

SHORT REPORT

Neurofibrillary tangles in Gerstmann-Sträussler-Scheinker syndrome with the A117V prion gene mutation

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Abstract

One patient of a French family with Gerstmann-Sträussler-Scheinker syndrome with the mutation in codon 117 of the prion protein (PrP) gene displayed unexpected neuritic degeneration around PrP plaques and numerous diffuse neurofibrillary tangles, whereas other members did not. The tau profile in this patient's brain was analysed and compared with one from another member of the Gerstmann-Sträussler-Scheinker family as well as with the Alzheimer's tau profile. A panel of well characterised antibodies against both normal tau protein and paired helical filaments-tau protein was used on immunoblots of brain proteins resolved by mono and two dimensional gels. The tau profile in the patient with Gerstmann-Sträussler-Scheinker syndrome without neurofibrillary tangles was normal. The tau profile from the patient with Gerstmann-Sträussler-Scheinker syndrome and neurofibrillary tangles was characterised by a hyperaggregation state of tau protein. This case illustrates the phenotypic heterogeneity of the GSS117 mutation not only from one family to another, but also between members of the same family. In this family, the presence of neurofibrillary tangles is still unexplained, but could be correlated with either the protracted duration of the disease or the old age of the patient.

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Gerstmann-Sträussler-Scheinker disease is an inherited human progressive neurodegenerative disease which has been associated with various mutations in the prion gene.¹ The clinical picture may associate dementia, ataxia, extrapyramidal, and pyramidal signs or pseudobulbar syndrome. Neuropathological findings in the brain include neuronal loss, spongiform change, and gliosis as well as unicentric or

more often multicentric amyloid plaques. Neurofibrillary tangles are consistently present in patients with mutation at codon 198² or 217³ of the prion protein (PrP) gene, each found in a single family, and in a Japanese patient with a stop mutation at codon 145.⁴

Neurofibrillary tangles correspond to intraneuronal changes, of which the most important components are phosphorylated tau proteins.⁵ They were first described in Alzheimer's disease, in which immunoblot labelling in brain homogenates using the highly specific and sensitive monoclonal antibody AD2, which binds to a phosphorylation site located at the carboxy terminal of the tau protein molecule,⁶ showed the presence of a typical tau triplet (55, 64, 69 kDa).⁷

We report here the unpublished case of one patient from the previously described French Alsatian Gerstmann-Sträussler-Scheinker syndrome family with the codon 117 mutation,⁸⁻⁹ who, on neuropathological examination, showed unexpected and numerous neurofibrillary tangles. A biochemical study of tau proteins was performed in this patient and in another case of the Gerstmann-Sträussler-Scheinker syndrome family with the same PrP mutation but devoid of neurofibrillary tangles. In parallel, biochemical results were compared with those obtained in typical cases of Alzheimer's disease.

Material and methods

PATIENTS

The two patients were from two generations of an Alsatian family in which 13 members had already developed the disease (fig 1).

Patient 1 (IV.4) was healthy until the age of 64. At this time, she was admitted to hospital because of gait impairment and tremor. Neurological examination disclosed parkinsonian signs with rest tremor, hypertonia, and akinesia in her right upper and lower limbs. Neuropsychological tests showed difficulties in memory as well as constructive apraxia and agraphia. The patient was dysarthric and had a bilateral grasping reflex. Despite treatment with levodopa, her neurological state worsened progressively. The patient was not seen again until she was 70, at which time she had become bedridden because of extrapyramidal and pyramidal

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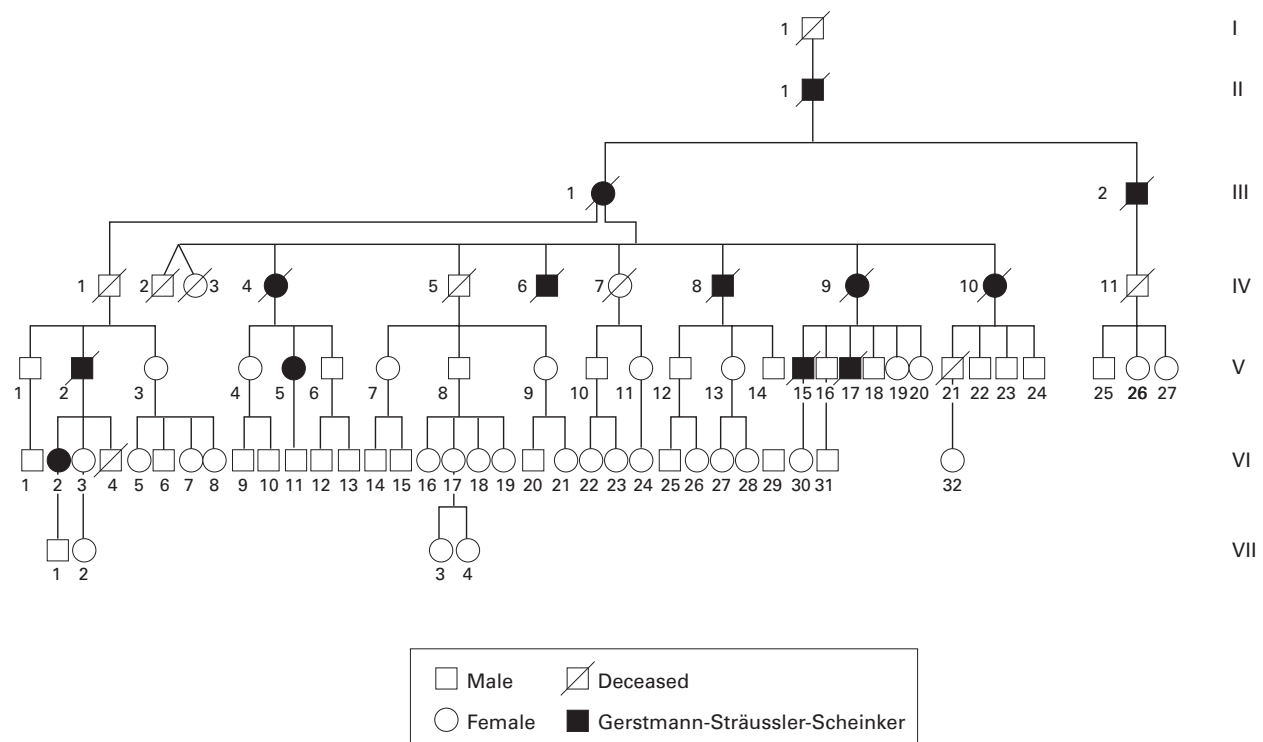


Figure 1 Genealogical tree of the French Alsatian family with Gerstmann-Sträussler-Scheinker disease.

signs (right hemiplegia). She had developed epileptic fits. Her speech was extremely reduced. Pseudobulbar signs and global dementia appeared later and she died at the age of 73.

Patient 2 (V.15) developed, at the age of 33, fatiguability of his right lower limb which progressively worsened. His voice became hoarser and a few months later, he had to leave his job because of difficulties in concentration and lack of attention. At the age of 34, neurological examination showed pyramidal signs in all four limbs, axial akinesia, and cerebellar signs which increased the gait difficulties. Facial diplegia, dysphagia, and dysarthria were present and the patient became extremely emotional. At 35, he was bedridden with pyramidal, extrapyramidal, and cerebellar symptoms. Pseudobulbar syndrome was significant. Cognitive dysfunction increased slowly and the patient died at the age of 39.

The genetic study performed by Tateishi *et al* has been previously reported.⁸⁻⁹ All the patients had the same mutation in the PrP gene: they carried a silent A to G transition at the third position of the alanine codon at 117, a C to T transition at the second position of the same codon leading to an alanine to valine (val) change (val 117), and an A to G transition at the first position of codon 129 that results in a methionine (met) to valine (val) substitution (val 129). In these two patients, the A to G transition at the first position of codon 129 was absent in the normal allele, and both were heterozygous met-val at codon 129. Apolipoprotein E genotype was E3/E3 in patient 1.

ANTIBODIES

Antiglial fibrillary acidic protein (GFAP) antibody (Dako), antineurofilament antibody reacting with 70 kDa, 60 kDa, and 200 kDa

polypeptide subunits (Dako), antiubiquitin antibody (Dako), anti-tau antibody reacting with phosphorylated and non-phosphorylated forms of the protein (Dako), antipaired helical filaments antibody (ICN), anti-A β antibody (Dako), and anti-PrP antibody (kindly provided by Dr S Prusiner) were used for immunohistochemical staining.

The antibody AD2 was used for the specific immunoblot staining of pathological tau protein.⁶ AD2 is a monoclonal antibody raised against a paired helical filament preparation isolated from Alzheimer tissue. It has a high specificity and sensitivity for phosphorylated tau proteins^{6 10} and it recognises an epitope located at the carboxy terminal part of the tau molecule including phosphorylated serines 396 and 404. Indeed, AD2 is able to directly detect pathological tau proteins in crude sodium dodecyl sulphate brain tissue homogenates.⁶ M19G is a polyclonal antibody directed against the synthetic peptide corresponding to the amino terminal part of the tau molecule^{6 11} and labels both normal and pathological tau proteins. Other antibodies against tau proteins were also used: a polyclonal antibody 133¹² which is raised against the first 16 amino acids of all tau proteins, and two other polyclonal antibodies 304 and 189 which recognise the 29 or 58 amino terminal inserts respectively, and therefore specifically detect the adult variants of tau proteins.⁵ Adult tau proteins are composed of six isoforms, and antiserum 304 recognises four isoforms and antiserum 189 recognises only the two longest isoforms.

NEUROPATHOLOGY

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At the time of necropsy, which was limited to the brain and spinal cord, one cerebral hemisphere from each of the two patients was frozen at -80°C for neurochemical, molecular genetic, and transmission studies. The remaining parts of the CNS were fixed in 10% formalin. Multiple brain tissue blocks were

embedded in paraffin and sections were stained with haematoxylin and eosin, cresyl violet, Congo red, Luxol fast blue/cresyl violet, and Bodian's silver method. Immunohistochemical staining was performed on selected sections. A standard peroxidase-antiperoxidase technique was used. Spinal cord sections were stained with haematoxylin and eosin, cresyl violet, and Luxol fast blue/cresyl violet.

An electron microscopic study was performed in cases 1 and 2. Samples of frontal cortex and cerebellar cortex were fixed in 3% buffered glutaraldehyde at the time of necropsy, then postfixated in 1% osmium tetroxide and embedded in Epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate. They were examined with an EM 300 Philips electron microscope.

BIOCHEMISTRY

Immunoblots

Several brain area samples from patients 1 and 2 were studied, using a previously described semiquantitative western blot approach.⁷ Brain tissue samples were heat treated in Laemmli sample buffer at a ratio of 1 g/10 ml. Homogenates (10 μl) were then loaded on SDS-PAGE (10–20% slab gel). Proteins were transferred on to nitrocellulose membranes (0.45 μm pore size, Schleicher and Schuell) for 90 minutes. (current: 0.8 mA/cm²) using an LKB Multiphor II Nova Blot (Pharmacia) according to the manufacturer's instructions. Blocking was carried out with tris buffered saline containing 5% (w/v) dry milk and 0.05% (w/v) Tween 20. The blotted proteins were incubated with monoclonal antibody AD2 at a final concentration of 0.3 $\mu\text{g/ml}$ for two hours at room temperature. They were then revealed with horseradish peroxidase labelled sheep anti-mouse or anti-rabbit immunoglobulins (Diagnostics Pasteur) and detected with the ECL western blotting system (Amersham).

Two dimensional gel analysis

A more detailed analysis of the tau profile from patient 1 was performed using two dimensional gel electrophoresis. Its pattern was compared with the one from a patient with Alzheimer's disease. Two dimensional gels were obtained according to a modified O'Farrell's method,¹³ as described by Sergeant *et al.*¹⁴ The first dimension gel contained 9.5M urea, 2% Triton X100, 5% (vol/vol) (Pharmacia) with 4% pH 3 to 10 and 1% pH 4 to 6.5. Brain homogenates (15 μl) in Laemmli sample buffer were heat treated for five minutes at 100°C and centrifuged at 10 000 g for 10 minutes at 4°C . Thirty five microlitres of a solution containing 8 M urea and 4% Triton X-100 were added to the supernatant. Samples were run for 20 hours at 500 V and at 700 V for 30 minutes on 10 cm

long, 3 mm diameter capillary tubes. The first dimensional gels were then equilibrated for five minutes in 5 ml equilibrium buffer containing 10% glycerol, 0.5% dithio treitol, 3% SDS, and 0.25 M tris at pH 6.8. The second SDS-PAGE dimension and western blot analysis were performed as described for immunoblotting.

Isoelectric points were determined using the Carbamylite™ calibration kit (Pharmacia). Five microlitres of carbamylated creatine phosphokinase was added to the brain homogenate, as recommended by Pharmacia. GFAP immunostaining was also used for calibration, after AD2 staining. M19G was used either as the first antibody, the blot being then stripped in accordance with the Amersham ECL western blotting system, and lastly stained with AD2, or vice versa (AD2 staining first, stripping followed by M19G staining).

Results

NEUROPATHOLOGICAL DATA

Patient 1 (No 40743-IPS) (fig 2)

The left cerebral hemisphere was severely atrophic. Microscopy disclosed PrP amyloid plaques that were numerous in the cerebral cortex, basal ganglia, thalamus, and the cerebellar cortex where they also affected the granular layer. They were often unicentric and large. Most of them were accompanied by many neuritic degenerations which were immunolabelled with anti-tau antibodies, including AD2, and which ultrastructurally consisted of dense bodies and paired helical filaments. Numerous PrP amyloid deposits were scattered throughout the cerebral and cerebellar white matter. In the cerebral grey matter swollen neurons were seen and astrocytic reactions were pronounced. Spongiform change varied in intensity and was often associated with PrP amyloid deposits. Numerous neurofibrillary tangles, immunolabelled with anti-tau and anti-paired helical filament antibodies, were found in the cerebral cortex, amygdala, and thalamus. Ultrastructurally, they consisted of paired helical filaments. Senile plaques were absent. However, rare A β protein deposits, always associated with PrP deposits, were found in the cerebral cortex and lower striatum. The spinal cord was histologically normal.

Patient 2 (No 40683-IPS)

Microscopy disclosed, as in patient 1, numerous PrP amyloid plaques in the cerebral cortex, basal ganglia, and thalamus. Neuritic reactions were absent or mild. Spongiform change varied in intensity but on the whole was moderate. Neuronal loss was suspected in the frontal cortex and confirmed in the globus pallidus and thalamus. Neurofibrillary tangles and A β protein deposits were absent.

BIOCHEMICAL DATA

Monodimensional western blot analyses (fig 3)

AD2 immunostaining—For patient 2, AD2 immunoreactivity was absent whereas it was positive for a patient with Alzheimer's disease who displayed a characteristic pathological tau triplet. On the same immunoblot, AD2 immunostaining for patient 1 was extremely

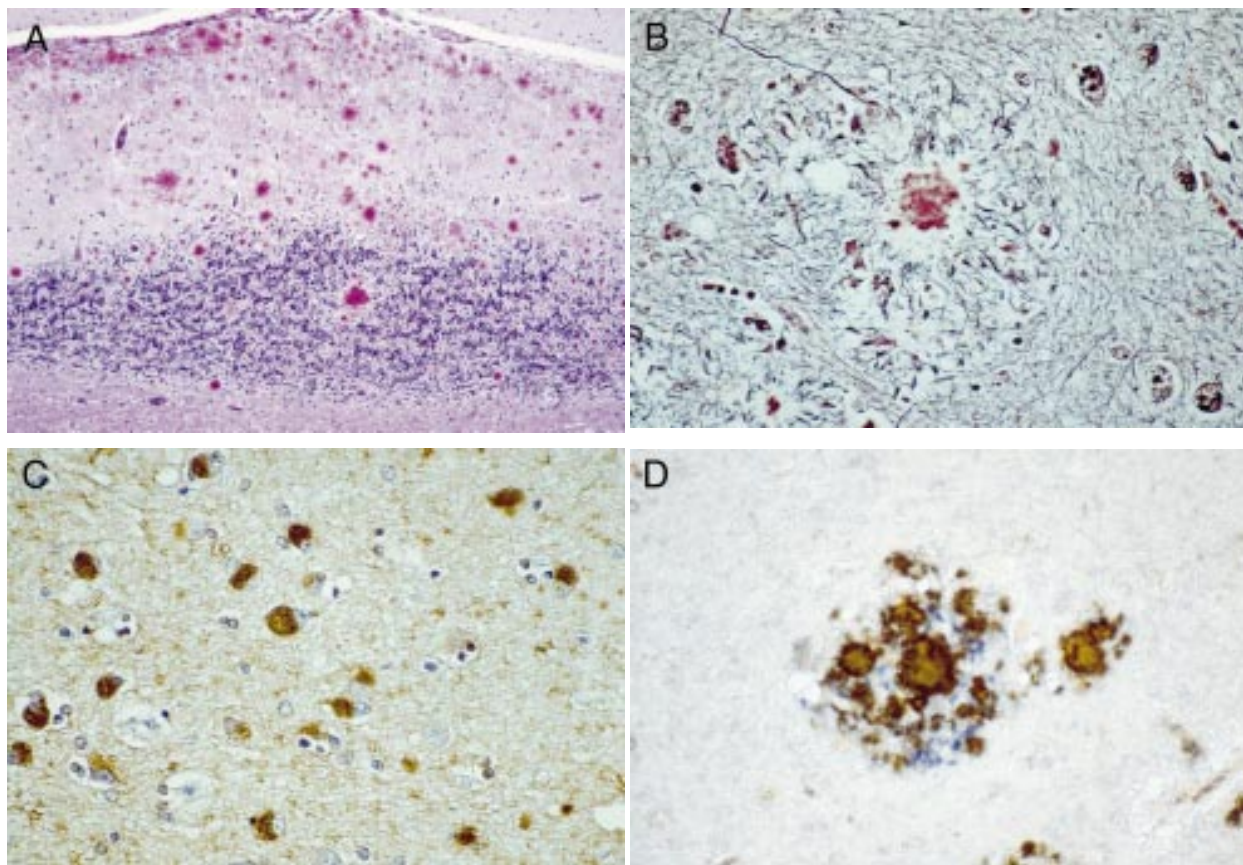


Figure 2 Neuropathological data in patient 1. (A) Cerebellar cortex: numerous amyloid plaques. Cresyl violet stain (originally $\times 25$). (B) Amygdala: amyloid plaque with neuritic reaction. Bodian stain (originally $\times 100$). (C) Amygdaloid nucleus: numerous anti-tau positive neurofibrillary tangles (originally $\times 100$). (D) Striatum: amyloid plaque. Double immunostaining for PrP (brown) and A β protein (blue) (originally $\times 100$).

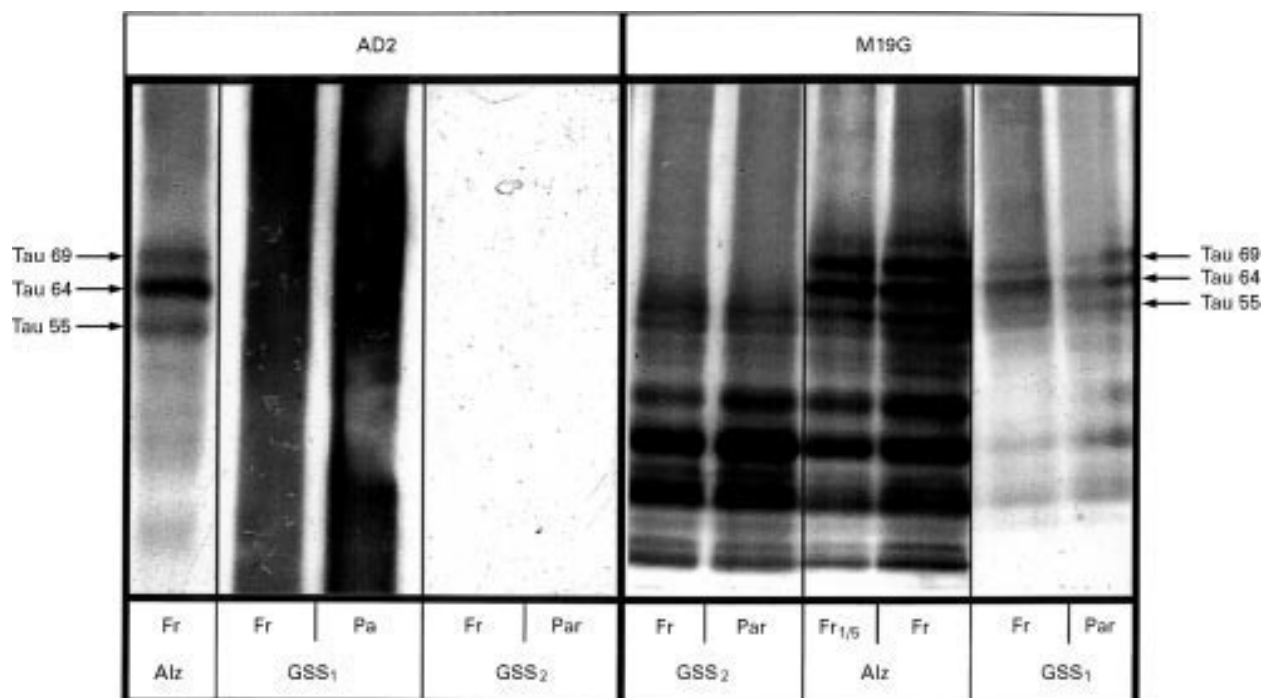


Figure 3 Western blot analysis of tau profiles in different brain areas from two cases with Gerstmann-Sträussler-Scheinker syndrome versus one typical case with Alzheimer's disease (Alz). Pathological tau proteins were detected with the monoclonal antibody AD2 or the polyclonal antibody M19G. Brain homogenates (15 μ l) were loaded in each well. AD2 immunodetected the 55, 64, 69 kDa triplet in the Alzheimer's disease cortical brain extract. Smears were heavily immunostained for Gerstmann-Sträussler-Scheinker syndrome case 1 whereas both AD2 immunoreactive smears and tau proteins were absent in Gerstmann-Sträussler-Scheinker syndrome case 2. M19G also detected the pathological tau protein triplet in the Alzheimer's disease brain extract and in small amount in Gerstmann-Sträussler-Scheinker syndrome case 1. Normal tau proteins were detected in Gerstmann-Sträussler-Scheinker syndrome case 2. Note that proteolytic products of tau proteins are rare for Gerstmann-Sträussler-Scheinker syndrome case 1, whereas they are numerous for Gerstmann-Sträussler-Scheinker syndrome case 2 as well as for the Alzheimer's disease case.

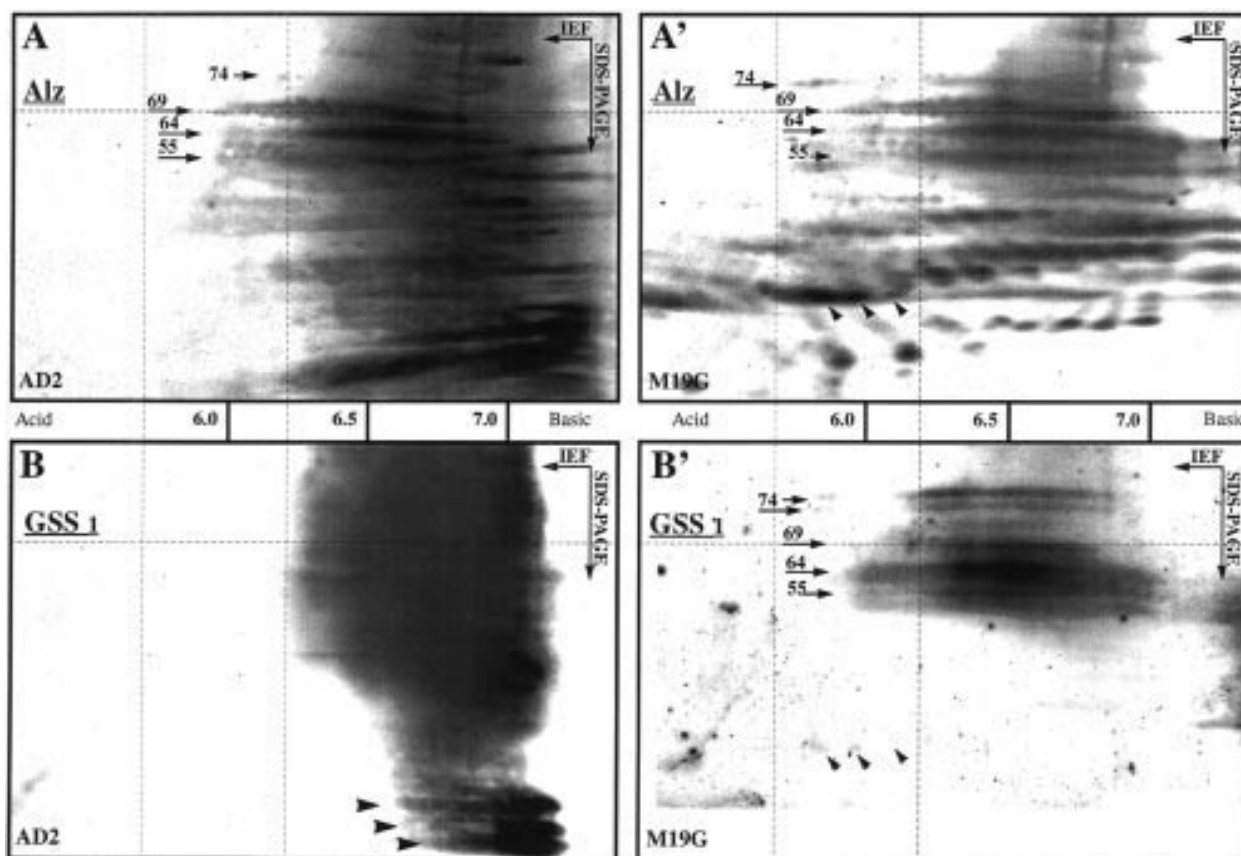


Figure 4 Two dimensional analysis of tau proteins in brain homogenates from Gerstmann-Sträussler-Scheinker syndrome case 1 and an Alzheimer's disease case. The first (IEF) and second (SDS-PAGE) dimensions are indicated by arrows. The pH gradient, determined with the carbamylates TM (Pharmacia) is represented on the abscissa with the acidic end on the left and the basic end on the right. (A) Analysis of the frontal cortex homogenate from a patient with Alzheimer's disease. AD2 immunoreactive tau proteins are marked by arrows. Note that besides the 55, 64, and 69 kDa tau components, a 74 kDa variant was weakly detected. Low molecular weight tau polypeptides are indicated by arrowheads. (A') M19G immunolabelling on the same nitrocellulose membrane used for A, after removal of AD2 by stripping. The tau protein triplet with molecular weights of 55, 64, and 69 are indicated by arrows, as well as the 74 kDa component. More acidic spots of the 74 kDa protein were immunodetected with M19G when compared with AD2. Low molecular weight tau catabolic products are marked by arrowheads. (B) AD2 analysis on a frontal homogenate from Gerstmann-Sträussler-Scheinker syndrome case 1. Smears were strongly detected whereas the tau triplet was not. Three small proteins with a molecular weight of 26, 27, and 29 kDa were strongly detected (arrowheads). (B') M19G immunolabelling on the same nitrocellulose membrane used for B, after removal of AD2 by stripping. Tau proteins from isoelectric point 7.0 to 6.0 were detected. The acidic part on the left of the smears (and of the dotted line) was labelled by M19G, but was not stained with AD2. Note that catabolic products are rare (arrowheads), when compared with the A' experiment.

strong, with smears all along the electrophoretic migration tracks. Pathological tau proteins were not detectable because they were masked by the smears.

M19G immunostaining—Using M19G, the group of normal tau proteins with molecular weight ranging from 45 to 64 kDa was detected for patient 2. Bands with a low molecular weight corresponding to tau catabolic products were also detected. For patient 1, tau proteins had a molecular weight in the range of Alzheimer's disease pathological tau proteins. However, tau proteins as well as their catabolic products were only present in small amounts.

Two dimensional western blot analysis (fig 4)

AD2 immunostaining—In the resolved Alzheimer's disease brain extract, AD2 immunodetected the triplet of pathological tau proteins, with an isoelectric point between 6.9 and 5.8. A higher component with a molecular weight of 74 kDa as already described,¹⁴ was also present. For patient 1, huge amounts of smears were found. Smears were in the isoelectric point range of 7.1 to 6.2. Low molecular weight components corresponding to catabolic products were absent, with the exception of three

characteristic low molecular weight components detected at 26, 27, and 29 kDa and with an isoelectric point ranging from 7.2 to 6.6. All brain areas studied were affected, and especially the limbic structures.

M19G staining—In the Alzheimer's disease brain extract, the triplet of pathological tau proteins and a 74 kDa component were immunodetected. A very acidic isoelectric variant at isoelectric point 5.8 corresponding to the 74 kDa component was detected with M19G, antibodies 133, 304, and 189 (data not shown), but not with AD2. These results show that the 74 kDa band corresponds to an adult tau protein isoform with exon 2 and 3. Numerous catabolic products were found. They were widely distributed all over the pH gradient. In brain extract from patient 1, the triplet of 55, 64, and 69 kDa tau proteins, and a 74 kDa component were detected, whereas the smears were faintly immunolabelled. However, a stronger band with a higher molecular weight of 78 kDa was also present. A very acidic component of the 74–78 double band was present at isoelectric point 5.8. Catabolic products were almost undetectable.

Mutations of the PrP gene in Gerstmann-Sträussler-Scheinker syndrome with their neuropathological and clinical expressions

Mutation	Codon 129 genotype		PrP deposits	NFT	A4 deposits	Duration	Clinical signs
	Mutant all	Normal all					
P102L ²³	Met or Val	Met or Val	++	-	-	6 y	Ataxia, late dementia
P105L ²⁴	Val	Met?	++	-	-	9 (3) y	Spastic paraparesia, late dementia
A117V							
French family	Val	Met	++	±*	±* Around PrP plaques	1 to 14 y	Pyramidal and extrapyramidal signs, ataxia, dementia
American family ²⁰	Val	Met	++	-	-	2 to 6 y	Dementia, extrapyramidal signs
American family ²⁵	Val	Val	++	-	-	2.5 y?	Ataxia+dementia
Y145 stop ^{*† 28}	Met	?	++	+	-	20 y	Dementia
F198S ^{† 16}	Val	Val or Met	++	++	±* Around PrP plaques	6 (3) y	Ataxia and dementia
Q217R ^{† 3}	Val	Met	++	++	++ Senile plaques, angiopathy amyloid	4 y	Dementia and ataxia

* Present in only one patient; † mutation described in only one family.

+ = Present; - = absent; ± = inconstant; ? = unknown; NFT = neurofibrillary tangles; PrP = prion protein.

Discussion

Gerstmann-Sträussler-Scheinker syndrome has been associated with six mutations of the PrP gene. The presence of neurofibrillary tangles, in addition to PrP immunoreactive amyloid deposits, is one of the neuropathological distinctive features of Gerstmann-Sträussler-Scheinker syndrome families with mutations at codon 198, 217, or 145 (table). In the codon 217 mutation,¹⁵ carried by two patients from a Swedish family, pathological studies showed numerous A β deposits besides PrP immunoreactive deposits. Some of them were found around PrP plaques but true senile plaques as well as A β blood vessel deposits were also detected, so that the association of Gerstmann-Sträussler-Scheinker syndrome and Alzheimer's disease could not be ruled out.³ The codon 145 mutation was found in one Japanese patient⁴ in whom amyloid deposits were immunolabelled with antibodies to PrP but not with antibodies to A β protein. Finally, codon 198 mutation has been described in a large American Indiana family;¹⁶⁻¹⁷ pathological studies showed numerous neurofibrillary tangles and, around PrP amyloid deposits, abnormal neurites which were immunostained by tau protein and ubiquitin antibodies, and which were composed as in Alzheimer's disease of paired helical filaments. Tagliavini *et al*¹⁸ showed that A68, an abnormally phosphorylated form of tau proteins and a major component of paired helical filaments in Alzheimer's disease, is present in the paired helical filaments of GSS198. No A β deposits were found except in the older patients, in whom A β immunoreactivity was seen only around some PrP deposits.¹⁹

As in mutations at codon 102 and 105, neurofibrillary tangles are usually absent in the mutation at codon 117. Mutation 117 has been described in three families. The lack of cerebellar involvement in the first reported American family²⁰ led to the definition of the 117 genotype as the "telencephalic form" of Gerstmann-Sträussler-Scheinker syndrome. However, the neuropathological findings in these French cases, with numerous PrP plaques in the cerebellum and presence of neu-

rofibrillary tangles illustrate the phenotypical heterogeneity of mutation 117. In this Alsatian family, neurofibrillary tangles and neuritic degeneration have been found only in patient 1 (among three who were necropsied, and another one with cerebral biopsy). Using immunochemical analyses, pathological tau proteins were not detected in brain homogenates from patient 2 but were found in huge amounts in almost all cortical areas for patient 1, in good agreement with the neuropathological findings. The tau protein pattern was characterised by the presence of smears all along the migrating track as visualised by AD2 immunostaining. Smears have already been reported in Alzheimer's disease but this phenomenon is by far more important for patient 1; they correspond to the carboxy terminal part of the tau protein²¹ or, as they were also detected in the range of low molecular weight, to small fragments of the tau protein which are still aggregated, even after SDS solubilisation. Low amounts of variants with a molecular weight similar to the pathological Alzheimer's disease tau proteins (55, 64, and 69 kDa) and a 74 kDa band which was more intense than in Alzheimer's disease extracts were found with M19G. Another higher band of 78 kDa was detected as well. Together, the analysis of tau proteins shows a hyperaggregation state of tau proteins in patient 1 compared with Alzheimer's disease.

The inconstant presence of neurofibrillary tangles in GSS 117 led us to ask why some patients develop neurofibrillary tangles whereas others do not. From a clinical point of view, as proposed in Alzheimer's disease,²² neurofibrillary tangles could be correlated with severity of the dementia. Indeed, cognitive impairment usually occurs only in the latest stage of the disease in patients with mutation at codon 102²³ or 105,²⁴ but is one of the first signs in patients with mutation at codons 145, 198, or 217 where neurofibrillary tangles are constant. However, in patients with mutation at codon 117, dementia is a cardinal feature of the disease in the French family and in one of the two American families,^{20 25} but neurofibrillary tangles were found in only one patient.

Although the codon 129 genotype has been shown to influence phenotypic expression of some prion diseases,²⁶ it cannot be linked to neurofibrillary tangle formation in Gerstmann-Sträussler-Scheinker syndrome. A val genotype in codon 129 was present in the mutant allele of all patients with the codon 117 mutation (either with neurofibrillary tangles or not) and the table confirms the absence of correlation between the presence of neurofibrillary tangles and codon 129 genotype in various PrP gene mutations. Because A β protein increases the phosphorylation of tau proteins *in vitro*,²⁷ coexistence of A β deposits in patient 1 could have contributed to paired helical filament formation. However, A β deposits are inconsistent in other PrP mutations with the presence of neurofibrillary tangles (table). In patient 1, coexistence of Gerstmann-Sträussler-Scheinker syndrome and Alzheimer's disease, as proposed in the codon 217 mutation, seems also to be excluded as A β deposits were too rare and always located around PrP deposits. Finally, at least in this mutation, neurofibrillary tangles could be correlated with either the old age of the patient (the oldest in this family), or the protracted duration of the disease, as the clinical course of our patient was the longest in the Alsatian family (nine years).

In conclusion, this case, with cerebellar involvement and neurofibrillary tangles, illustrates the phenotypical heterogeneity of GSS 117 not only from one family to another, but also between cases from the same family. Factors other than PrP genotype, still unknown, should be implicated in the phenotypic expression of the disease.

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