Brain Pathology 8: 515-520 (1998)

Clinical Features of Fatal Familial Insomnia: Phenotypic Variability in Relation to a Polymorphism at Codon 129 of the Prion Protein Gene

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Fatal Familial Insomnia is a hereditary prion disease characterized by a mutation at codon 178 of the prion protein gene cosegregating with the methionine polymorphism at codon 129 of the mutated allele. It is characterized by disturbances of the wake-sleep cycle, dysautonomia and somatomotor manifestations (myoclonus, ataxia, dysarthria, spasticity). PET studies disclose severe thalamic and additionally cortical hypometabolism. Neuropathology shows marked neuronal loss and gliosis in the thalamus, especially the medio-dorsal and anterior-ventral nuclei, olivary hypertrophy and some spongiosis of the cerebral cortex. Detailed analysis of 14 cases from 5 unrelated families showed that patients ran either a short (9.1+ 1.1 months) or a prolonged (30.8 + 21.3 months) clinical course according to whether they were homozygote met/met or heterozygote met/val at codon 129. Moreover, homozygotes had more prominent oneiric episodes, insomnia and dysautonomia at onset, whereas heterozygotes showed ataxia and dysarthria at onset, earlier sphincter loss and epileptic Grand Mal seizures; they also displayed more extensive cortical involvement on PET and at postmortem examination. Our data suggest that the phenotype expression of Fatal Familial Insomnia is related, at least partly, to the polymorphism at codon 129 of the prion protein-gene.

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Introduction

The clinical features of Fatal Familial Insomnia (FFI) were already described by us in individual patients from two unrelated Italian kindreds (11). We summarize here the clinical features of the disease as observed in 14 cases from three Italian families and in two additional families from France (9). We relate here the symptoms and signs to the clinical course of the disease and to the genetic background, in particular the 129 codon polymorphism of the prion protein gene (PRNP).

Material and Methods

All patients were directly monitored in hospital for extended periods of time, sometimes throughout illness duration until death, and as early as possible from onset. In order to ensure clinical consistency, patients and relatives were interviewed by the same physicians, and clinical notes, diaries and indirect reports from other institutions, though available and scrutinized, were not used for this reconstruction of the clinical features of the disease.

Three Italian unrelated families (FFI 1-3), coming from different regions of the country (Tuscany, the Venetian and Neapolitan areas), were characterized. FFI 1 comprised 11 certainly affected individuals, while FFI 2 and 3 included 3 and 1 respectively. The disease was transmitted in an autosomal dominant way, affecting males and females equally. A total of 10 Italian patients were examined, 6 belonging to FFI 1, 3 to FFI 2 and 1 to FFI 3. All of them except two, still living, died and underwent post-mortem studies. Four additional patients coming from 2 families, unrelated to the Italian ones, were observed in France. They also died and underwent post mortem examination.

Severe intractable insomnia was considered the initial symptom of disease. Patients were admitted as early as possible from onset, and were then readmitted at regular intervals of two to three months in order to monitor the disease course. On admission, after a detailed physical examination, patients were genetically characterized with respect to the 178 codon mutation characteristic of FFI (12) and the 129 codon polymorphism of the PRNP gene, and underwent standard blood and CSF studies. EEG, 24-hour videopolygraphic recordings,

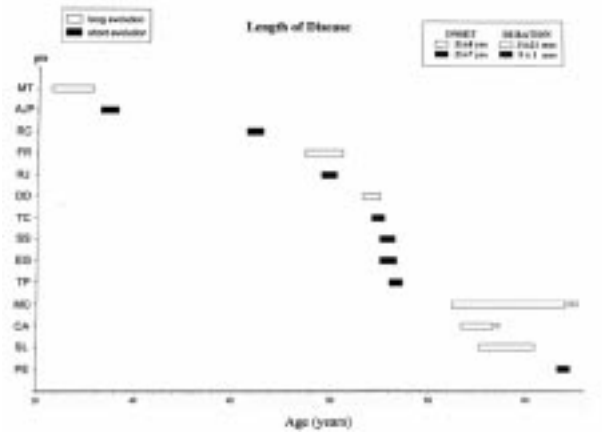

Figure 1. Onset and duration of disease in 14 patients with FFI arranged according to 129 codon polymorphism (black bars: 129 met/met; open bars: 129 met/val). Patients MC and CA are still living.

aimed at the definition of sleep patterns, and studies of autonomic and endocrine functions exploring the regulation of cardiovascular reflexes and catecholamines and hormonal (GH, PRL, ACTH, cortisol, LH and FSH, melatonin) incretions, throughout the wake-sleep cycle, were performed under polygraphic monitoring. Neuropsychological investigations included mental deterioration batteries and behavioral tests. All the patients underwent brain CT/MRI scans, and 7 Italian patients PET studies performed using (18F)-2-fluoro-2-deoxy-D-glucose (FDG). Clinical symptoms and signs and laboratory findings were analyzed for each patient according to a standardized protocol at three observation times: at onset (within the first month of disease), midway (at half of the total disease course) and terminally (within 1 month from death or last follow-up if still alive).

Results

All 14 patients had the 178 codon mutation of the PRNP gene, with the 129 codon specifying for methionine (met) on the mutated allele. Of them, 8 were 129 met/met homozygotes (i.e. met in the non mutated allele), while 6 were 129 met/val heterozygotes (i.e. val in the non mutated allele).

Clinical course and neurological symptoms and signs. In the 14 patients analyzed, the disease arose at a mean age of 51 ± 7.1 years (range 36-62), and there was no significant difference between patients who were met/met homozygotes (50.9 ± 6.8 yrs, range 38-62) or met/val heterozygotes (51.2 ± 8.1 yrs, range 36-58) at codon 129. In contrast, disease duration (18.4 ± 17.3 months as a mean) differed significantly between the two groups, being 9.1 ± 1.1 months (range 8-11) until death in the eight 129 met/met cases versus 30.8 ± 21.3 months (range 11-72) in the six 129 met/val patients (Fig. 1). Two of the latter are still living, one 72 and the other 22 months after onset. These differences could not be accounted for by different medical care since hospitalization time was comparable in both groups and actually longer for the 129 homozygotes patients.

Clinical symptoms and signs were comparable among the different patients and families, including sleep-wake and vigilance disturbances (insomnia, oneiric stuporous episodes with hallucinosis and episodic confusion), altered autonomic functions (hypertension, irregular breathing, diaphoresis, pyrexia, urinary and fecal incontinence, impotence), and somatomotor manifestations (diplopia, dysarthria and dysphagia, ataxia/abasia, dysmetria, spontaneous and evoked myoclonus, spasticity); tonic-clonic seizures were observed in some cases. We analysed clinical symptoms and signs for each patient according to the 129 codon polymorphism and found that: 1) 129 met\met patients were the only ones to display prominent oneiric episodes with hallucinosis and episodic confusion upon disease onset, and, at the same time, spontaneous and evoked myoclonus and more obvious autonomic alterations (irregular breathing, hypertension); these features occurred rather later in the 129 met/val group. 2) Patients 129 met/val were characterized by ataxia and dysarthria already at disease onset; these somatomotor disturbances worsened subsequently and remained always more severe in these patients, leading to a bedridden state midway in the disease course; 129 met/val patients were the only ones to need artificial feeding already at this stage of the disease. 3) Patients 129 met/val also suffered sphincter impairment (urinary and fecal incontinence) earlier that the 129 met/met ones, already midway in the disease, and were nearly the only ones to display tonic-clonic seizures, which were observed in only one out of the 8 129 met/met cases.

Neuropsychological disturbances. Patients were studied in regard both of mood and behavioural alterations, and with neuropsychological tests exploring vigilance, attention, intelligence, memory, and constructional, visuoperceptive and motor abilities(6).

The social behavior of the patients remained normal, cooperation was good and personal relationships were maintained until late in the disease. Intellectual functioning also remained normal, with good results at IQ

tests as long as these could be performed (6, 7). The main disturbances consisted in altered vigilance with frequent and spontaneous intrusions of dream-like states into normal wakefulness (oneiric stupors), from which the patients could be easily awakened early on in the disease, but which later led to progressively decreased vigilance levels with stupor and eventually coma and terminal akinetic mutism state. Attention was especially impaired, in the early stages, even more than vigilance, and all patients displayed difficulty in sustained attention, being easily distracted with fluctuations in performance, especially in more complex tasks. Tests also showed altered encoding and defective manipulation of information and incorrect ordering of events (chronoataraxia). Therefore working memory was particularly impaired, whereas semantic, retrograde and procedural memory remained unaffected. There was no significant difference in neuropsychological tests between 129 met/met homozygotes and 129 met/val heterozygotes patients.

Given the normal intellective tests and preserved behaviour, and the prominent vigilance alterations, our patients did not fulfill standard criteria for the diagnosis of dementia. Thus, we argue that FFI should not be defined as a dementing, but rather as a confuso-oneiric illness (7).

Autonomic and endocrine disturbances. Patients were studied in a temperature controlled (23+1°C) room with continuous monitoring of systemic blood pressure (BP), heart rate (HR) and respiratory rate. The head-up tilt test (10 min at 65°) was performed after 30 min of supine rest and blood samples drawn to measure norepinephrine (NE) and adrenaline (A) by high performance liquid chromatography with electrochemical detection. Pharmacological tests included infusion of scalar doses of NE (12.5 to 150 ng/kg/min) and clonidine (2 μg/kg).

In all patients, BP, HR and NE resting plasma levels were higher than in normal age and sex matched controls. Likewise, BP and NE plasma levels increased disproportionately during the head-up tilt test. Intravenous NE infusion induced a blunted pressor response with a normal HR response, suggesting an adrenoreceptor down-regulation with intact baroreflex function; the depressor effect of clonidine as well as its ability to reduce the spill-over of NE were reduced. Overall these results indicated sympathetic overactivity in all patients. Follow-up studies documented increasing changes throughout the disease course (4). A comparison of four 129 met/met versus five 129 met/val patients studied in greater detail showed that the former usually had more severe sympathetic hyperactivity, especially higher basal NE levels (675 ± 220 vs 390 ± 240 pg/ml) upon head-up tilt test; given the small numbers, however, the differences were not statistically significant.

Circadian studies of BP and HR rhythms showed that the nocturnal blood pressure fall was lost early on in the disease, while the physiological bradycardia was still preserved and the rhythmic component persisted, although with a reduced amplitude and shifted phase, even in the absence of recorded sleep. Rhythmicity was abolished in the terminal stages only. Body core temperature was likewise persistently elevated (10) (Fig. 2).

Circadian catecholamine rhythms were essentially preserved in the early stages of FFI, but increasing mean plasma levels and decreasing circadian amplitudes marked the progression of the disease, up to a total loss of rhythms in the terminal stages. Cortisol levels were high, whereas ACTH remained at normal levels; abnormal nocturnal peaks were detected in the circadian rhythms of these hormones, all findings suggesting a condition of hypercortisolism added to a functional dysregulation of the hypothalamic-pituitary-adrenal axis. Melatonin incretion showed a gradual decrease in circadian amplitude and a shift in phase, until an eventual complete rhythm loss (Fig. 2). Somatotropin also showed a reduced or even absent rhythmicity, which, however, paralleled the loss of deep sleep, whereas prolactin rhythmicity remained unaltered.

Neuroradiological and PET studies. All patients underwent standard brain CT and MRI studies, which disclosed cerebral and cerebellar atrophy and ventricular dilatation only in those patients with a prolonged course. (18F)-FDG Pet studies were performed in seven patients, four of whom were 129 met/met homozygotes. Glucose utilization was severely reduced in the thalamus and to a milder degree in the cingular cortex in all cases. Additional glucose hypometabolism was also found in the basal and lateral frontal and middle and inferior temporal cortices, and in the caudate nuclei, in six patients. 129 met/met homozygotes cases had less metabolic involvement, restricted to the thalamus, basal frontal and cingular cortex, whereas 129 met/val cases showed additional severe hypometabolism of the hippocampus, putamen and caudate (5). Since the latter were examined at later stages, the more widespread involvement may reflect diffusion of the disease process with time.

Neurophysiological and polysomnographic studies. Routine EEGs did not show specific alterations in any of

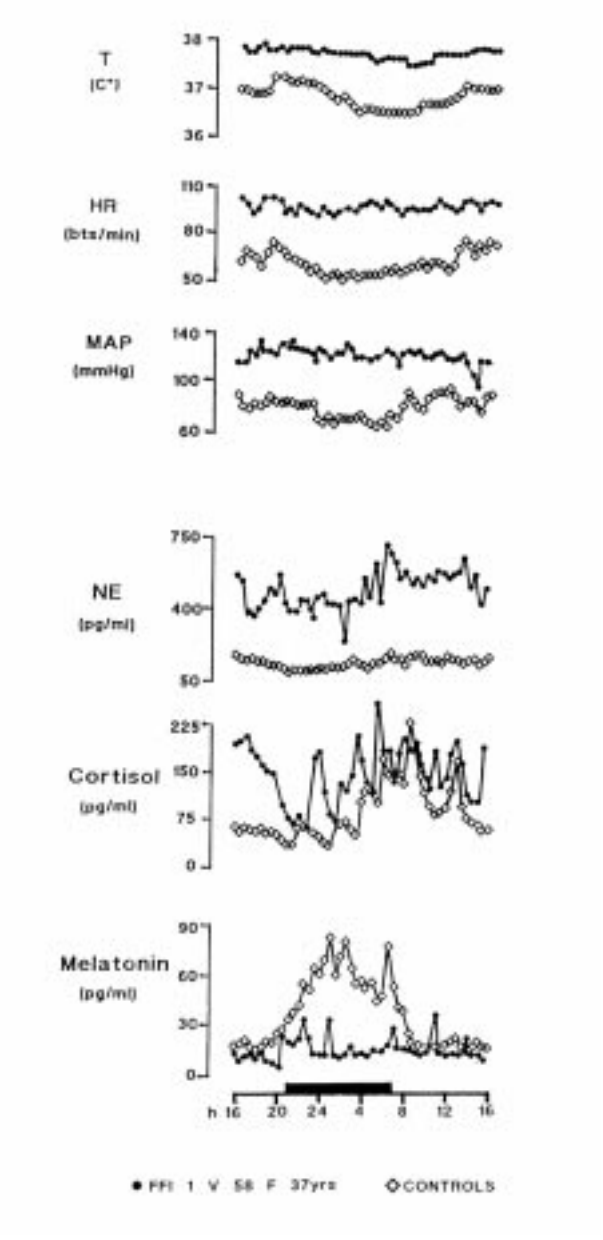

Figure 2. 24-hour circadian rhythms of body core temperature (T), HR, mean arterial pressure (MAP), norepinephrine (NE), cortisol and melatonin in a FFI 129 met/val patient compared to a normal sex and age-matched control. The black bar indicates light-out time during night.

the patients at onset. As the disease progressed, especially in the terminal stages, the background EEG activity progressively changed to a monomorphic flat activity. Several months after onset and almost exclusively in the long duration cases (except one 129 met/met case, 20 days before death), bursts of repetitive diffuse 1-2 Hz periodic sharp waves appeared, associated with clinically and polygraphically evident periodic myoclonias. On 24-hour videopolysomnographic recordings, two patterns emerged:

1. absence of normal sleep since the very onset of the disease with substantially normal wakefulness activity; the latter was, however, interrupted by periods during which patients were unresponsive and manifested peculiar motor activity in the form of rapid twitching of one or more limbs, utterance of words or sentences, and finalistic complex gestures such as sitting-up in bed, combing the hair, giving a military salute, sewing or threading a needle which, when the patient was subsequently awakened by an external random or provoked stimulus, could be seen to correspond to an intrinsic oneiric content reported by the patient during the episode. When fully awake and in contact, patients complained of increasing generalized fatigue, and would try to fall asleep or could be seen to occasionally nod or close their eyelids, as if trying to doze or sleep, but in vain. Deep lasting sleep could not, in fact, be attained. Such a behaviour explains why patients could be defined as “sleepy” in other settings. On polygraphy, patients displayed an EEG pattern characterized by brief and continuous oscillations between a diffuse alpha activity, typical of relaxed wake (W), and bursts of desynchronized theta activity. The hallmarks of stage two sleep, spindles and K-complexes, disappeared since the early stages of the disease. In this transitional state the patients presented prolonged oneiric behaviors, and when stimulated, they often looked drowsy and confused. Non-REM sleep was abolished and only brief REM sleep episodes occurred, often in clusters, and associated with oneiric behavior. The patients spent most of their time in a non-wake-non-sleep subwakefulness state characterized by a mixture of alpha and theta EEG activity similar to stage I non-REM sleep (Fig. 3 subW). REM sleep could present a normal pattern, with desynchronized low voltage theta activity on EEG in the presence of physiological EMG atonia. However, short, 10 to 20 seconds, REM sleep periods could abnormally emerge directly from wakefulness and recurred periodically, every 10-30 seconds (Fig. 3). REM sleep could otherwise present with a dissociated pattern, with persistent axial tone, and flow into a complex behavioral state due to the absence of physiological muscle atonia, increase in muscle tone, and intensified myoclonic jerks, during which the patients enacted their dream. This state resembled the REM sleep behavior disorder or status dissociatus seen in some other neurodegenerative diseases. Spectral analysis performed on the 24-

hour EEG recordings, confirmed that, in these cases, there was nearly total, or total, absence of deep sleep, that is of EEG activity in the range below 4 Hz, throughout the 24 hours. Progression of the disease was marked by more and more prolonged episodes of abnormal REM sleep, during which the patients became ever less arousable and progressively more unable to report any oneiric activity. Thereafter, patients could die suddenly or lapse instead into a short terminal coma with slow flat monomorphic EEG activity preceding death.

2. In other cases, 24-hour recordings at the onset of the disease were characterized by a relative preservation of the cyclic structure of nocturnal sleep (Fig. 3) and by the persistence of slow (<4 Hz) EEG activity, typical of slow-wave sleep. However, REM sleep often showed the characteristic lack of physiological muscle atonia, and oneiric activity was present, though less prominent. Sleep spindles and K-complexes, preserved on the earlier stages of disease, went on, however, to progressively disappear, and EEG progressively slowed to an unreactive monomorphic theta/delta in the terminal stages.

All of the patients displaying the first type of sleep-wake alterations were found to be 129 met/met homozygotes, whereas the second pattern was typical of the 129 met/val heterozygotes patients.

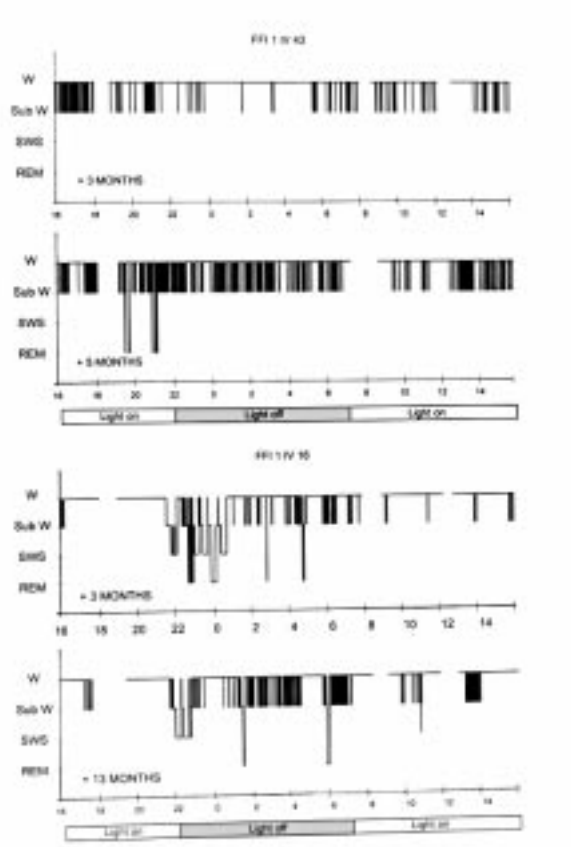

Figure 3. 24-hour wake-sleep histograms of 2 FFI patients (FFI 1 IV-43 129 met/met, and FFI1 IV-16 129 met/val), recorded 3 and 5, and 3 and 13 months after onset respectively. W indicates wake, Sub W subwakefulness, SWS slow-wave sleep and REM rapid eye movement sleep.

Conclusion

We have shown that FFI patients may run either a short, less than 1 year, or a long clinical course until death. Clinically, the patients with a short-lasting disease course are also characterized by more prominent oneiric episodes, insomnia, and vigilance and autonomic disturbances at onset, while patients with a long disease duration show ataxia and dysarthria at onset, earlier sphincterial impairment and are almost the only ones to suffer epileptic grand mal seizures and to show periodic spikes on the EEG. These different clinical features are related to differences in brain metabolism and pathological findings. In particular, PET scans disclosed a more severe and widespread glucose hypometabolism of the cerebral cortex and basal ganglia in cases with long disease duration. Since the latter patients also had more severe spongiosis, gliosis and neuronal loss in the cerebral cortex and cerebellum at postmortem examination (8), this more widespread cortical involvement may explain why patients with long disease duration have periodic EEG spikes and epileptic seizures, and earlier and more obvious cerebellar clinical signs (dysarthria, ataxia). It remains, however, clinically puzzling why those patients with less metabolic and neuropathological alterations, nearly restricted to the thalamus, are the ones who die earlier.

Overall, the two clinical pictures in FFI may be said to involve the same type of clinical and laboratory changes, but to a different degree of severity and especially in a different disease duration between the two groups of patients. Though some findings may just be the consequence of the more prolonged clinical course, why the duration of disease differs so much remains unexplained. A possible explanation came from the genetic characterization of the patients in regard to the 129 codon polymorphism, showing that all the cases with short disease duration were homozygotes met/met whereas cases with long disease duration were heterozygotes met/val.

Codon 129 of the PRNP gene is polymorphic: in the normal Caucasian population 37% are met/met, 12% val/val homozygotes and 51% met/val heterozygotes

(13). Codon 129 has been shown to influence the susceptibility to iatrogenic CJD (3); met or val homozygosity are respectively 1.6 and 2 times more prevalent, while met/val heterozygoty is 3 times less prevalent in sporadic CJD (14). Codon 129 has also been shown to influence the age of onset of disease in families with the 198 PRNP mutation and with the 144 bp PRNP insertions (1).

Codon 129 has been hypothesized to influence the interallelic conversion of PrP into PrPres: conversion would occur more readily in 129 homozygotes because of higher allelic homology and more rapid accumulation of the pathogenic PrPres. Furthermore, in CJD with insertional octapeptide repeat mutations, PrPres consists of both mutant and wild-type PrP (2), an indication that the non mutated PRNP allele also participates in the pathogenesis of the disease. However, PrPres accumulation in FFI is actually less in 129 met/met homozygotes (15), and only PrP deriving from the mutant allele is accumulated as either detergent-insoluble or PrPres (2). Thus, the participation of wild-type PrP in the formation of PrPres seems unable to fully account for the phenotypic variability of FFI that we have shown in our cases. Other pathogenic mechanisms, implicating the PRNP normal allele, must be sought.

Acknowledgement

We wish to thank Ms A. Laffi and Ms A. Collins for assistance with the manuscript. Supported by Biomed 2 BMH4 - CT96 - 0856 and CNR 96/5253 grants.

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