Prominent psychiatric features and early onset in an inherited prion disease with a new insertional mutation in the prion protein gene

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Summary
In five generations of the French M-E kindred, 11 members are now known to be or have been affected by a form of spongiform encephalopathy previously recorded as Gerstmann–Straussler–Scheinker disease. Mean age at onset was 28 years (range 21–34 years). In six instances, these patients were hospitalized in psychiatric institutions with various diagnoses, the most frequent being mania or mania-like symptoms. Dementia occurred progressively after a lengthy course. Histological studies showed atrophy of the cerebellar molecular layer, which contained kuru and multicentric plaques labelled with anti-prion protein antibodies. Spongiosis was not prominent and remained largely limited to the periphery of plaques; it was more marked in the thalamus, where plaques were scarce. A 192 base pair (bp) insert (eight extra repeats of 24 bp) in the octapeptide coding region of the prion protein gene (PRNP) within a codon-129 methionine allele was found in four symptomatic subjects. Early age at onset, the prominence of psychiatric symptoms and the long course of the disease are noticeable clinical features in this family with an inherited prion disease due to a new insertional mutation in PRNP.

Keywords: prion diseases; Gerstmann–Sträussler–Scheinker disease; prion protein gene; psychiatry

Abbreviations: bp = base pairs; CJD = Creutzfeldt–Jakob disease; GSS = Gerstmann–Sträussler–Scheinker disease; MMS = Mini-Mental State; PRNP = prion protein gene; PrP = prion protein; WAIS-R = Weschler Adult Intelligence Scale—Revised

Introduction
The rare inherited disease known under the eponym of Gerstmann, Sträussler and Scheinker (GSS) has been defined by these authors as a hereditary–familial condition characterized clinically by cerebellar symptoms followed by dementia and anatomically by cerebellar amyloid plaques (Gerstmann et al., 1936). It has been shown to be a transmissible spongiform encephalopathy (Masters et al., 1981), and is also called prion disease (Prusiner, 1982). Prion diseases are identified by the accumulation in the brain of an abnormal form of the host-encoded prion protein (PrP) (for review, see Prusiner and Scott, 1997). The normal isoform of the prion protein (PrPN) is a cell membrane-anchored glycoprotein expressed in the brain and in almost all peripheral tissues of mammals. Its normal function remains elusive; it has been suggested that copper ions (Cu2+) can bind in vivo to octapeptide repeats near the N-terminus of PrP (Brown et al., 1997), but the functional significance of this binding remains unclear.

The genotype of affected persons is now of primary importance for the classification of the inherited prion diseases. The GSS phenotype has been shown to be linked or associated with point mutations at codons 102, 105, 117, 145, 198 and 217, and also with specific inserts between codons 51 and 91 of the prion protein gene (PRNP) (Hsiao et al., 1989; Owen et al., 1989; Goldfarb et al., 1996; Collinge and Palmer, 1997; Tranchant and Warter, 1998). Insert mutations occur in a portion of the gene that encodes one nonapeptide and four copies of a repeating octapeptide, and these mutations involve extra copies of this last motif. Mixed clinical and neuropathological features of Creutzfeldt–Jakob disease (CJD) and GSS are often seen in patients carrying these insertions. A family with an insert of eight extra octapeptide-encoding repeats in PRNP has been reported with the diagnosis of GSS (Brown et al., 1992).

In this paper, we present new clinical, neuropathological
and molecular data concerning a kindred (M-E) with an inherited prion disease; some of the data have already been published as illustrating an instance of GSS (Foncin et al., 1982). They focus on a peculiar clinical presentation (early age at onset, prominence of psychiatric symptoms and lengthy course) which can be related to an eight-octapeptide insertional mutation in the \textit{PRNP} gene.

**Patients and methods**

**Family analysis**

The family investigation, starting from data provided by relatives of the patients, has been conducted mostly through a study of municipal registers. Recorded members of the M-E kindred now number 228. The earliest recorded date of birth is 1763. Within the M-E kindred, 11 persons are classified as affected with GSS (Fig. 1): one by clinical and pathological evaluation (the primary proband, no. 37, reported by J.F.F. and K.H.E.); four by personal clinical examination by some of the authors (nos 56, 187, 188 and 190, reported by I.D., P.T., L.D. and A.D.); five by evaluation of hospital records of deceased patients; and two by second-hand evidence. We had knowledge of seven sibships at first-degree risk, five escapees (at-risk persons aged $>60$ years who were symptom-free or died symptom-free after the age of 65 years) and 13 persons of unknown status. Male-to-male, female-to-male and female-to-female transmissions were observed. No instance of skipping a generation was noted.

**Patients and relatives**

Patient 190 was a female (the secondary proband) whose first years were unremarkable. A slow learner at elementary school, she was deemed unable to attend a regular high school. She left school at the age of 17 years, before completing vocational training as a service employee, to care for her demented mother (no. 53). A year later, she took up a job as a dishwasher, which she carried out to her employer’s and her own satisfaction until the age of 24. At that time, she complained of headache, ill-de

and emotional lability were obvious. Neurological examination showed akinesia and rigidity, possibly due to neuroleptic medication, and severe dysarthria in the absence of other cerebellar signs. Cognitive impairment was evident, with paraphasia, understanding difficulties, agraphic alexia and global apraxia. On the Wechsler Adult Intelligence Scale—Revised (WAIS-R), verbal IQ was 52, performance IQ was $<45$, global IQ was 51 at first testing and 58, 48 and 58, respectively, on repeated testing; the Mini-Mental State (MMS) score was 21. Her mental status improved, and she resumed employment a few months later, but soon had to give it up, allegedly because of irritability and aggressiveness towards her fellow workers. She complained of loss of balance and difficulty in buttoning her clothes. Neurological examination disclosed ataxic gait and left upper limb dysmetria. Examination in the following year, when she was 25, showed a slightly worse status, with impairment of upper limb co-ordination and deteriorated handwriting (she wrote in the script style, i.e. using lower-case print letters), and her global IQ score was 50. Various biological investigations designed to show evidence of metabolic pathway abnormalities were non-contributive. MRI was interpreted as showing a mild cerebellar atrophy. The patient was still alive 4.8 years after the first signs of the disease.

Patient 56 was a sister of the secondary proband; she had a normal childhood. After normal elementary and middle schooling, she completed vocational training as a pastry-cook and was employed as such from the age of 16 years. When she was 28, her family noted some gait disorders with frequent falls; her mood became unpredictable and her speech was slurred. At 29, her handwriting changed to script. A year later, she became pregnant for the third time and diabetes was detected, but she refused medical advice. She was again examined when she was 32 and displayed slurred speech, ataxic gait, and dysmetria that was more marked at the left upper extremity. Cognitive deterioration was apparent, with impaired judgement, reasoning, understanding and short-term memory. As scored with WAIS-R, her verbal IQ was 55, performance IQ 52 and full-scale IQ 52, and her MMS score 24. The patient was alive 4 years after the onset of the disease.

Patient 187 was a brother of the secondary proband. He was a slow learner at elementary school and started middle school at the age of 13 years, 2 years beyond the normal age, and at that time he was referred to the school psychologist for dysorthographia and ‘clumsiness’. His intellectual status was deemed ‘correct’, but a propensity to aggressiveness was noted. He completed vocational training as a pipe-fitter but afterwards took various odd jobs, and at 18 he was discharged from military service because of ‘behavioural difficulties’. He was admitted to a mental hospital at 21 years with presenting symptoms of agitation, anxiety and impulsive behaviour and the diagnosis of ‘atypical mania’ was considered. Further examination revealed ambivalence, reasoning disturbances, inappropriate mood and feelings of strangeness, and the diagnosis of schizophrenic syndrome was considered. No neurological symptoms were recorded. The patient was given neuroleptic therapy and was discharged as improved. At the age of 23 he was again admitted to a mental hospital, where he presented in a delirious state with agitation and both auditory and visual hallucinations with persecutory content. Within 3 weeks, he had improved under therapy and was discharged on probation, but he moved soon
Fig. 1 Genealogical tree of the family M-E. Individuals are identified by their original record number. Generation 6, comprising persons at risk but not yet affected, has been omitted for confidentiality reasons.
afterwards to another part of France and was lost to follow-up. He was again examined at the age of 30, when his speech was nearly unintelligible because of severe dysarthria. No history of recent illness could be obtained. All aspects of memory were severely impaired. WAIS-R scores were uniformly under 45 and the MMS score was 17. Language was affected, with paraphasia, incoherent speech, loss of understanding and morphological paralexia, and he wrote in the script style. Neurological examination evidenced atactic gait, severe lack of co-ordination of the upper extremities and posture tremor. Myotactic and plantar reflexes were normal. Ocular motility examination showed hypometric saccades, square waves on fixation and the cogwheel phenomenon on pursuit, which was interpreted as indicative of associated cerebellar and extrapyramidal syndromes. MRI was interpreted as showing cortical and subcortical atrophy. The patient is alive 12 years after the first signs of the disease.

Patient 188 was another brother of the second proband and is now 31 years old. Three years ago, he presented with behavioural and cognitive disorders, mainly impaired attention, of which he was not conscious, and a specimen of his handwriting at the age of 29 showed an awkward, script style of writing. Recent neurological examination revealed a minor kinetic cerebellar syndrome with dysarthria. His MMS score was 23, his memory was severely impaired and there was limb-kinetic and ideational apraxia.

Patient 53 was the mother of the secondary proband, and she had previously performed her chores as a housewife satisfactorily. At the age of 42 years, she became unable to program a washing machine, to iron clothes and to cook. Walking and cycling became difficult and she fell frequently. Disorientation, impaired memory and emotional lability with inappropriate giggling were noted. A CT scan was interpreted as showing mild cortical and subcortical atrophy and the diagnosis of Alzheimer’s disease was considered. Deterioration progressed rapidly and she became quasi-mute, with occasional, aggressive speech, and was bedridden, with infrequent deambulation. On examination, she displayed a perplexed facies; dysarthria was massive, no contact could be established, and detailed cognitive evaluation was impossible. Neurological examination showed spastic and opposition rigidity, possibly due in part to neuroleptic medication. Tendon and mid-line reflexes were brisk and plantar reflexes were in extension. No cerebellar signs could be identified and biological investigations were non-contributive. She developed hyperthermia and died at the age of 43 years, 15 months after the first recorded symptoms. An autopsy was not performed.

Patient 57 was a brother of no. 51. He was examined aged 64 years, when he displayed neither neurological nor psychiatric symptoms. His MMS score was 30.

The medical histories of the patients described by Foncin and colleagues (Foncin et al., 1982) are summarized below; the alphanumeric between the brackets refers to the patient number in that paper.

Patient 37 (1) was the primary proband. His childhood was unremarkable. An able pupil, he qualified as a linotypist. His first symptoms were observed at the age of 32 years (difficulty in pointing to an object) and soon afterwards he displayed some gait disorders and memory loss. When he was aged 34 years, a psychologist diagnosed hypomania with an organic mood syndrome. He lost his employment as a linotypist and worked as a gardener’s assistant for 1 1/2 years. At the age of 35, a major cerebellar syndrome, with ‘motor discharges’ causing falls, was observed. EEG showed irregular paroxysmal discharges. Pneumoencephalography revealed a diffuse atrophy with cerebellar involvement. He was institutionalized when he was 36 and he became profoundly demented, with flexor rigidity of the upper extremities and extensor rigidity of the lower extremities. He died in cachexia with terminal hyperthermia at the age of 38 years, 7 years after the onset. A suspension of his formalin-fixed brain was inoculated intracerebrally into a rhesus monkey in April 1979 (Laboratory of Central Nervous System Studies, Bethesda, Md., USA). The animal showed no signs of neurological disease 3 years later, in April 1982.

Patient 33 (2) was the father of the primary proband and was affected with ‘the same disease’ as his son, as reported by his daughter-in-law. At age 43 years he was committed to a mental hospital, where he died 3 years later. Relevant medical records were destroyed by a flood. A clinical diagnosis of Pick’s disease was found in the autopsy room register, but the neuropathology records and preparations had been destroyed.

Patient 22 (5) was the mother of the preceding patient. She died at the age of 47 years, and was reportedly affected with ‘the same disease’.

Patient 45 (2e) was an aunt of the primary proband. She was committed to a mental hospital when she was aged 33 years, with the diagnosis of manic state. Massive dysarthria caused the diagnosis to be changed to that of general paresis, although the Wassermann reaction was negative and her pupillary reflexes were not documented. She died in cachexia at the age of 36 years and an autopsy was not performed.

Patient 43 (2c) was an aunt of the primary proband and great-grandmother of the secondary proband. She was committed to a mental hospital at the age of 34 years with the diagnosis of long-standing schizophrenia with motor instability. No mention of a neurological examination can be found in her records. She did not improve after electroconvulsive therapy, and died from pulmonary tuberculosis and cachexia 1 year later. An autopsy was not performed.

Patient 51 (2ca) was a daughter of the preceding patient and first cousin of the primary proband. She was a slow learner and left elementary school at the age of 14 years without a certificate of achievement. At the age of 17 years she gave birth to a daughter (father unknown), who was to be the mother (no. 53) of the secondary proband. She then worked on a succession of farms but her medical and occupational history are unknown between the ages of 29 and 33 years. At that time, she fell on the rail-tracks in the Paris subway; tentative suicide was suspected, and she was
committed to a suburban mental hospital. No personal or familial history could be obtained at this point and a diagnosis of mental deficiency was considered. Dysarthria was prominent and rigidity and frequent falls were noted. She became bedridden with flexor hypertonia of the upper extremities and extensor rigidity of the lower extremities. She died in cachexia with terminal hyperthermia at the age of 38 years, which was 5 years after the first recorded symptoms. An autopsy was not performed.

**Immunohistochemical analysis**

Serial sections of 7 µm were cut from blocks of formalin-fixed and paraffin-embedded cerebral and cerebellar brain tissues from patient 37, and were treated according to simple or double immunostaining methods. Pretreatment with 95% formic acid for 10 min, alone or followed by hydrated autoclaving at 120°C for 10 min, was used to enhance the immunostaining of PrP deposits. Two rabbit anti-PrP antisera, one raised against the hamster scrapie strain 263K (gift of P. Brown, National Institutes of Health, Bethesda, Md., USA) and the other against a synthetic peptide corresponding to residues 90-102 of the human PrP (gift of O. Bugiani, Istituto Neurologico Carlo Besta, Milan, Italy) were used at a dilution of 1:400. A third antibody, the monoclonal mouse antibody 3F4 (Senetek, Maryland Heights, Mo., USA), raised against the hamster scrapie strain 263K, was used at a dilution of 1:2000. Monoclonal mouse anti-GFAP (glial fibrillary acid protein) (Sigma, St-Quentin Fallavier, France), polyclonal rabbit anti-ferritin (Dako, Glostrup, Denmark) and monoclonal mouse anti-calbindin (Swant, Bellinzona, Switzerland) antibodies were used at dilutions of 1:300, 1:500 and 1:20000, respectively. For single-antibody immunohistochemistry, anti-PrP was visualized by the PAP (peroxidase–antiperoxidase) system and DAB (3,3′-diaminobenzidine) as a chromogen. For double immunostaining, anti-calbindin was visualized using the same PAP/DAB system, and anti-PrP was visualized using ABC (the avidin–biotin complex) (Vectastain, Vector Laboratories, Burlingame, Calif., USA) with biotinylated anti-rabbit or mouse antibody, alkaline phosphatase-conjugated streptavidin (1:100) (Vectastain; Vector Laboratories) and 5-bromo-4-chloro-3-indolyl phosphate and 4-nitroblue tetrazolium chloride as blue chromogen. Specificity of immunostaining was controlled by incubating sections with Tris buffer, or preimmune serum as a primary antibody, or by absorption of primary antibodies with synthetic peptides.

**Molecular genetic analysis**

After informed consent had been obtained, genomic DNA was extracted by standard techniques from the blood of four symptomatic members of the fifth generation (patients 56, 187, 188 and 190) and a healthy, 64-year-old first-degree relative, considered as an ‘escapee’ (no. 57) of family M-E. Several attempts to extract DNA from the archival formalin-fixed, paraffin-embedded brain tissue of patient 37 were unsuccessful. The coding sequence of the PRNP gene was amplified by the polymerase chain reaction (PCR) with the following two sets of primers:

- **PrP1** (5′-AACACATTCTCGACATTCTCTTTCA-3′, forward);
- **PrP2** (5′-AAGGATCCCTCAAGCTGGAAAAAGA-3′, reverse); and
- **PrP8** (5′-GATGCTGGTTCTTTGGCG-3′, forward)
- **PrP3** (5′-GATGGCGCTGCATCAGC-3′, reverse).

PrP1 and PrP2 were located 5′ and 3′ with respect to the coding sequence of the PRNP gene (Hsiao et al., 1989), whereas PrP8 and PrP3 were internal primers flanking the octapeptide-coding region (Laplanche et al., 1995). Thirty cycles of amplification were performed under the following conditions: initial denaturation at 94°C for 5 min then 94°C for 1 min/50°C (PrP1-PrP2) or 64°C (PrP8-PrP3) for 1 min/72°C for 1 min; and a final extension at 72°C for 5 min (Hybaid, Teddington, UK). The sizes of the amplified fragments were checked on a 6% polyacrylamide gel after staining with ethidium bromide and UV light transillumination. The amplified PRNP fragments from patient 190 were eluted from a 1% low-melt agarose gel and directly sequenced on both strands by the dideoxy chain-termination method (Epicentre Technology, Madison, Wis., USA) using an automated sequencer (ALFexpress; Amersham-Pharmacia Biotech, Uppsala, Sweden). Allele-specific amplifications of PRNP using primers with a 3′ end complementary to either codon-129 methionine or valine were conducted to confirm the genotype at this polymorphic codon.

**Results**

**Clinical studies**

The overall mean age at onset of symptoms was 28 years (range 21–34 years); in the last (fifth) generation the mean was 25 years (range 21–28 years). Remarkably, this fifth generation was younger at onset and had a longer psychiatric course before the manifestation of neurological symptoms (the total duration of the illness is still unknown; all the patients are still alive) than the previous generations. Neurological symptoms signalled the onset of the disease in only three instances, but were a constant feature at a later stage; they were mostly defined under the loose terms of ‘ataxia’ and ‘dysarthria’, but specific cerebellar symptoms, such as dysmetria, were recorded when the neurological examination was thorough. In one case (no. 43) the predominance of psychiatric symptoms induced a psychiatrist to dismiss a cerebellar syndrome as psychogenic ‘motor instability’. It was not clear whether the falls recorded in four of the cases should be ascribed to ataxia as part of the cerebellar syndrome or to ‘motor discharges’, as recorded in the primary proband. In these patients, rigidity dominated the terminal phase, with flexor hypertonus in the upper
extremities and extensor hypertonia in the lower extremities. Myoclonus, as observed in classical CJD, was not recorded in the M-E kindred, neither were periodic spikes in the EEG, although abnormal EEG was frequent. A peculiar feature, found in four instances, was the reversion of handwriting to the script style, which was previously taught in the first years of elementary school in France.

The most striking feature of the phenotype displayed by affected members of the M-E kindred is the importance of psychiatric symptoms, which led in six cases out of 11 to commitment to a mental hospital. Psychiatric diagnoses were diverse: mania (with or without qualification) has been considered at one time or another in four patients and schizophrenia was mentioned once. As a rule, the first psychiatric symptoms preceded the appearance of dementia by a long period (12 years in case 187), whereas dementia was the initial diagnosis in only two instances. The regular occurrence of psychiatric symptoms in the affected members of the M-E kindred indicates that they are a constituent of the phenotype; on the other hand, underachievement at school, possibly due to early cognitive deficiency, is present in three affected members of the M-E kindred but does not co-segregate with the overall phenotype and therefore seems unrelated.

Neuropathological studies
The neuropathological diagnosis of GSS in the M-E kindred still rests on observations made on the primary proband, no. 37. Further study of the material, aided by modern immunohistochemical techniques, affords new points of view, which are briefly described below. The classical neuropathology was characterized by lesions found mainly in the cerebellar cortex. Atrophy and gliosis of the molecular layer was moderate. The cerebellar cortex displayed kuru plaques and a few multicentric plaques without a neuritic component. These formations were best evidenced by periodic acid–Schiff staining. The ultrastructural study of post-mortem material showed uni- or multicentric plaques made of radiating amyloid-like fibrillary bundles, generally surrounded by microglial cells (Fig. 2A and B). Plaques appeared to be present in much larger numbers in the cerebellum when visualized by PrP immunohistochemistry than with conventional staining (Fig. 3A and B). They were associated with abundant microglial cells, as evidenced by an antiferritin antibody (Fig. 4B). Further, whereas classical morphology failed to reveal them, many plaques, mostly multicentric, were found in the neocortex by immunohistochemistry (Fig. 3C and D). Except for the plaques and some gliosis seen with GFAP immunostaining (Fig. 4A), cerebral cortical lesions were not prominent, and in particular there was no major cell loss. Spongiosis was mostly restricted to spherical bubbles in contact with plaques, with an occasional ‘daisy flower’ aspect (Fig. 4D), although it was more marked in the thalamus (Fig. 4C), where plaques were scarce.

Molecular genetics
Analysis on a 6% acrylamide gel of the expected 864-base pair (bp) (PrP1-PrP2) or 394-bp (PrP8-PrP3) PRNP amplified fragments revealed a one-allele insertion of ~200 bp in all subjects tested except no. 57 (Fig. 5). Direct sequencing of the longer allele from patient 190 revealed an insert of 192 bp
in the octapeptide coding region, introducing eight extra octapeptide coding repeats in addition to the five that are normally present (Fig. 6). In the normal allele, small differences at the nucleotide level identified the five regular repeats, which have been be abbreviated as R1-R2-R2-R3-R4 (Kretzschmar et al., 1986). R1 encodes a nonapeptide, whilst R2 to R4 each encode an octapeptide of the form P(H/Q)GGG(−G)WGQ. According to Goldfarb’s nomenclature (Goldfarb et al., 1991), the repeats of the mutant allele found in the M-E family were arranged as follows: R1-R2-R2-R3-R4.
R2-R2-R2-R2a-R2-R2-R3-R4. R2a was a variant of R2 with a silent G→A change at position 21 in the repeat. No known or new point mutations were found elsewhere in the PRNP coding sequence. Allele-specific amplifications confirmed that the insert was present within the codon-129 methionine allele. All patients examined were homozygous, with methionine/methionine at codon 129.

Discussion

The generic diagnosis of spongiform encephalopathy in the M-E family is defined by the neuropathological study of the primary proband, through the association of spongiform changes and PrP-immunoreactive plaques, in the absence of landmarks of Alzheimer’s disease (El Hachimi et al., 1996). A neuropathological phenotype of GSS, within the quasi-
continuum of spongiform encephalopathies, rests on: (i) abundant, often multicentric, PrP plaques; (ii) sparse spongiosis, seen mainly in the vicinity of plaques, with occasional daisy flower aspect, and in the thalamus in the near absence of plaques; and (iii) overall predominance of lesions in the cerebellum. Using immunohistochemistry, we failed to detect the elongated deposits that seem to be the hallmark of the four to seven extra repeats (Vital et al., 1998).

As intimated at the conclusion of this family analysis, dominant Mendelian transmission of the disease seems fairly straightforward in the M-E kindred, although the small number of patients precludes a quantitative discussion. Consistent with the dominant transmission of the disease, we found a one-allele, eight-octapeptide coding repeat insert (192 bp) in PRNP in four affected members of the M-E family, predicting the addition of 64 amino acids in PrP. Eighteen different insert mutations (with one or three to nine extra repeats), differing in the arrangement of the repeats or with nucleotide variations, have already been linked or associated with human inherited prion diseases (for reviews, see Goldfarb et al., 1996; Capellari et al., 1997). An eight-repeat insert has previously been described in two families, one with French ancestry (family Che from Brittany) and the other with Dutch ancestry (family A) (Goldfarb et al., 1991, 1992; van Gool et al., 1995), suggesting that the insert mutation was also pathogenic in the M-E family (Table 1).

Further, brain tissue from a patient of the Che family, with the same octapeptide motif inserted in PrP, caused transmission of the disease to a chimpanzee (Goldfarb et al., 1991). Although the nucleotide changes between the three inserts did not modify the amino acid sequencing of the octapeptides, DNA sequencing established that members of the M-E family carried a novel 192-bp insert in their PRNP gene and were unrelated to the two previous kindreds. This was confirmed by the fact that in families Che and A the inserted allele encodes a valine at codon 129, whereas it encodes a methionine in the M-E family.

The main characteristics of the disease in the three known families with an eight-octapeptide coding repeat insert in their PRNP gene are summarized in Table 2, and they highlight the prominent psychiatric symptoms seen in the present kindred. They were also different from the 'premorbid personality disorder' (aggressiveness, irritability, antisocial behaviour and hypersexuality) and the episodes of aggressive

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**Fig. 5** Acrylamide gel analysis of amplified fragments of the PRNP gene containing the octapeptide-coding region in five members of the M-E family (PrP5-PrP3 fragment; expected size 394 bp). Numbers refer to individuals identified in the genealogical tree in Fig. 1. M is a size marker (ΦX174/HaeIII).

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**Fig. 6** Nucleotide and amino acid sequences of the mutant PRNP octapeptide-coding region in the M-E family, starting at codon 51.
Table 1  Eight-octapeptide coding repeat insert mutations in PRNP

<table>
<thead>
<tr>
<th>Family</th>
<th>Origin</th>
<th>Insert</th>
<th>Amino acid 129</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Che</td>
<td>France</td>
<td>R1-R2-R3-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2a-R4</td>
<td>Valine</td>
<td>Golfarb et al., 1991, 1996</td>
</tr>
<tr>
<td>A</td>
<td>Netherlands</td>
<td>R1-R2-R3g-R3-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R3-R4</td>
<td>Valine</td>
<td>van Gool et al., 1995</td>
</tr>
<tr>
<td>M-E</td>
<td>France</td>
<td>R1-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R3-R4</td>
<td>Methionine</td>
<td>This paper</td>
</tr>
</tbody>
</table>

Coding repeats in the normal allele are R1-R2-R2-R3-R4. Extra repeats are in bold. In the M-E family it was not possible to determine if the insert introduced the first or the last R3 repeat. R3g = CCCCCATGGTGGTGGTTGGGGGCAG according to the nomenclature of Goldfarb and colleagues (Goldfarb et al., 1991). For the nucleotide sequence of R2a, see Fig. 6.

Table 2  Summary of the clinical and neuropathological features of the three families with an eight-octapeptide coding repeat insert in PRNP

<table>
<thead>
<tr>
<th>Family</th>
<th>Age at onset (years)</th>
<th>Disease duration</th>
<th>Clinical course</th>
<th>Neuropathology</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Che</td>
<td>45 (35–55)</td>
<td>2.3 years (3 months to 5 years)</td>
<td>Abnormal behaviour; cerebellar signs; mutism; pyramidal signs; hyper-reflexia; myoclonus; periodic EEG in one case</td>
<td>Spongiosis, gliosis; neuronal loss; multicentric (GSS) plaques</td>
<td>Goldfarb et al., 1991</td>
</tr>
<tr>
<td>A</td>
<td>43 (21–54)</td>
<td>3.3 years (5 months to 6 years)</td>
<td>Personality changes; gait disturbances; memory disorders; hypokinesia; rigidity</td>
<td>Mild spongiosis and gliosis; mild neuronal loss; multicentric plaques</td>
<td>van Gool et al., 1995</td>
</tr>
<tr>
<td>M-E</td>
<td>28 (21–34)</td>
<td>Patients deceased: 3.8 years (1–7 years) Patients alive: &gt;2 years &gt;4 years &gt;4.8 years &gt;12 years</td>
<td>Psychiatric symptoms; (mania or mania-like); cerebellar signs; dementia</td>
<td>Mild spongiosis; multicentric (GSS) plaques; microglial proliferation around plaques</td>
<td>This paper</td>
</tr>
</tbody>
</table>

behaviour and/or depression that have been observed in some patients from families with inherited prion diseases carrying six (Collinge et al., 1992) or eight extra repeats (Goldfarb et al., 1992a) in their PRNP gene. Psychiatric symptoms are also prominent in other forms of prion disease that are described as depression, such as new-variant CJD (Will et al., 1996; Zeidler et al., 1997).

Interestingly, the sole difference at the prion protein level between the three known families carrying an insert with eight extra repeats is the polymorphic residue 129 on the mutant allele, the M-E family having a methionine instead of a valine. The polymorphism at codon 129 is known to have an important bearing on inherited prion disease phenotypes. Spectacular instances in which codon 129 determines the phenotype are fatal familial insomnia and a particular familial form of CJD, both linked to the point mutation at codon 178 of PRNP (D178N) (Goldfarb et al., 1992b). Fatal familial insomnia and D178N CJD families carry the mutation on a methionine and a valine allele, respectively.

We noted an apparently earlier onset of the disease in the last (fifth) generation. In favour of a possible, but not statistically demonstrated, anticipation is the onset at the age of 32 years of a rapidly progressive dementia with neurological symptoms in patient 37, compared with the longevity of subjects 15 and 21 (the parents of patient 22, the first-generation patient), who died at the age of 77 and 74 years, respectively. This, however, could be due to non-paternity or to a de novo mutation in patient 22. It could also be explained by chance or better diagnosis in the later generations, or by the large stochastic variance of the age of manifestation of disease, as has been described in familial CJD linked to the E200K PRNP gene mutation in Libyan Jews (Goldfarb et al., 1990). The genotype at codon 129 may also play a part in the causation of the phenotypic variability, as reported in other inherited prion disease kindreds (Baker et al., 1991; Goldfarb et al., 1992; van Gool et al., 1995). It must be noted that up to now all the M-E kindred patients in whom this polymorphism is known have been homozygous for methionine/methionine and have displayed an early onset and a psychiatric phenotype.

From all these data, we may conclude that the spongiform encephalopathy, transmitted within the M-E kindred is indeed an instance of inherited prion disease associated with an
eight-octapeptide coding repeat insert (192 bp) in the PRNP gene. This family with the features of GSS (cerebellar symptoms and PrP multcentric plaques) is nevertheless characterized, in the last affected generation, by juvenile onset with psychiatric, often ‘maniac’, symptoms and a protracted course, and by the presence in the brain of occasional daisy flower plaques. Similarity of the phenotype, and particularly the psychiatric features, as seen in the fifth generation of the M-E family, to those described in new-variant CJD (Will et al., 1996) is striking (Foncin, 1996) and re-emphasizes the need for screening the PRNP gene for mutations in such a clinical context.

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