The German FFI Cases

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The German national CJD surveillance study was started in 1993. The primary aim of this study was to obtain reliable statistical data on sporadic CJD in Germany in order to assess possible changes in the incidence or prevalence of the disease and to identify possible new variants. Clinical and pathological diagnoses were made according to established diagnostic criteria (2). Analysis of the prion protein gene (PRNP) was performed in order to identify cases with hereditary prion disease. Various pathogenic mutations were found in 30 individuals. The constellation D178N-129M was identified in 8 individuals of 7 families. In addition, pathology and clinical changes in one additional case were typical of FFI. A detailed account of clinical and pathological features of this group will be published elsewhere (Zerr *et al*., submitted).

Materials and Methods

A total of 587 patients with clinically suspected CJD were seen in the German national CJD surveillance study between June 1993 and August 1997. These cases underwent a detailed neurological examination, data on the clinical course and technical investigations were gathered from hospital records.

Genetic analysis was performed in 424 cases from the CJD surveillance study and 72 cases were referred from elsewhere. Genomic DNA was extracted from blood or brain tissue according to standard procedures. The coding region of PRNP was amplified in the polymerase chain reaction (PCR) as described previously (9). The PCR product was inspected on a 1% agarose gel to detect potential insertion mutations and deletions. Potential point mutations were revealed by the single-strand conformation polymorphism (SSCP) technique (10, 12). Additionally, codons 129 and 178 of PRNP were examined by digestion with the restriction endonucleases *Nsp*I and *Tth*111I. The final sequence confirmation was obtained by solid-phase direct sequencing of the complete coding region of PRNP using an automated system (Model 4000L; LI-COR, Lincoln, NE). In the cases with heterozygosity at codon 129, and the pathogenic D178N mutation on one of the alleles, the amino acid at codon 129 on the mutated allele was determined by the simultaneous digestion of the complete coding region of PRNP with *Mae*II and *Tth*111I.

H&E stains and immunohistochemistry for PrP using monoclonal antibodies 3F4 and Gö138 (2) were performed in all cases where brain autopsy or biopsy material was available. The whole brain was available in only two cases, whereas in four cases a limited number of paraffin embedded tissue blocks was available.

Results

Of the 587 suspect cases collected in the German CJD surveillance study from June 1993 to August 1997, the diagnosis of definite or probable CJD was made in 251 cases, 113 were classified as possible CJD, and in 200 suspect cases the diagnostic criteria for CJD were not met.

Genetic analysis identified mutations in the PRNP gene in 30 cases. Among these, a D178N mutation was found in combination with homozygosity for methionine at codon 129 in six cases as revealed by digestion with *Nsp*I and direct DNA sequencing. Two other cases with a D178N mutation were heterozygous at codon 129. Methionine in coupling with D178N was shown by simultaneous digestion of the amplified PRNP fragment with *Mae*II and *Tth*111I. A fragment of 152 bp was diagnostic for the normal allele having valine at codon 129, and was detectable in the digestion products of both patients. In addition, pathology and clinical changes in one patient in which genetic analysis could not be performed were typical of FFI. The nine cases of FFI belonged to eight apparently unrelated families (see table 1). D178N-129M is the most common pathogenic PRNP mutation found in the German CJD surveillance study.

The clinical features observed in these patients will be described in more detail elsewhere (Zerr *et al*., submitted). In contrast to the FFI cases first reported (5) none of the patients described here complained of severe insomnia in the early stages of the disease. Autonomic disturbances such as hyperhydrosis, hyperthermia, tachycardia and hypertension were observed in varying degrees in most patients. These were sufficiently prominent to lead to further clinical investigation in only one case. Serial EEG tracings were studied from each

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Case	Year of Onset	Age at Onset (years)	Duration of Disease (Months)	Sex	Codon 129	Family History as Reported by Relatives	Pathology
1	1993	59	10	m	Met/Val	no	unusual (see text)
2	1993	51	12	f	Met/Met	no	typical of FFI
3	1994	57	16	f	Met/Met	yes (affected brother died at age of 53	typical of FFI
4	1995	44	9	m	Met/Met	no	N.A.
5	1996	67	9	m	Met/Met	possibly affected sister	typical of FFI
6*	1996	53	8	m	N.A.	no	typical of FFI
7	1996	70	9	f	Met/Met	yes (belongs to same large family as case 8	typical of FFI
8	1996	48	N.A. (alive)	f	Met/Val	yes (c.f. case 7)	N.A.
9	1996	59	N.A.	f	Met/Met	no	N.A.

* genetic analysis was not possible in case 6

Table 1.

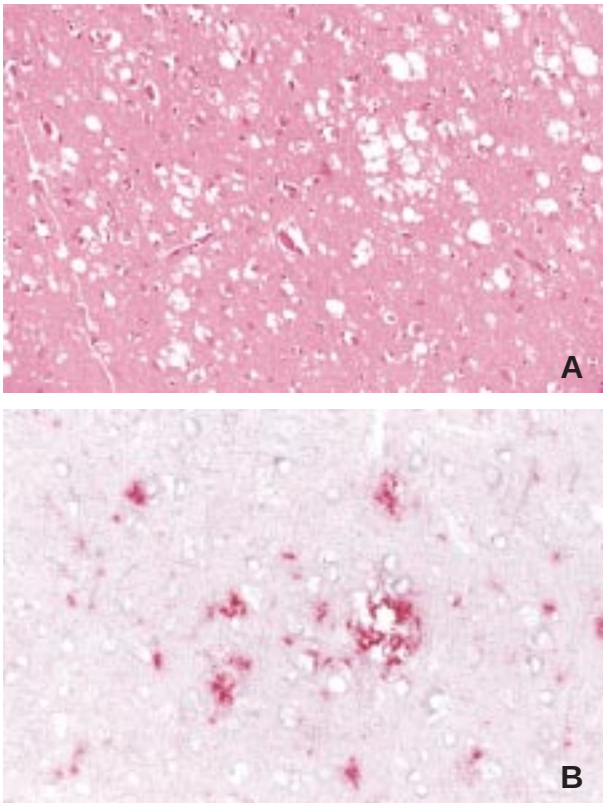

Figure 1. A. Medium-sized and in parts coalescing vacuoles are seen in the deep cortical layers of patient 1 who had a D178N mutation and was heterozygous at codon 129. H&E, x 200. **B.** Immunohistochemistry with antibody Gö138 shows intense coarse and perivacuolar staining in this case. Prion protein deposits were most prominent in cortical layers 2/3 and 6, x 400.

patient. Periodic sharp wave or slow wave complexes were not noted in any of the patients.

Neuropathological studies were performed in 6 cases (table 1). A consistent histological picture was found in 5 cases which corresponded very well with the typical changes described in the literature. These included severe nerve cell loss and astrocytic gliosis in the thalamus and the inferior olivary nucleus. In the inferior olive, neuronal loss was virtually complete in some cases, while in others it was more pronounced in the dorsal part. Mild spongiform changes were noted consistently in the entorhinal cortex and subiculum. In the neocortex, most cases showed mild astrocytic gliosis. However, spongiform change was either sparse or absent. The striatum did not show significant changes. The cerebellum was unremarkable. Immunohistochemistry for PrP did not show unequivocal staining. In one case (patient 1) neuropathological examination showed changes that were more reminiscent of forms of sporadic CJD (2, 11). There were marked spongiform changes and gliosis in all cortical areas investigated with most intense changes in the temporal and frontal cortex. Spongiform change exhibited a distinct laminar distribution with large and often confluent vacuoles in the deep cortical layers (Figure 1A). Immunohistochem-istry showed intense coarse and perivacuolar staining for PrP (Figure 1B) which was pronounced in cortical layers 2/3 and in cortical layer 6. Additionally, coarse PrP staining was found in the dentate gyrus and to a lesser extent in the hippocampus proper. In the cerebellum, no pathological changes apart from mild proliferation of Bergmann glia were found in H&E stained sections. Immunohistochemistry for PrP showed rare coarse deposits of PrP in the molecular layer. The thalamus and the brainstem were not available for analysis. In this case, no family history was reported by the relatives, however, the patient's father died at the age of 34 from a sepsis.

Discussion

Among 30 patients with different mutations of the prion protein gene that were identified in the German CJD surveillance study, 8 had the constellation D178N-129M which is typical of FFI. This mutation is the most

common PRNP mutation in the German population. The cases of FFI described by us were identified in the course of a study aimed primarily at identifying cases of non-hereditary CJD. It can therefore be speculated that the incidence of patients carrying the D178N-129M mutation in Germany is even higher. A D178N-129V case, which is associated with familial CJD, was only identified in a historic case from the 1920s (3). This was the first patient with a reported familial form of CJD (6).

A family history of neurodegenerative disorder was reported by family members in only four of the nine patients from eight families reported here. The clinical presentation was thought to be typical of sporadic CJD in five patients and the identification of a PRNP mutation came as a surprise. The clinical diagnosis of FFI was considered in only one case. Sleep disturbances in general were mild and were most often recognized only in retrospect by detailed questioning of the family or reinvestigation of the hospital records. Single cases with the typical PRNP gene constellation and only minimal sleep disturbance have also been reported by others (1, 4, 8). Taken together with our findings, this suggests that sleep disturbances may be inconspicuous in many cases and may become apparent only in retrospect by detailed questioning. It is noteworthy that in the cases of sporadic CJD identified in the German CJD surveillance study, sleep disturbances were noted at some stage of the disease in nearly 30%. In later stages of the disease, all patients with a detailed clinical history developed dementia and neurological signs and symptoms which are typical of sporadic CJD.

We were able to study brain autopsy material in 6 cases. The typical FFI- associated pathology was seen in five brains. One brain displayed changes that were more typical of sporadic CJD, i.e. widespread spongiform change with confluent vacuoles and perivacuolar PrP deposition. Unfortunately, frozen brain material is not available from this patient, so that it will not be possible to perform a Western blot study to identify the PrP type in this case and confirm whether it corresponds to the typical PrP pattern observed in FFI or to one of the PrP forms found in sporadic CJD (7, 11).

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