlow RH, et al. Autosomal dominant subcortical gliosis presenting as frontotemporal dementia. *Neurology.* 2009; 72:260–7. [PubMed: 19153373]
11. van der Knaap MS, Naidu S, Kleinschmidt-Demasters BK, Kamphorst W, Weinstein HC. Autosomal dominant diffuse leukoencephalopathy with neuroaxonal spheroids. *Neurology.* 2000; 54:463–8. [PubMed: 10668715]
12. Van Gerpen JA, et al. Insights into the dynamics of hereditary diffuse leukoencephalopathy with axonal spheroids. *Neurology.* 2008; 71:925–9. [PubMed: 18794495]
13. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature.* 2001; 411:355–65. [PubMed: 11357143]
14. Stanley ER, et al. Biology and action of colony--stimulating factor-1. *Mol Reprod Dev.* 1997; 46:4–10. [PubMed: 8981357]
15. Pixley FJ, Stanley ER. CSF-1 regulation of the wandering macrophage: complexity in action. *Trends Cell Biol.* 2004; 14:628–38. [PubMed: 15519852]
16. Hamilton JA. CSF-1 signal transduction. *J Leukoc Biol.* 1997; 62:145–55. [PubMed: 9261328]
17. Sengupta A, et al. Identification and subcellular localization of proteins that are rapidly phosphorylated in tyrosine in response to colony-stimulating factor 1. *Proc Natl Acad Sci U S A.* 1988; 85:8062–6. [PubMed: 2460861]
18. Yeung YG, Stanley ER. Proteomic approaches to the analysis of early events in colony-stimulating factor-1 signal transduction. *Mol Cell Proteomics.* 2003; 2:1143–55. [PubMed: 12966146]
19. Akiyama H, et al. Expression of the receptor for macrophage colony stimulating factor by brain microglia and its upregulation in brains of patients with Alzheimer's disease and amyotrophic lateral sclerosis. *Brain Res.* 1994; 639:171–4. [PubMed: 7514086]
20. Raivich G, et al. Regulation of MCSF receptors on microglia in the normal and injured mouse central nervous system: a quantitative immunofluorescence study using confocal laser microscopy. *J Comp Neurol.* 1998; 395:342–58. [PubMed: 9596528]
21. Wang Y, Berezovska O, Fedoroff S. Expression of colony stimulating factor-1 receptor (CSF-1R) by CNS neurons in mice. *J Neurosci Res.* 1999; 57:616–32. [PubMed: 10462686]
22. Ridge SA, Worwood M, Oscier D, Jacobs A, Padua RA. FMS mutations in myelodysplastic, leukemic, and normal subjects. *Proc Natl Acad Sci U S A.* 1990; 87:1377–80. [PubMed: 2406720]
23. Soares MJ, et al. CSF1R copy number changes, point mutations, and RNA and protein overexpression in renal cell carcinomas. *Mod Pathol.* 2009; 22:744–52. [PubMed: 19377443]
24. Browne L, Sweeney BJ, Farrell MA. Late-onset neuroaxonal leukoencephalopathy with spheroids and vascular amyloid. *Eur Neurol.* 2003; 50:85–90. [PubMed: 12944712]
25. Keegan BM, et al. Sporadic adult-onset leukoencephalopathy with neuroaxonal spheroids mimicking cerebral MS. *Neurology.* 2008; 70:1128–33. [PubMed: 18287567]
26. Levin N, et al. Leukoencephalopathy with neuroaxonal spheroids presenting as frontotemporal dementia. *Isr Med Assoc J.* 2008; 10:386–7. [PubMed: 18605367]
27. Mascalchi M, et al. CT and MR imaging of neuroaxonal leukodystrophy presenting as early-onset frontal dementia. *AJNR Am J Neuroradiol.* 2006; 27:1037–9. [PubMed: 16687539]
28. Moro-de-Casillas ML, Cohen ML, Riley DE. Leukoencephalopathy with neuroaxonal spheroids (LENAS) presenting as the cerebellar subtype of multiple system atrophy. *J Neurol Neurosurg Psychiatry.* 2004; 75:1070–2. [PubMed: 15201379]
29. Yamashita M, Yamamoto T. Neuroaxonal leukoencephalopathy with axonal spheroids. *Eur Neurol.* 2002; 48:20–5. [PubMed: 12138305]
30. Akiguchi I, Tomimoto H, Suenaga T, Wakita H, Budka H. Alterations in glia and axons in the brains of Binswanger's disease patients. *Stroke.* 1997; 28:1423–9. [PubMed: 9227695]

31. Lassmann H. Axonal and neuronal pathology in multiple sclerosis: what have we learnt from animal models. *Exp Neurol*. 2010; 225:2–8. [PubMed: 19840788]
32. Smith TW, DeGirolami U, Henin D, Bolgert F, Hauw JJ. Human immunodeficiency virus (HIV) leukoencephalopathy and the microcirculation. *J Neuropathol Exp Neurol*. 1990; 49:357–70. [PubMed: 2362185]
33. Klunemann HH, et al. The genetic causes of basal ganglia calcification, dementia, and bone cysts: DAP12 and TREM2. *Neurology*. 2005; 64:1502–7. [PubMed: 15883308]
34. Paloneva J, et al. CNS manifestations of Nasu-Hakola disease: a frontal dementia with bone cysts. *Neurology*. 2001; 56:1552–8. [PubMed: 11402114]
35. Tanaka J. Nasu-Hakola disease: a review of its leukoencephalopathic and membranlipodystrophic features. *Neuropathology*. 2000; 20(Suppl):S25–9. [PubMed: 11037183]
36. Paloneva J, et al. Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. *Nat Genet*. 2000; 25:357–61. [PubMed: 10888890]
37. Paloneva J, et al. Mutations in two genes encoding different subunits of a receptor signaling complex result in an identical disease phenotype. *Am J Hum Genet*. 2002; 71:656–62. [PubMed: 12080485]
38. Otero K, et al. Macrophage colony-stimulating factor induces the proliferation and survival of macrophages via a pathway involving DAP12 and beta-catenin. *Nat Immunol*. 2009; 10:734–43. [PubMed: 19503107]
39. Chouery E, et al. Mutations in TREM2 lead to pure early-onset dementia without bone cysts. *Hum Mutat*. 2008; 29:E194–204. [PubMed: 18546367]
40. Bianchin MM, Martin KC, de Souza AC, de Oliveira MA, Rieder CR. Nasu-Hakola disease and primary microglial dysfunction. *Nat Rev Neurol*. 2010; 6:2. following 523. [PubMed: 20836191]
41. Gnirke A, et al. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. *Nat Biotechnol*. 2009; 27:182–9. [PubMed: 19182786]
42. Bentley DR, et al. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature*. 2008; 456:53–9. [PubMed: 18987734]
43. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009; 25:1754–60. [PubMed: 19451168]

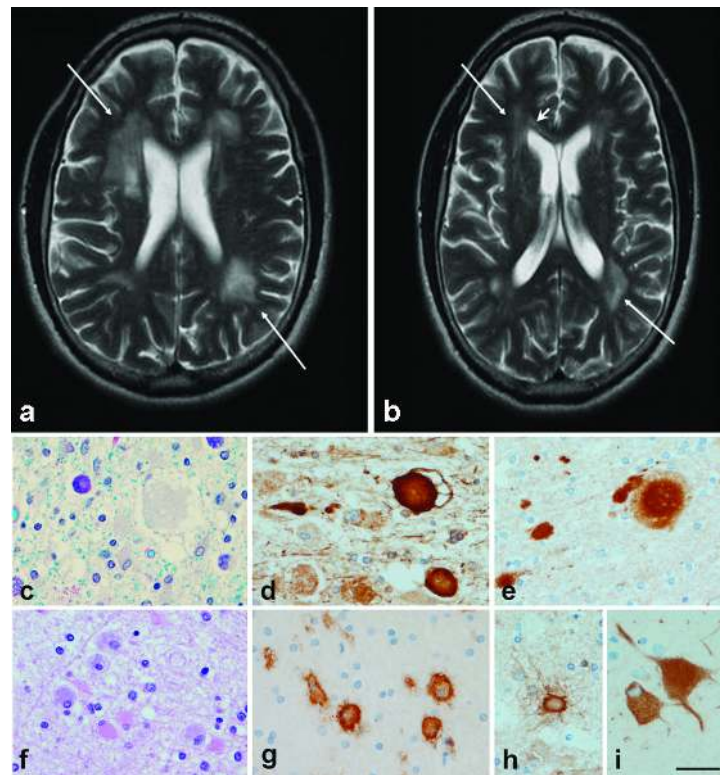


Figure 1. Neuroimaging (a-b) and neuropathological (c-i) findings in HDLS patient FL2-1 Patient FL2-1 (Table 1 and Fig. 2) developed a mild depression followed shortly by forgetfulness at the age of 50 years. Two years later he had a flat affect, inappropriate behavior, poor concentration, executive dysfunction, restless legs syndrome, and insomnia. Examination 3 years after the onset of symptoms demonstrated psychomotor slowing, and ideomotor and constructional apraxia. The Mini-Mental State Examination (MMSE) 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 MR images showed localized hyperintense foci in both frontal and parietal lobes (long arrows), involving the periventricular, deep and subcortical white matter, sparing the subcortical U-fibers. Hyperintense focus in the right forceps minor (arrowhead) 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 (H&E). **(g)** White matter macrophages with HLA-DR immunohistochemistry. **(h)** Bizarre white matter astrocytes. **(i)** Ballooned cortical neurons with alpha-B-crystallin immunohistochemistry. Bar (c-i) = 30 μ m

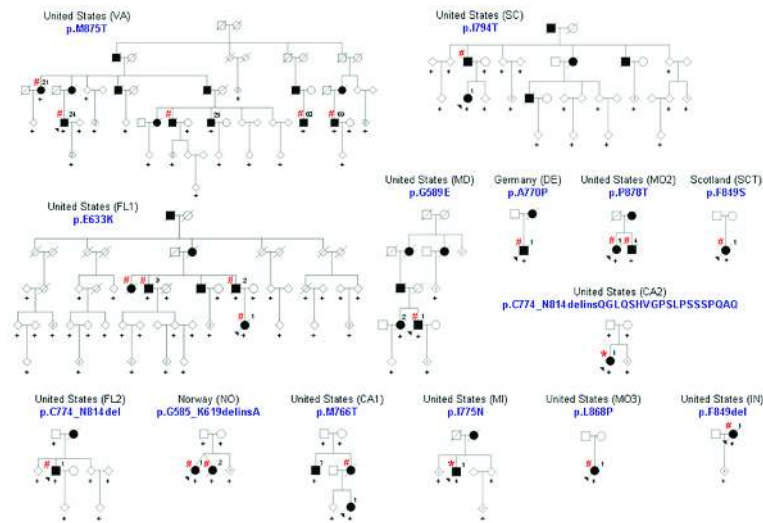


Figure 2. Families with HDLS and *CSFR1* mutations

Abbreviated pedigrees of all families with HDLS included in this study. Filled symbols indicate affected individuals. An arrowhead indicates the proband. To protect confidentiality some individuals are not shown and sex is portrayed using a diamond for all individuals except affected individuals and their spouse. In each family, at least one affected family member received an autopsy (red pound sign) or biopsy (red star) confirmation of HDLS. A '+' sign indicates that DNA was included in the *CSFR1* sequencing analyses to confirm that mutations segregated with disease. For each patient with DNA available for genetic studies, a unique patient number (UPN) corresponding to Table 1 is included above the patient.

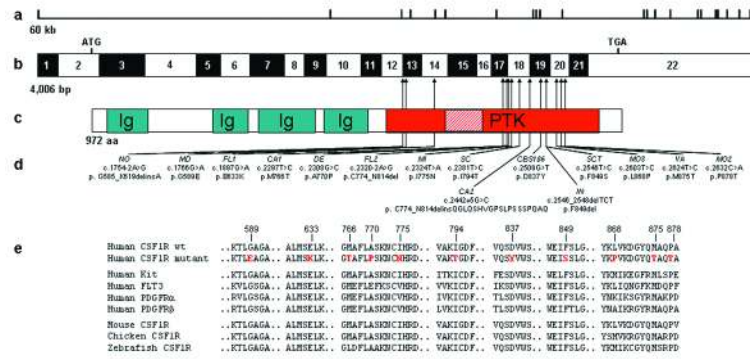


Figure 3. Genomic organization and protein domain structure of CSF1R with summary of CSF1R mutations

(a) The *CSF1R* gene extends over 60kb 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. For mutations detected, arrows indicate positions 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 heterozygote *CSF1R* mutations detected in 14 families with autopsy or biopsy proven HDLS and in one patient 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 human CSF1/PDGFR family members. Comparison of human CSF1R (NP_005202.2), mouse CSF1R (NP_001032948.2), chicken CSF1R (XP_414597.2), Zebrafish CSF1R (NP_571747.1), human KIT (NP_000213.1), human FLT3 (NP_004110.2), human PDGFRα (NP_006197.1) and human PDGFRβ (NP002600.1). Amino acid positions of the mutations are indicated above the alignment.

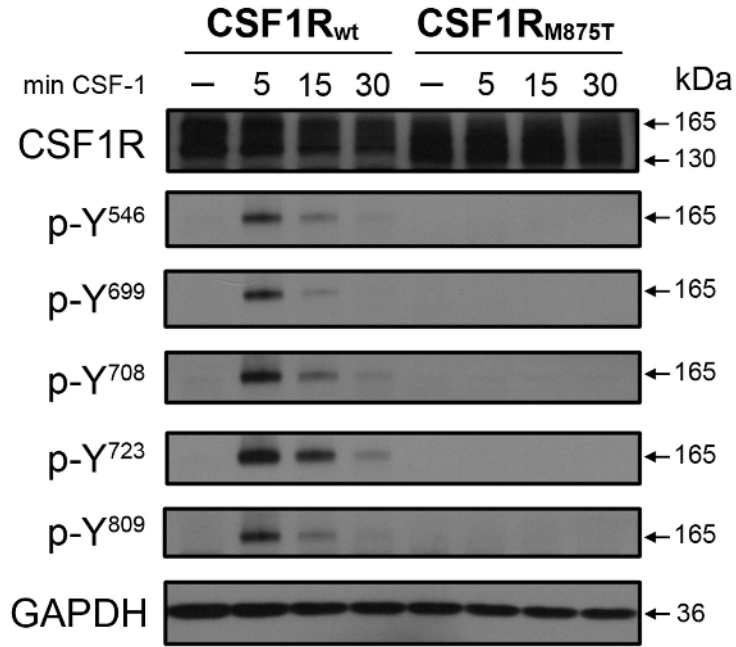


Figure 4. CSF-1 induces autophosphorylation of wild-type but not mutant CSF1R

Autophosphorylation of several tyrosine residues within the kinase domain of CSF1R is critical 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. A representative Western blot of lysates from CSF1R_{WT} or mutant CSF1R_{M875T} transfected HeLa cells treated with CSF-1 for 5, 15, or 30 minutes is shown. Lysates from untreated CSF1R-transfected cells are included as a control and GAPDH immunoreactivity is shown 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 minutes of CSF-1 treatment, which decreased over the course of 15 and 30 minutes, as determined by immunoblotting using CSF1R phospho-specific tyrosine (p-Y) 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 outcome. Comparable results were obtained using CSF1R mutants CSF1R_{E633K} and CSF1R_{M766T} (Supplementary Fig. 5).

Table 1

Clinical characteristics of 24 patients from 14 families with genetically confirmed *CSF1R* mutations.

Family (individual)	Origin	Sex	Onset age	Death age	Initial symptoms	Clinical Features during course of the illness				Clinical Diagnosis	Reference			
						Personality/Behavior changes	Dementia	Depression	Parkinsonism			Seizures		
NO (NO-1)	Norway	F	36	40	Dizziness/Cognitive impairment Depression	+	+	+	+	MS	new			
		F	38	41								+	+	MS
MD (MD-1)	US	M	58	61	Cognitive impairment Spasticity	+	+	-	+	FTD	new			
		F	47	58								+	+	Epilepsy
FL1 (FL1-1)	US	F	42	46	Speech problems/Alien limb Cognitive impairment Cognitive impairment	+	+	+	+	CBS	4			
		M	67 ^a	74								+	+	Parkinsonism
		M	78	84								+	+	AD
CA1 (CA1-1)	US	F	18	-	Depression Facial pain	+	+	+	-	HDL ^S ^b	new			
		M	43	-								+	+	Psychiatric disease/NPH
DE (DE-1)	Germany	M	52	63	Cognitive impairment	+	+	+	-	FTD ^c /atypical CADASIL ^d	new			
FL2 (FL2-1)	US	M	50	55	Depression	+	+	+	+	FTD	new			
		M	48	-								+	+	MS/ atypical PD
SC (SC-1)	US	F	35	-	Cognitive impairment	+	+	+	-	Atypical CADASIL ^d	12			
CA2	US										9			

Family (individual)	Origin	Sex	Onset age	Death age	Initial symptoms	Clinical Features during course of the illness					Clinical Diagnosis	Reference
						Personality/ Behavior changes	Dementia	Depression	Parkinsonism	Seizures		
(CA2-1)		F	23	-	Dysarthria/apraxia/gait impairments	+	+	+	+	-	Leukoencephalopathy with unknown etiology	
<i>IN</i> (IN-1)	US	F	63	67	Depression/balance issues	-	+	+	+	+	Atypical dementia	new
<i>SCT</i> (SCT-1)	Scotland	F	46	51	Depression/epilepsy/cognitive impairment	+	+	+	+	+	FTD/cerebrovascular disease	new
<i>MO3</i> (MO3-1)	US	F	55	63	Depression	+	+	+	+	+	FTD	new
<i>VA</i> (VA-21)	US	F	n/a	77	n/a	n/a	+	n/a	n/a	n/a	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 ^d	
<i>MO2</i> (MO2-1)	US	F	39	49	Cognitive impairment	+	+	-	-	+	FTD	new
(MO2-4)		M	33	43	Cognitive impairment	+	+	+	-	+	FTD	

^aPatient had an isolated seizure at age 47.

^bClinical diagnosis of HDLS was made after autopsy confirmation of HDLS in an affected family member.

^cClinical diagnosis was Pick's disease.

^dMutations in *NOTCH3* were excluded. MS, multiple sclerosis; FTD, frontotemporal dementia; CBS, corticobasal syndrome; AD, Alzheimer's disease; PD, Parkinson's disease; HDLS, hereditary diffuse leukoencephalopathy with spheroids; NPH, normal pressure hydrocephalus; n/a, not available.