Brain Pathology ISSN 1015-6305

RESEARCH ARTICLE

Enlarging the Nosological Spectrum of Hereditary Diffuse Leukoencephalopathy with Axonal Spheroids (HDLS)

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Keywords
ALSP, *CSF1R*, HDLS, POLD.

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Received 14 November 2013
Accepted 10 January 2014
Published Online Article Accepted 16 January 2014

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doi:10.1111/bpa.12120

Abstract

Hereditary diffuse leukoencephalopathy with axonal spheroids (HDLS) is an autosomal dominant disease clinically characterized by cognitive decline, personality changes, motor impairment, parkinsonism and seizures. Recently, mutations in the colony-stimulating factor-1 receptor (*CSF1R*) gene have been shown to be associated with HDLS. We report clinical, neuropathological and molecular genetic findings of patients from a new family with a mutation in the *CSF1R* gene. Disease onset was earlier and disease progression was more rapid compared with previously reported patients. Psychiatric symptoms including personality changes, alcohol abuse and severe depression were the first symptoms in male patients. In the index, female patient, the initial symptom was cognitive decline. Magnetic resonance imaging (MRI) showed bilateral, confluent white matter lesions in the cerebrum. Stereotactic biopsy revealed loss of myelin and microglial activation as well as macrophage infiltration of the parenchyma. Numerous axonal swellings and spheroids were present. Ultrastructural analysis revealed pigment-containing macrophages. Axonal swellings were detected by electron microscopy not only in the central nervous system (CNS) but also in skin nerves. We identified a heterozygous mutation (c.2330G>A, p.R777Q) in the *CSF1R* gene. Through this report, we aim to enlarge the nosological spectrum of HDLS, providing new clinical descriptions as well as novel neuropathological findings from the peripheral nervous system.

INTRODUCTION

Hereditary diffuse leukoencephalopathy with axonal spheroids (HDLS) is an autosomal dominant disease with the pathological hallmark of white matter changes with axonal spheroids leading to progressive behavioral, cognitive and motor dysfunction (3). HDLS was first described in 1984 in a large Swedish kindred (2). Since the first description, over 26 kindreds with considerably variable clinical presentations have been reported (6–9, 11, 17, 18). Median age at onset is 39 ± 15 years (range, 8–78) and disease duration is 9 ± 10 years (range, 1–34) (20). HDLS is considered a rare disease, but actual prevalence rates are not available. Recently, it has been suggested that HDLS and pigmentary orthochromatic leukodystrophy (POLD) belong to the same disease spectrum or might even be a single entity called adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) (14, 20). Furthermore, it is assumed that HDLS/ALSP is often misdiagnosed, suggesting the likelihood of higher prevalence of the disease. HDLS/ALSP "mimics" include dementia (frontotemporal dementia, Alzheimer's disease), atypical parkinsonism (corticobasal degeneration, multisystem atrophy), progressive multiple sclerosis, cerebral autosomal dominant arteriopathy with subacute infarcts and leukoencephalopathy (CADASIL) and the wide range of leukodystrophies (18). Until recently, caused by the lack of pathognomonic clinical and magnetic resonance imaging (MRI) features, diagnosis of HDLS/ALSP could only be reliably established by histopathological examination (10). In 2011, Rademakers *et al* identified the colony-stimulating factor-1 receptor gene (*CSF1R*) as the mutated gene in HDLS/ALSP (17)

Here, we present the clinical, imaging and neuropathological results of affected individuals from a German family. They had a mutation in the *CSF1R* gene and presented early with a more rapid progression of their disease than in most reported patients (6).

METHODS

Histopathological examination

Four-micrometer-thick paraffin sections were stained with routine stains. Immunohistochemistry illustrated T- and B-cells, microglia/macrophages CD68 (clone KP1, DAKO) and HLA-DR (clone CR3/43, DAKO), leukocytes expressing CD45/leucocyte

common antigen (LCA) (clone 2B11, DAKO, Hamburg, Germany), amyloid precursor protein (APP) (clone 22L11, M.P., Santa Ana, CA, USA), glial cells expressing CD115 (CSF-1R) (clone 2-4A5-4, ACRIS, Herford, Germany), neurofilament protein (NFP) (clone 2F11, DAKO), myelin basic protein (MBP) (clone 7H11, Leica, Wetzlar, Germany) by using the *i*view-Ventana diaminobenzidine (DAB) Detection Kit (Ventana, Tucson, AZ, USA) with appropriate biotinylated secondary antibodies and DAB visualization of the peroxidase reaction product with a Benchmark XT immunostainer (Ventana) in a standardized manner. Omission of primary antibodies resulted in the absence of any cellular labeling. Double labeling of MBP or OLIG2 with CD68 by immunofluorescence and autofluorescence of pigment was evaluated on cryosections mounted with nonfluorescing medium with a Zeiss Observer Z1 microscope and Axiovision software (Zeiss, Oberkochem, Germany). Photomicrographs were taken with the Olympus BX50 microscope, the digital camera DP25 and cell D software (Olympus, Hamburg, Germany).

Electron microscopy

Ultrastructural analysis of the central nervous system (CNS) and the skin biopsy specimens was performed after fixation in 2.5% glutaraldehyde for 48 h at 4°C, postfixation in 1% osmium tetroxide and embedding of the brain tissue in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate. Electron microscopy was performed with a Zeiss P902 electron microscope.

Molecular genetic analysis

Written informed consent for genetic analysis was obtained from all investigated family members or their legal representatives. Genomic DNA was extracted from fresh peripheral blood leukocytes, and in one case, from paraffin-embedded tissue of the skin (patient 1, III:2), as described in Murrell *et al* (12). All exons and surrounding intronic regions of *CSF1R* were analyzed by amplification followed by direct sequencing. Sequencing products were run on a CEQ 8000 GeXP Genetic Analysis System (Beckman Coulter, Pasadena, CA, USA). For patient 1 (III:2) from whom fixed tissue was used for analysis, only exon 18 was amplified and sequenced.

MRI studies

MRI examinations were performed using a 1.5-T MRI scanner (Avanto, Siemens, Erlangen Germany). Axial T2-weighted and diffusion-weighted imaging (DWI) was performed and corresponding decreased apparent diffusion coefficient (ADC) was measured. Spectroscopy and contrast-enhanced studies were performed in patients II:8 and III:2.

RESULTS

The pedigree of the family is shown in Figure 1. We have reliable information on generations II and III. However, with two affected individuals in generation II, it is compelling to assume that one of their parents was the gene carrier. The mother (I:2) of patient 2 (II:8) died of renal failure at the age of 46 years. The father (I:1) of patient 2 (II:8) died of bleeding esophageal varices at the age of 64 years. He exhibited chronic alcohol abuse and aggressive behavior. Interestingly, a cerebral computed tomography (CT) showed considerable periventricular hypodensities.

The index patient was a 22-year-old pregnant (18th week) woman with a history of migraine. She presented with an 8-month course of a progressive bulbar syndrome including dysarthria, dysphagia and masticatory difficulties. Handwriting was blurred because of disturbed fine motor skills of the right hand and gait was unstable. Family members reported poor concentration and progressive loss of short-term memory. Neurological examination revealed a cerebellar syndrome, pyramidal signs and bradydiadochokinesia of both arms. Neuropsychological analysis disclosed borderline processing speed, limited attentional capacity and increased interference susceptibility. MRI showed confluent supratentorial white matter lesions with partially increased signal in DWI and decreased ADC signal (Figure 2A). 1H-MR spectroscopy demonstrated a significant loss of N-acetyl-aspartate (NAA)

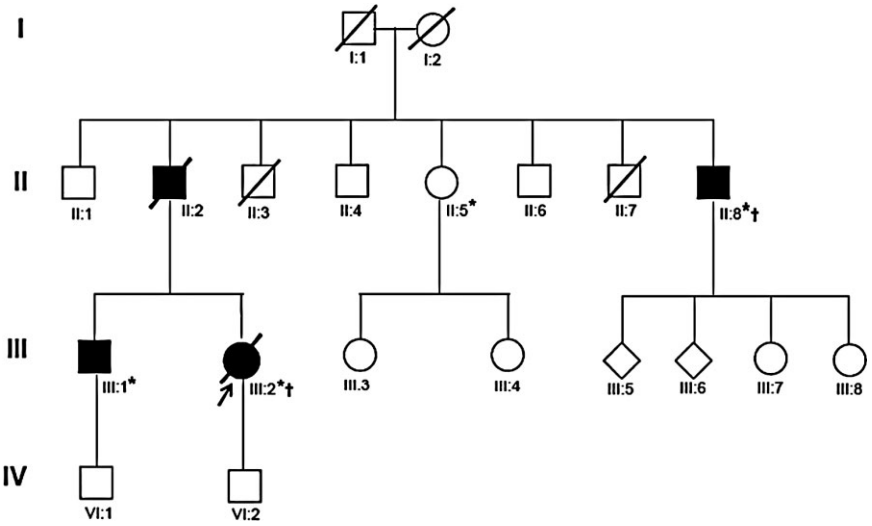

Figure 1. *Family pedigree*. Squares represent men and circles represent women. Filled symbols represent affected individuals and slashed symbols represent deceased individuals. * = individuals tested for mutation in *CSF1R*, † = neuropathologically investigated individuals, → = index patient.

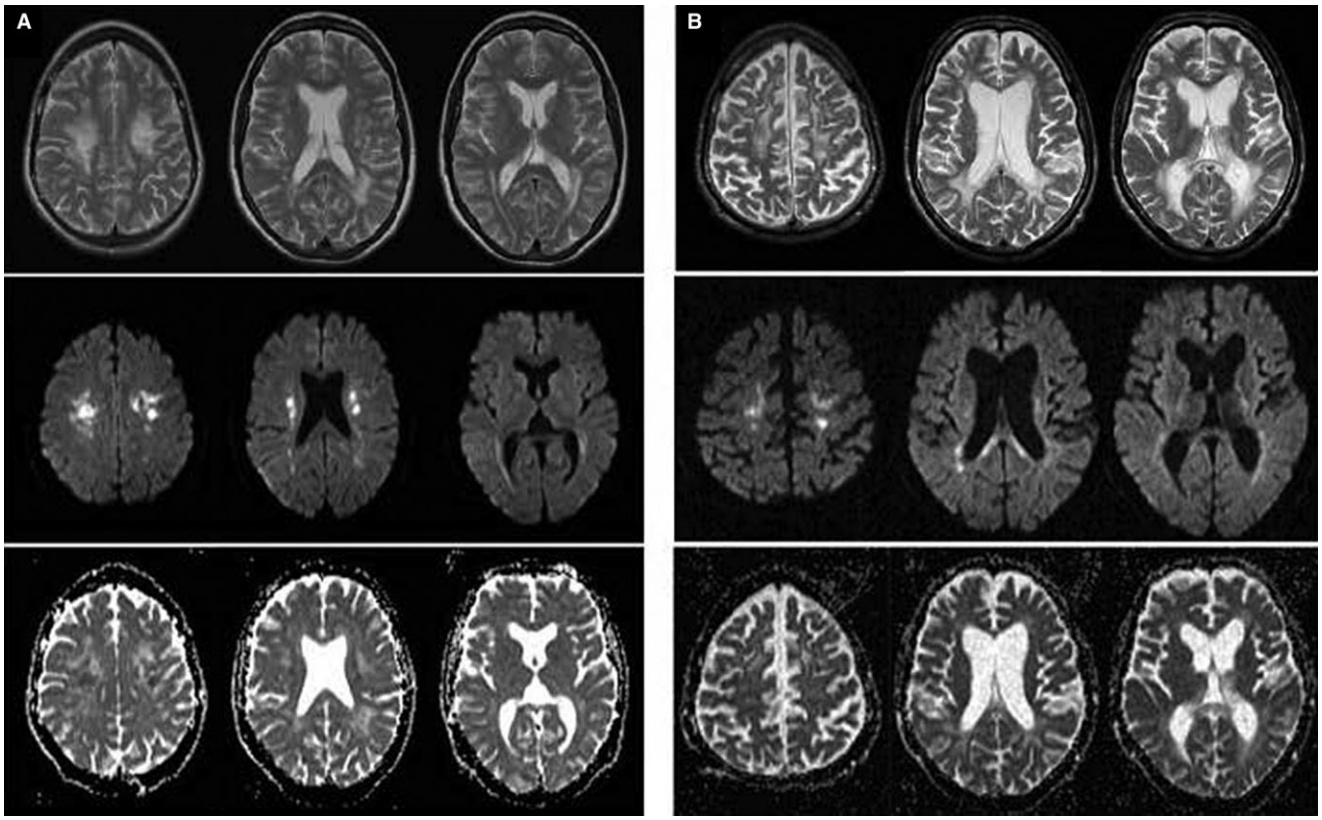

Figure 2. Magnetic resonance imaging (MRI) of patient III:2 (**A**) and patient II:8 (**B**). Both show confluent supratentorial white matter lesions with partially increased signal in diffusion-weighted imaging (DWI) and corresponding decreased apparent diffusion coefficient (ADC) signal.

in these lesions (not shown). Spinal MRI was unremarkable and electroencephalography (EEG) was normal. Extensive laboratory studies were normal. A skin biopsy intended to search for CADASIL by electron microscopy showed no pathognomonic granular osmiophilic material (GOM) in the basement membrane layer of vascular smooth muscle cells; however, the dermal nerve fascicles showed axonal swelling with accumulation of organelles degenerating mitochondria, lysosomal remnants and so-called "dense bodies" (Figure 3L). A healthy baby was born during the 36th week of pregnancy via caesarean section. Death from bacterial pneumonia occurred 18 months after disease onset.

Her father (II:2) died at age 29 after 1 year of rapidly evolving personality changes, speech disturbances and tetraparesis. A suspicion of herpes simplex encephalitis could not be verified: unfortunately, autopsy material was not available for reexamination. He had seven siblings of whom one brother (patient II:8) was ill and lived in a nursing home. The other brothers and the sister (II:5) were reported to be healthy or had died by accident.

The 40-year-old uncle (II:8) of the index patient was bedridden with tetrarigidospasticity and showed a minimal response state, suffering from infantile behavior, alcohol abuse and speech disturbances for 3 years. Vascular parkinsonism had been discussed earlier. MRI revealed significantly more extensive confluent supratentorial white matter lesions as compared with those of the index patient, with otherwise identical features (see Figure 2B).

The elder brother (III:1) of the index patient became symptomatic during his sister's illness, presenting with severe depression at the age of 30 years. Neuropsychological examination revealed cognitive dysphasia and very high scores (4) for hostility, phobic anxiety and psychosis. MRI showed marked confluent white matter lesions similar to those of his sister (III:2) and his uncle (II:8) (data not shown). Six months after very rapid progression, his condition had deteriorated towards severe dementia.

Neuropathological investigations of brain tissue (II:8) and skin (III:2)

A stereotactic brain biopsy of the right frontal cortex and adjacent white matter was performed on the uncle (II:8) of the index patient. The tissue revealed notable loss of myelin (Figure 3A) and MBP deposition in microglia/macrophages (Figure 3B,N) as well as activated microglia/macrophages (Figure 3C) in the white matter. In addition, numerous neurofilament- (Figure 3D) and APP-positive (Figure 3E) axonal swellings were detectable in the white matter. Inflammatory infiltrates were rare and diffusely scattered throughout the lesions (data not shown). Macrophages containing lipopigment were visible by autofluorescence (Figure 3F) and by Sudan black (Figure 3G) as well as by cresyl violet (Figure 3H). CSF-1R immunoreactivity was not detectable on glial cells, while a control specimen from a patient with multiple

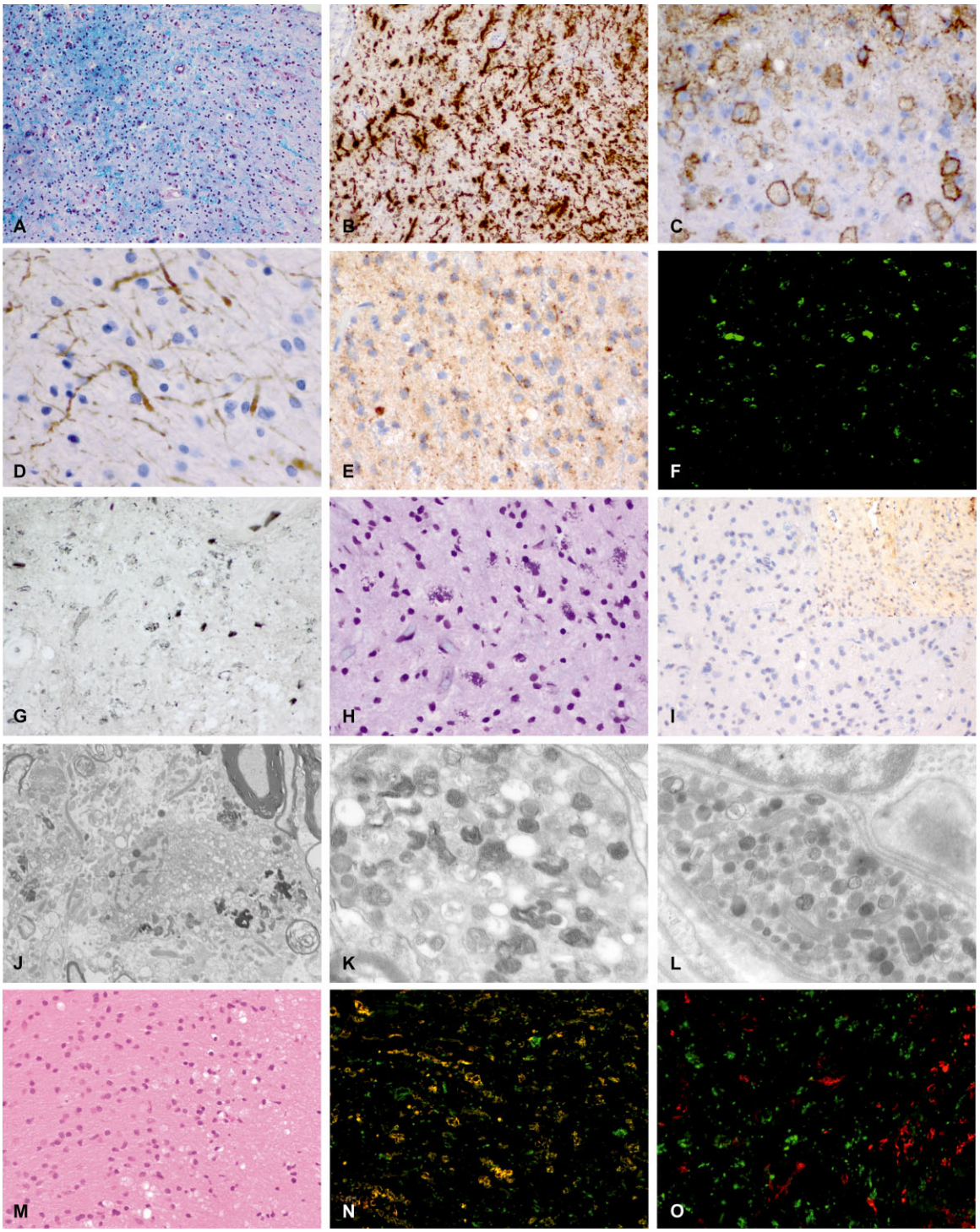

Figure 3. Histopathological investigation of a brain biopsy from patient 2, II:8 (uncle of the index patient) reveals loss of myelin in the white matter in a Luxol fast blue stain (**A**) with prominent glial cells illustrated by MBP (**B**) and voluminous macrophages in an HLA-DR stain (**C**). NFP- and APP-positive axonal swellings are also present (**D**,**E**) as well as autofluorescent, Sudan black and cresyl violet-positive lipopigment (**F–H**). CD115 (*CSF1R*) was negative on glial cells, but proved to be positive on microglia in a control patient with multiple sclerosis (**I**). Lipopigment was osmiophilic within macrophages (**J**). Additionally, axonal swellings are demonstrated ultrastructurally in the CNS (**K**) and the PNS, skin (patient 1, III:2) (**L**). Conventional hematoxylin and eosin (H&E) staining does not illustrate morphologically abnormal macrophages (**M**). Double immunofluorescence with MBP (red) and CD68 (green) show significant colocalization (**N**), while OLIG2$^+$ cells (green) do not colocalize with MBP (red) (**O**). Abbreviations: APP = amyloid precursor protein; CNS = central nervous system; MBP = myelin basic protein; NFP = neurofilament protein; PNS = peripheral nervous system.

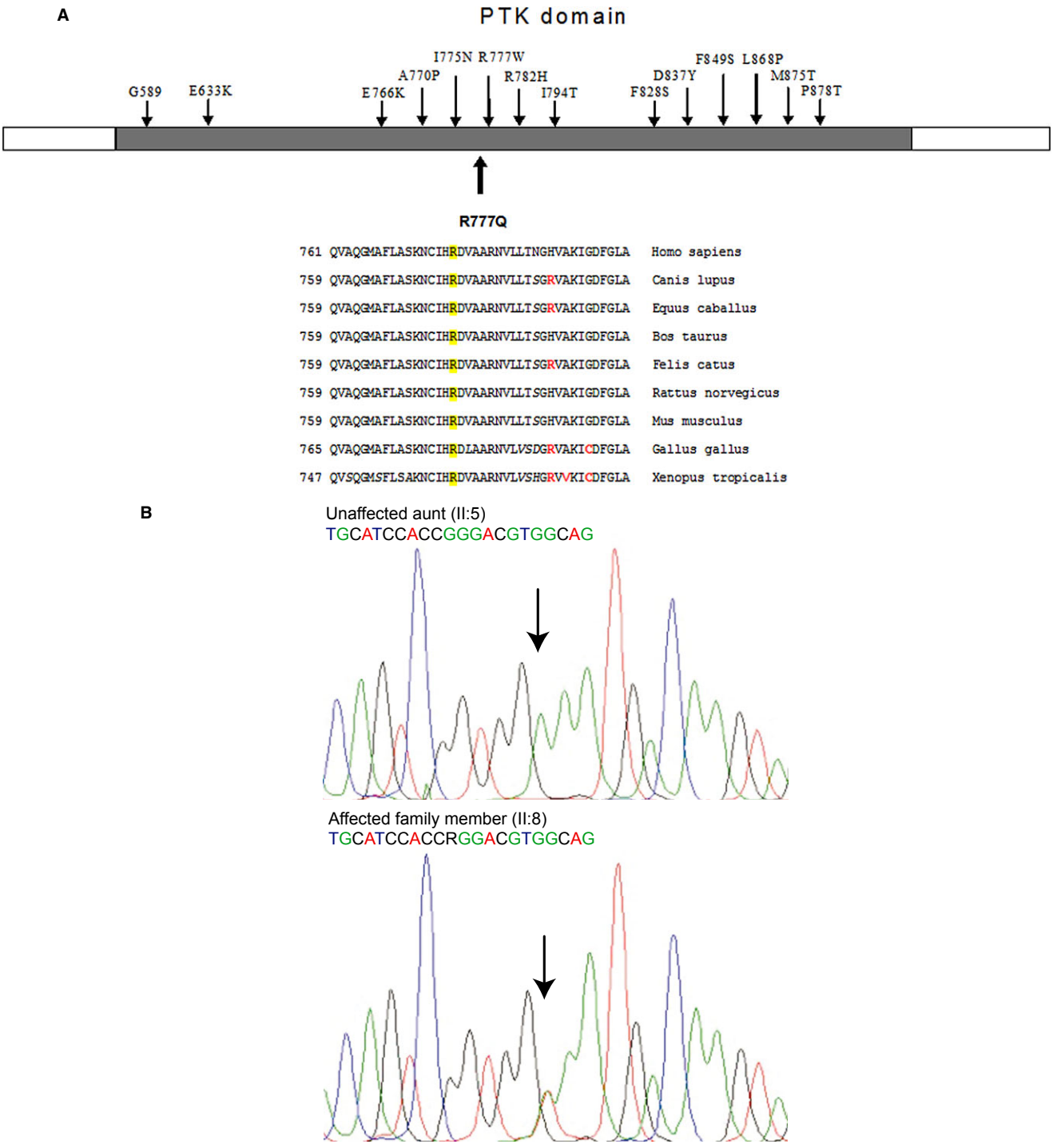

Figure 4. A. Location of protein tyrosine kinase domain (PTK) of *CSF1R*, including the mutation found in this pedigree (R777Q) and those identified in previous studies (8, 9, 11, 17). Comparative amino acid alignment shows evolutionary conservation of altered residues. **B.** Direct sequencing of *CSF1R* exon 18 from the unaffected aunt (II:5) and the affected proband (III:2). This nucleotide substitution (lower panel) results in a glutamine-for-arginine amino acid change (c.2330G>A, p.R777Q).

sclerosis showed CSF-1R$^+$ glial cells (Figure 3I). Glia from normal unaffected CNS tissue was negative for CSF-1R immunoreactivity. Ultrastructural analysis revealed occasional pigment-containing microglia/macrophages (Figure 3J) as well as enlarged axons containing dense bodies (Figure 3K), but no needle-like, tuffstone-like or lamellar deposits in lysosomes. Few unmyelinated axons enlarged by mitochondria and dense bodies were identified in ultrathin sections of the skin tissue, perhaps representing

degenerated mitochondria (Figure 3L and Supporting Information Figure S1).

Mutation detection in the *CSF1R* gene

Molecular genetic testing was performed on the three affected family members III:2 (patient 1, index patient), II:8 (patient 2, uncle) and III:1 (patient 3, elder brother) and an unaffected aunt of patient 1, II:5. Direct sequencing revealed a single nucleotide (guanine-to-adenine) substitution at the second position of codon 777 in exon 18 in one allele of *CSF1R* in the affected family members (Figure 4B) but not in the unaffected aunt (II:5). This mutation was also identified in the index patient's skin tissue, which had been formalin-fixed and paraffin-embedded. The nucleotide change results in a glutamine-to-arginine amino acid change (c.2330G>A, p.R777Q). This mutation is located within the conserved intracellular tyrosine kinase domain of the protein (Figure 4). This particular mutation has recently been reported in a French (6) and another German family (7).

DISCUSSION

We report a family with HDLS, showing early onset and rapidly progressive neuropsychological decline with two fatal cases within 1.5 years after clinical onset. Investigation of the biopsied skin by electron microscopy revealed axons expanded by mitochondria and dense bodies, that is spheroids, similar to the ultrastructure of CNS spheroids, suggesting that the peripheral nervous system (PNS) is also affected. This important observation illustrates that nerve and skin biopsies can be used for diagnosis.

Median age at onset in the herein described kindred was 29 years and death occurred after 1.5 years. This is at variance with the hypothesis that "an earlier disease onset seems to be associated with a longer disease duration." Wider *et al* reviewed 12 hereditary HDLS families and found the average age at onset to be 39 years with an average disease duration of 10 years (20). Thus, there seems to be important intrafamilial, but also interfamilial, variability of disease duration and signs.

CSF1R is a cell-surface receptor for the cytokines CSF-1 and IL-34, which regulate survival, proliferation, differentiation and function of mononuclear phagocytic cells, including microglia of the CNS (15). There is evidence that CSF1R is also expressed by neuronal cells (19). It is unclear whether peripheral monocytes/macrophages are involved in the disease process, whether neuronal or microglial lack of CSF1R function plays the leading role in HDLS/ALSP, and similarly, whether deficits in CSF-1 or IL-34 signaling (or both) are preferentially implicated in HDLS/ALSP pathogenesis.

Expression of mutant *CSF1R* in cultured cells abrogates the CSF1R kinase activity impeding the phosphorylation of downstream targets (17). It has been demonstrated that HDLS-associated mutations in CSF1R result in loss of function (16). Brains of *Csf1r*-mutant mice are smaller in size, display an expansion in size of lateral ventricles and have a thinner neocortex than wild-type mice, with a general reduction in the thickness of all cortical layers (13). CSF1R expression in the brain decreases dramatically during development and is expressed at low levels in adult brains (13). This finding might explain onset of the disease in adulthood. HDLS/ALSP patients are heterozygous for *CSF1R* mutations and might become symptomatic when the decrease of CSF1R expression reaches a critical threshold.

Of note, we report the first HDLS/ALSP patient diagnosed during pregnancy. Interestingly, in a murine model it has been shown that CSF1R expression is high in mature unfertilized oocytes and decreases rapidly in the preimplantation phase (1). These authors show the purported importance of CSF-1/c.fms paracrine signaling during the preimplantation period, yet our patient had a normal pregnancy with delivery of a "healthy" child, illustrating the peculiar issue of the obvious brain-specific mechanism, given that it is a germline mutation and CSF1R-signaling should also be important in the periphery.

Employing an antibody against CSF1R, we identified CSF1R-positive microglial cells in a pathological control tissue (multiple sclerosis), but not in the HDLS brain biopsy specimen. Whether this limited finding indicates a dominant negative effect of the mutant CSF1R protein on expression of normal CSF1R, or if downstream signaling is affected, while expression is preserved (16), remains to be substantiated. However, in experimental autoimmune encephalitis, induced in a neuronal IkappaB-deficient mouse, CSF1R expression was reduced (5).

Brain biopsy specimen from the patient described in this study exhibited both spheroids and accrued lipopigments. As spheroids are a hallmark of HDLS and lipopigment deposits a hallmark feature in POLD, and because the clinical symptoms of both conditions are similar and their molecular backgrounds are identical, our findings support and expand the suggestion to rename HDLS and POLD as one entity, namely ASLP (9, 20).

In summary, we present individuals from a family with a mutation in the *CSF1R* gene with ALSP/HDLS exhibiting an earlier disease onset and a more rapid disease progression than in most other reported kindreds (6). With the identification of the causative gene and available mutational analysis of *CSF1R*, a diagnosis can be established more rapidly. We show that this can be done even from paraffin-embedded tissue from the skin, which can be used for electron microscopy and molecular analysis. Through these advances, diagnoses may become more frequent, revealing a higher incidence rate of HDLS than previously believed.

ACKNOWLEDGMENTS

We thank Petra Matylewski and Hanna Plueckhahn for help with electron microscopy and immunohistochemistry.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Several unmyelinated axons from different parts of a skin biopsy and Schwann cells with their nucleus are illustrated (**A–C**). Some axons are enlarged by numerous dense bodies, degenerated mitochondria and lysosomal remnants (**D**).