SHORT REPORT

# Inherited prion disease with A117V mutation of the prion protein gene: a novel Hungarian family

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**Abstract**

**Three members of a family with inherited prion disease are reported. One additional family member had a progressive neurological disease without details. Two developed symptoms of ataxia, dementia, myoclonus, rigidity, and hemiparesis, and one had a different phenotype with the combination of lower motor neuron deficit, parkinsonism, intellectual decline, and ataxia. In this last patient cell loss of the anterior horn motor neurons and chronic neurogenic muscle atrophy was evident. Immunostaining for the prion protein disclosed unicentric and multicentric plaques, and coarse and fine granular positivity. Genetic analysis of the prion protein gene of the propositus showed a 117 codon alanine to valine mutation and homozygous 129 valine/valine genotype.**

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Human prion diseases are mostly sporadic and rarely acquired, but about 10% are inherited.[1] There are three human hereditary disease types: familial Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, and fatal familial insomnia.[1] Point mutations and insertions within the prion protein gene (PRNP) form the genetic background, but polymorphisms are also described.[1] The best known polymorphism at codon 129 (methionine/valine) is a susceptibility factor and influences the clinicopathological presentation.[2 3] Recently, pathogenic mutations in four different genes (including PRNP) were found in patients with familial early onset dementia, and further PRNP mutations and polymorphisms are also registered.[4 5] Here we present a Hungarian family with the rarely reported alanine to valine mutation at PRNP codon 117, including one case with a distinct clinicopathological phenotype.

## Methods

PEDIGREE

The pedigree, provided by the propositus' mother, is shown in figure 1.

PRION PROTEIN GENE ANALYSIS

This was done with written permission. High molecular weight genomic DNA was prepared from peripheral blood cells of the propositus (III/2). The PRNP open reading frame was amplified by polymerase chain reaction (PCR) in 50 µl reactions containing 100 ng DNA, 200 µM each dNTP, 15 mM MgCl2, 50 mM KCl, 10 mM TrisCl (pH 8.3), 1 U *Taq* polymerase, and 0.1 µM of primers (forward primer: TGATACCATTGCTATGCACTCATTC, reverse primer: GACACCACCACTAAAAGGGCTGCAG). Cycling conditions were 35 cycles of 94°C for 1 minute, 60°C for 1 minute, 72°C for 1 minute, with a final step at 72°C for 10 minutes. The amplified product (956 bp) was sequenced using a ThermoSequenase dye primer 7-deaza cycle sequencing kit (Amersham Pharmacia Biotech) in four overlapping segments using the fluorescently labelled (5'FAM) primers CTATGCACTCATTCATTATG, GCAGCCCTGGAGGCAACCGC, AGGTGGCACCCACAGTCAGT, and

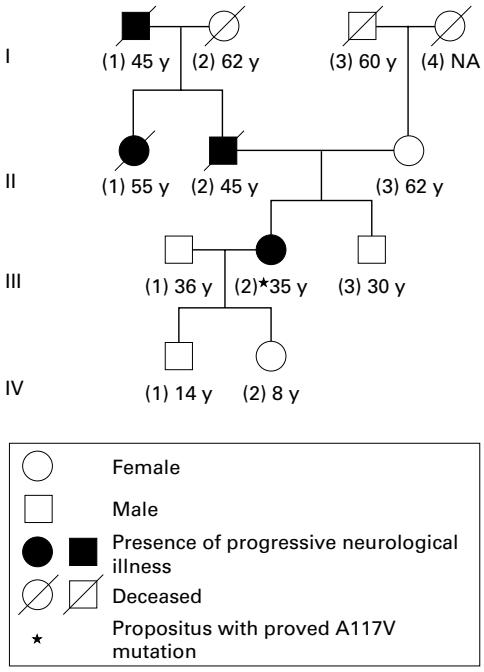


*Figure 1 Pedigree of the family. Generations are indicated with Roman, the family members with arabic numerals. NA=data not available.*

ACTTTGTGCACGACTGCGTCA. Sequencing reactions were analysed on an ALF Express DNA analysis system (Amersham Pharmacia Biotech).

NEUROPATHOLOGY

Postmortem was carried out 24 hours after death. Formalin fixed, paraffin embedded blocks of the spinal cord and several regions of the brain, brainstem, and cerebellum of patient II/1 were investigated. Sections were stained using haematoxylin and eosin, Klüver-Barrera, PAS, and Congo red methods. Prion protein (PrP) immunocytochemistry after a consensus pretreatment protocol[6] was performed using five monoclonal antibodies (3F4/epitope: amino acids 109–112/, 1:300, SENETEK, Maryland Heights, MO, USA; 6H4/epitope: amino acids 144–152/, 1:500, Prionics, Zürich, Switzerland; 12F10/epitope:amino acids 142–

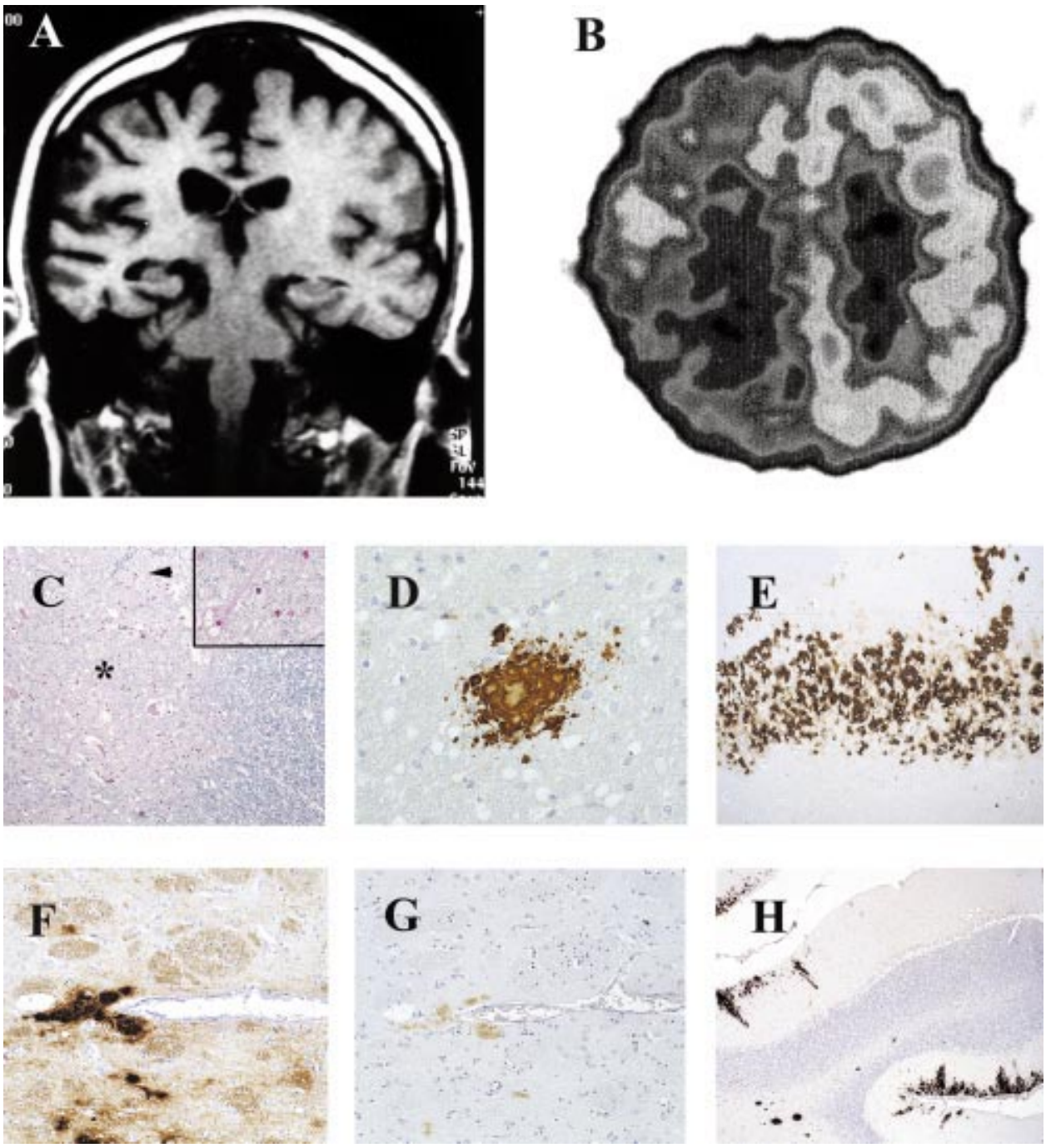


*Figure 2(A) Coronal T1 weighted cranial MRI of the propositus showing atrophy of the right hemisphere. (B) fluorodeoxyglucose positron emission tomography (PET) of the propositus showing reduced metabolism in the right hemisphere. (C) Loss of motor neurons and intraneuronal vacuolation (arrowhead, enlarged in the right upper corner) in the anterior horn (*) of the cervical spinal cord (Klüver-Barrera, originally×10 and ×40). (D) Multicentric amyloid plaque in the white matter of the cerebellum (immunocytochemistry for prion protein with 12F10, originally×20). (E) Diffuse granulofibrillar depositions in the subiculum with immunostaining for prion protein with 3F4 (originally×10). (F) Prion protein deposition adjacent to a vessel in the striatum (immunocytochemistry for prion protein with 3F4, originally×20). (G) Immunopositivity for the prion protein around the same vessel as seen on F is weaker using 6H4 antibody (originally×20). (H) Overview of the cerebellum: plaques are seen in the white matter, and along the surface in the molecular layer (immunocytochemistry for prion protein with 3F4, originally×10).*

160/, 1:1000, CEA, Service de Pharmacologie et d'Immunologie, Saclay, France; BG4/ epitope: N-terminus/, 1:1000, and KG9/ epitope: amino acids 140–180/, 1:1000, TSE Resource Centre, Birkett CR, Compton, UK). Immunostaining for glial fibrillar acidic protein (GFAP, 1:4000 DAKO, Denmark), Ubiquitin (1:200, DAKO, Denmark), Tau (AT8, 1:200, INNOGENETICS, Belgium), Beta A4 (1:50, DAKO, Denmark), α-synuclein (1:2000, CHEMICON INT INC,Temecula, CA, USA), and neurofilament protein (NFP, 1:800, DAKO, Denmark) was also done. As a secondary system we used the ChemMateTM detection kit (DAKO, Denmark).

## Results

### CASE REPORTS

The propositus (III/2) developed depression at the age of 29 years. At the age of 32, progressive ataxia and left sided weakness appeared. Now at the age of 35, left sided hemiparesis, exaggerated tendon reflexes, pyramidal signs, rigidity, dysdiadochokinesis, limb and gait ataxia, myoclonus in all limbs, and intellectual decline were seen. Repeated cranial MRI showed progressive atrophy of predominantly the right hemisphere (fig 2 A). An EEG showed a reduced alpha activity and increased theta activity over the right side. Transcranial magnetic stimulation demonstrated a mild lesion of the right sided motor pathways. Fluorodeoxyglucose positron emission tomography (PET) showed reduced tracer uptake over the whole right hemisphere (fig 2 B), with contralateral cerebellar diaschisis.

The propositus' father (II/2) died at the age of 45. For 3 years, he had been affected by progressive left sided hemiparesis with Babinski's sign, rigidity, resting, and intention tremor, ataxia, lack of spontaneity, primitive reflexes, depression, and intellectual decline. At postmortem his brain weight was 1140 g. Marked cortical atrophy with right sided accentuation was described, but histological examination was not performed.

The propositus' paternal aunt (II/1) died at the age of 55 in 1974. She had been a heavy drinker for years, but for the last 6 years she was abstinent. Her symptoms started at the age of 53 with impairments in social behaviour, lack of initiative, memory failure, and personality change. Six months before her death, disordered gait was reported. Rigidity, hypokinesia, and cerebellar signs were also noted. She developed atrophy in the interosseal, shoulder girdle, thigh, and tibial muscles, and increased upper limb tendon reflexes, but there was Achilles areflexia. Fasciculation, but not Babinski's sign, was seen. An EMG was not performed. Chronic neurogenic atrophy was seen in an anterior tibial muscle biopsy. An EEG showed diffuse slowing, later sharp waves in the right-frontocentral region.

The propositus' paternal grandfather (I/1) died at the age of 45 after a progressive neurological illness. No further clinical or neuropathological data are available.

### GENETIC ANALYSIS

In the propositus (III/2) an alanine to valine mutation was found at PRNP codon 117, with valine homozygosity at codon 129.

### NEUROPATHOLOGY OF PATIENT II/1

Focal spongiform change, neuronal loss, and reactive astrogliosis was seen in the putamen and medial thalamus. Cell loss and intraneuronal vacuolation were evident in the anterior horn of the spinal cord (fig 2 C). Prion protein immunopositive plaques were striking; they were already visible in haematoxylin and eosin sections, and were variably PAS and Congo red positive. The plaques were most frequent in the subiculum and hippocampal CA1, putamen, and medial thalamus, less in the white matter and molecular layer of the cerebellar hemispheres (with relative sparing of the vermis), and in deeper layers and adjacent white matter of the neocortex (with frontal dominance). The plaques showed variable morphology; mostly they had multicentric cores (fig 2 D), but unicentric and coreless, diffuse, granulofibrillar types were also noted. The major finding was plaques with a pale centre and distinctly strong surrounding positivity using the various antiprion protein antibodies. The plaque core was mainly stained with the 3F4 antibody. Interestingly, the diffuse plaque type in the subiculum (fig 2 E) and depositions adjacent to vessels were hardly, or not at all, labelled with 6H4 (fig 2F and G). Further immunostaining patterns were a coarse granular type (roughly following fasciculi) in the striatum; finely granular in the striatum, medial thalamus, and neocortex; and a granulofibrillar positivity along the surface of the molecular layer of the cerebellum (fig 2 H). These patterns were best seen with antibodies 3F4, KG9, BG4, and 12F10. Immunocytochemistry for Tau, β-A4 and α-synuclein was negative. Immunostaining for ubiquitin showed labelled dystrophic neurites around some of the plaques, that for NFP stained as sparse torpedoes in the cerebellum.

## Discussion

We describe three members of a Hungarian family with the A117V PRNP mutation. According to the dominant inheritance of a neurological disease, the PRNP mutation in one affected member, and the presence of multicentric plaques in the brain of another, the diagnosis of familial prion disease,Gerstmann-Sträussler-Scheinker disease type, was made.[7]

Previously one British-Irish, two French-Alsatian, and two American (one with German descent) families were described with this genotype.[8–16] In these, the age at onset varied between 20 and 64 years, the duration of illness 1 to 11 years; our patients were similar. Hsiao *et al* reported on two members of a family with intellectual decline in one, and with dementia, rigidity, myoclonus, and dysarthria in the other. The cerebellum was preserved; thus the term telencephalic variant was used.[14] In the other patients, the central clinical syndrome was progressive dementia and varying degrees of cerebellar ataxia, parkinsonian features, pyramidal signs, myoclonus, pseudobulbar

syndrome, and dysarthria, but behavioural, personality, and mood disturbance were also noted.[8–13 15 16] In the Alsatian family progressive hemiparesis was mentioned as well.[9]

In our patients, where detailed clinical data were available, intellectual decline, rigidity, and cerebellar signs were seen in all patients, whereas two (II/2 and III/2) had progressive hemiparesis (and unilateral cerebral atrophy in patient III/2). The clinical picture of patient II/1, consisting of motor neuron disease associated with dementia, parkinsonism, and ataxia, resembled frontotemporal dementia-parkinsonism-motor neuron disease.[17] Previously this combination of symptoms was not emphasised in A117V patients, although fasciculation and atrophy were mentioned.[9 18] Loss and intraneuronal vacuolation of spinal cord motor neurons in combination with neurogenic atrophy of muscle tissue were not described previously. Neuropathological investigation disclosed widespread accumulation of prion protein immunopositive plaques, as in earlier findings.[13 15 16 19]

The codon 129 polymorphism might account for the heterogeneity between reported cases. The genotype of our propositus showed valine on the mutated allele, as in previous cases.[8–10 12 14–16] As genetic information was available in only one of our patients, other causes for the motor symptoms might be also considered. However, the role of alcohol intake in the symptoms of patient II/1 is unlikely as she was abstinent for the past 6 years.

Prion immunocytochemistry of the plaques supports the notion that the amyloid core is strongly visible, with antibodies recognising the mid-region of the prion protein molecule, and that prion positive depositions might contain distinct prion protein fragments. It was already demonstrated that A117V Gerstmann-Sträussler-Scheinker disease shows western blot patterns different from those seen in Creutzfeldt-Jakob disease.[3 20]

In conclusion, one of our patients showed a distinct clinical phenotype with loss and vacuolation of lower motor neurons (motor neuron disease type). In other respects (genotype, clinical course of other patients), our family is in accordance with earlier reports. Our finding widens the range of familial prion disease phenotypes and further emphasises the need for detailed analysis of the prion protein gene in atypical familial neurodegenerative conditions.

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1 Prusiner SB. Prion diseases and the BSE crisis. *Science* 1997;**278**:245–51.
2 Alperovitch A, Zerr I, Pocchiari M, *et al*. Codon 129 prion protein genotype and sporadic Creutzfeldt-Jakob disease. *Lancet* 1999;**353**:1673–4.
3 Parchi P, Giese A, Capellari S, *et al*. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Ann Neurol* 1999;**46**: 224–33.
4 Windl O, Giese A, Schulz-Schaeffer W, *et al*. Molecular genetics of human prion diseases in Germany. *Hum Genet* 1999;**105**:244–52.
5 Finckh U, Müller-Thomsen T, Mann U, *et al.* High prevalence of pathogenic mutations in patients with early-onset dementia detected by sequence analyses of four different genes. *Am J Hum Genet* 2000;**66**:110–17.
6 Bell JE, Gentleman SM, Ironside JW, *et al.* Prion protein immunocytochemistry: UK five centre consensus report. *Neuropathol Appl Neurobiol* 1997;**23**:26–35.
7 Budka H, Aguzzi A, Brown P, *et al*. Neuropathological diagnostic criteria for Creutzfeldt-Jakob disease (CJD) and other human spongiform encephalopathies (prion diseases). *Brain Pathol* 1995;**5**:459–66.
8 Tateishi J, Kitamoto T, Doh-ura K, *et al*. Immunochemical, molecular genetic and transmission studies on a case of Gerstmann-Sträussler-Scheinker syndrome. *Neurology* 1990;**40**:1578.
9 Tranchant C, Doh-ura K, Warter JM, *et al*. Gerstmann-Sträussler-Scheinker disease in an Alsatian family: clinical and genetic studies. *J Neurol Neurosurg Psychiatry* 1992;**55**: 185–7.
10 Tranchant C, Sergeant N, Wattez A, *et al*. Neurofibrillary tangles in Gerstmann-Straussler-Scheinker syndrome with the A117V prion gene mutation. *J Neurol Neurosurg Psychiatry* 1997;**63**:240–6.
11 Mohr M, Tranchant C, Heldt N, *et al*. Alsatian variant of codon 117 form of Gerstmann-Sträussler-Scheinker syndrome: autopsy study of three cases. *Brain Pathol* 1994; **4**:524.
12 Heldt N, Boellaard JW, Brown P, *et al*. Gerstmann-Sträussler-Scheinker disease with A117V mutation in a second French-Alsatian family. *Clin Neuropathol* 1998;**17**: 229–34.
13 Mohr M, Tranchant C, Steinmetz G, *et al*. Gerstmann-Sträussler-Scheinker disease and the French-Alsatian A117V variant. *Clin Exp Pathol* 1999;**47**:161–75.
14 Hsiao KK, Cass C, Schellenberg GD, *et al*. A prion protein variant in a family with the telencephalic form of Gerstmann-Sträussler-Scheinker syndrome. *Neurology* 1991;**41**:681–4.
15 Mastrianni JA, Curtis MT, Oberholtzer JC, *et al*. Prion disease (PrP-A117V) presenting with ataxia instead of dementia. *Neurology* 1995;**45**:2042–50.
16 Malluci GR, Campbell TA, Dickinson A, *et al*. Inherited prion disease with an alanine to valine mutation at codon 117 in the prion protein gene. *Brain* 1999;**122**:1823–37.
17 Foster NL, Wilhelmsen K, Sima AAF, *et al*. Frontotemporal dementia and parkinsonism linked to chromosome 17: a consensus conference. *Ann Neurol* 1997;**41**:706–15.
18 Worrall BB, Rowland LP, Chin SS-M, *et al*. Amyotrophy in prion diseases. *Arch Neurol* 2000;**57**:33–8.
19 Ghetti B, Piccardo P, Frangione B, *et al.* Prion protein amyloidosis. *Brain Pathol* 1996;**6**:127–45.
20 Piccardo P, Dlouhy SR, Lievens PMJ, *et al.* Phenotypic variability of Gerstmann-Sträussler-Scheinker disease is associated with prion protein heterogeneity. *J Neuropathol Exp Neurol* 1998;**57**:979–88.