Pre-symptomatic diagnosis in fatal familial insomnia: serial neurophysiological and $^{18}$FDG-PET studies

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Knowing how and when the degenerative process starts is important in neurodegenerative diseases. We have addressed this issue in fatal familial insomnia (FFI) measuring the cerebral metabolic rate of glucose (CMRglc) with $2-[^{18}F]$fluoro-2-deoxy-D-glucose PET in parallel with detailed clinical, neuropsychological examinations and polysomnography with EEG spectral analyses. Nine asymptomatic carriers of the D178N mutation, 10 non-carriers belonging to the same family, and 19 age-matched controls were studied over several years. The CMRglc as well as clinical and electrophysiological examinations were normal in all cases at the beginning of the study. Four of the mutation carriers developed typical FFI during the study but CMRglc and the clinical and electrophysiological examinations remained normal in all cases before disease onset. The carrier whose tests were normal 32 months before disease onset was re-examined 13 months before the onset. At that time, selective hypometabolism was detected in the thalamus while spectral-EEG analysis disclosed an impaired thalamic sleep spindle formation. Following clinical disease onset, CMRglc was reduced in the thalamus in all 3 patients examined. Our data indicate that the neurodegenerative process associated with FFI begins in the thalamus between 13 and 21 months before the clinical presentation of the disease.

Keywords: fatal familial insomnia; $^{18}$FDG-PET; pre-symptomatic diagnosis; thalamus

Abbreviations: $^{18}$FDG-PET = $2-[^{18}F]$fluoro-2-deoxy-D-glucose PET; FFI = fatal familial insomnia; PrP = prion protein; rCMRglc = regional cerebral metabolic rate of glucose


Introduction

Fatal familial insomnia (FFI) is an autosomal dominant prion disease clinically characterized by alterations of the sleep-wake cycle, dysautonomia and motor signs (Lugaresi et al., 1986; Medori et al., 1992). The histopathological hallmark of FFI is severe neuronal loss especially in the anterior-ventral and medial-dorsal nuclei of the thalamus associated with a variable involvement of the inferior olive, striatum and cerebellum (Manetto et al., 1992). In addition, a mild to moderate spongiform degeneration is present in the cerebral cortex of subjects with the longest disease duration (Parchi et al., 1995). FFI is transmissible and is linked to a mutation at codon 178 of the prion protein gene (PRNP), replacing aspartic acid with asparagine, and to the presence of the methionine codon at position 129 of the mutant allele (D178N-129M haplotype) (Goldfarb et al., 1992). A sporadic form of FFI, named sporadic fatal insomnia, has also been reported (Mastrianni et al., 1999; Parchi et al., 1999; Montagna et al., 2003). FFI presents between 20 and 72 years of age (mean 49 years) and has either a ‘short’ duration (mean 11 ± 4 months) or a ‘long’ disease course (mean 23 ± 19 months) (Cortelli et al., 1991; Gallassi et al., 1992; Goldfarb et al., 1992; Manetto et al., 1992; Perani et al., 1993; Portaluppi et al., 1992).
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1994a; Portaluppi et al., 1994b; Portaluppi et al., 1995; Sforza et al., 1995; Gallassi et al., 1996; Cortelli et al., 1997; Harder et al., 2004; Kong et al., 2004). While the age at onset is unrelated to sex or PRNP codon 129 genotype (Goldfarb et al., 1992), the duration of the disease correlates with the genotype at codon 129 of the non-mutated allele, since patients who are methionine/methionine (M/M) homozygous at codon 129 have a shorter duration on average than those who are methionine/valine heterozygous (Goldfarb et al., 1992; Chen et al., 1997; Kong et al., 2004).

Tests using 2-[18F]fluoro-2-deoxy-D-glucose PET (18FDG-PET) have consistently demonstrated glucose hypometabolism in the thalamus and cingulate cortex of M/M homozygous patients in the early phase of the disease, while the involvement of other brain regions depends on disease duration (Perani et al., 1993; Parchi et al., 1995; Cortelli et al., 1997). Comparison between neuropathological and 18FDG-PET findings in six FFI patients showed that although hypometabolic areas and areas with neuronal loss co-distributed extensively, the hypometabolism was actually more widespread than neuronal loss and significantly correlated with the presence of protease-resistant prion protein (PrP) (Cortelli et al., 1997). The thalamic hypometabolism demonstrated by 18FDG-PET is also a marker of sporadic fatal insomnia (Mastrianni et al., 1999; Montagna et al., 2003).

A puzzling feature that FFI shares with other familial neurodegenerative diseases is that the clinical onset generally occurs at a mature or advanced age although the mutation is congenital, leaving an ample interval free of clinical signs during which the pathogenic effect of the mutation is unknown. In the present study, we made serial assessments of glucose metabolism and sleep electrophysiology along with detailed clinical evaluations in carriers of the D178N-129M haplotype linked to FFI, who were clinically asymptomatic at the time of the initial evaluation. Four subjects became affected in the course of the serial assessments. We report on the time and anatomical location of the initial detectable effect of the FFI-linked D178N-129M haplotype on brain metabolism and electrical function that may help in planning future preventive treatment.

Subjects and methods

Nineteen members of an Italian FFI family (family kindred and genealogical tree published in Cortelli et al., 1999) were included after approval of the study by the local ethics committee. All subjects gave informed consent to participate in the study according to the Declaration of Helsinki but declined to know their genetic status. Details of the clinical findings and genetic analysis of this family have already been published (Lugaresi et al., 1986; Medori et al., 1992; Montagna et al., 1998). The subjects were divided into two groups: non-carriers of the D178N-129M PRNP mutation comprising ten individuals (aged 44 ± 17 years, range: 21–69 years, 2 women) and asymptomatic carriers comprising nine individuals (aged 44 ± 13 years, range: 25–65 years, 3 women). We defined as asymptomatic subjects who did not report neurological complaints, reported normal ability to sleep and were normal upon neurological examination. All subjects had a normal brain CT scan performed with thin slices of 3 mm at the beginning of the study (1993). The demographics at this time and genetic findings are summarized in Table 1. In addition, we used a population of controls of 19 age-matched normal subjects (range 31–69 years) to compare 18FDG-PET data obtained at the beginning of the study (1993). We also recruited another control population of 16 age-matched normal subjects (range 24–65 years) whose data were compared with those obtained during the follow-up studies when the PET scanner was replaced (see Subjects and methods).

### Table 1

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex</th>
<th>Age</th>
<th>Codon 129</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-carriers of D178N PRNP mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>21</td>
<td>M/V</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>23</td>
<td>M/V</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>30</td>
<td>M/V</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>33</td>
<td>M/V</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>36</td>
<td>V/V</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>46</td>
<td>M/V</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>57</td>
<td>M/V</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>58</td>
<td>M/V</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>64</td>
<td>M/M</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>69</td>
<td>M/M</td>
</tr>
<tr>
<td>Carriers of D178N PRNP mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>25</td>
<td>M/M</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>31</td>
<td>M/M</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>32</td>
<td>M/M</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>35</td>
<td>M/M</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>48</td>
<td>M/V</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>48</td>
<td>M/V</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>51</td>
<td>M/M</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>59</td>
<td>M/V</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>65</td>
<td>M/M</td>
</tr>
</tbody>
</table>

M = methionine. V = valine.

All subjects underwent a general medical and standardized full neurological examination by the same neurologist (P.C.). The medical examination included laboratory tests (e.g. serum chemistry and haematology, thyroid, liver and kidney function tests). The neuropsychological testing included standard EEG and a polysomnogram collected by an Oxford-Medilog 9200 polygraph. The autonomic functions were evaluated with standardized cardiovascular reflex tests (head-up tilt test, Valsalva manoeuvre, deep breathing, isometric handgrip) performed with continuous non-invasive measurement of heart rate, blood pressure and breathing. In addition, the noradrenaline plasma values were measured at supine rest condition and after 10 min of orthostatic stress. The neuropsychological assessment included Rey’s 15 words immediate and delayed recall, Paired Associate Learning, Digit forward and reverse, Corsi’s cube test, the Weigl’s sorting test, Raven Coloured Progressive Matrices, Word fluency with phonemic cues, Tapping test and Temporal rules induction (Gallassi et al., 1996). In addition, we measured auditory reaction time (simple choice, complex choice and errors), and critical flicker fusion frequency (Gallassi et al., 2003).
2-deoxy-D-glucose (18FDG) and PET in a resting state. PET scans 5, 6 and 8 agreed to undergo neurophysiological, neuropsychological and 18FDG-PET examinations during the follow-up (see below for details).

PET acquisition method

The methods have been described previously in detail (Cortelli et al., 1997). Briefly, all the subjects were studied by 2-[18F]fluoro-2-deoxy-D-glucose (18FDG) and PET in a resting state. PET scans were performed with a whole-body positron emission tomograph (921/04-12; Siemens/CPS, Knoxville, TN) at the beginning of the study and with a GE-Advance 3D tomograph (General Electric Medical Systems, Milwaukee, WI) after 1994 during the follow-up. Quantitative measurements of regional cerebral metabolic rate of glucose (rCMRglc) were obtained in all cases. Each study started with administration of local anaesthesia and insertion of a 20-gauge Teflon catheter into a radial artery. The subjects were then positioned on the scanner bed with their heads immobilized by a customized head holder. Transmission scans were carried out with an external Ge ring source. Each subject then received an intravenous pulse of 250–300 MBq of 18FDG. Scanning was performed between 45 and 70 min after intravenous tracer administration. Timed arterial blood samples were collected continuously for the first minute following 18FDG administration and then at increasing intervals (2, 3, 5, 7.5, 10, 15, 25, 35, 45 and 60 min) up to the end of scanning. Glucose and 18FDG concentrations were assayed in the plasma.

PET data analysis

Image processing was performed using Analyze software (BRU/ Mayo Clinic, Rochester, MN) on SUN SPARC (Mountain View, CA) workstations. To obtain anatomic localization with reference to the stereotactic atlas (Talairach and Tournoux, 1988), images were axially reoriented parallel to the anterior commissure-posterior commissure line, according to the method described by Friston et al. (1989). Local cerebral metabolic values were obtained using circular regions of interest (ROIs) (diameter, 1.5 FWHM; equal to 9.6 mm) manually drawn on 16 cortical, subcortical, and cerebellar regions identified on the stereotactic atlas templates. Regions included the cingulate cortex; the frontal basal, frontal lateral and rolandic cortex; the parietal cortex; the temporal polar, superior, middle and inferior temporal cortex, and the hippocampus; the occipital lateral and calcine cortex; the caudate and the putamen; the thalamus; and the cerebellum. These brain regions were also used for the ROIs analysis in the subjects who developed the disease.

Average values of rCMRglc consumption were calculated from the multiple ROIs included in these regions to obtain a value representative of the limbic cortex (average of rCMRglc of cingulate cortex, frontal basal, inferior temporal cortex, and the hippocampus), basal ganglia (average of rCMRglc of caudate and putamen), neocortex (average of frontal lateral and rolandic cortex, parietal, anterior, superior and middle temporal cortex, and calcine cortex), thalamus and cerebellar cortex. These average values were used for the data analysis.

Statistics

The first step in the analysis was to verify the influence of sex, age and side (right or left hemisphere) on the rCMRglc values (SAS procedures GLM, SAS Institute, Cary, NC). None of these variables had a significant effect on rCMRglc values, thus, left and right values were averaged for each region.

Then, rCMRglc regional values were normalized to the rCMRglc values of the lateral occipital cortex (non-carriers n = 10, 6.88 ± 0.61 mg 100 g−1 min−1; carriers n = 9, 7.07 ± 1.09 mg 100 g−1 min−1; controls n = 19, 6.3 ± 0.7 mg 100 g−1 min−1). The occipital cortex was chosen because it had shown few or no abnormalities at post-mortem examination in affected patients (Kong et al., 2004; Parchi et al., 1995). The rCMRglc normalized values were considered hypometabolic if they were more than two standard deviations below the control mean. We used the values obtained from the first group of 19 control subjects to compare the rCMRglc of carriers and non-carriers at the beginning of the study. Data from a new control group of 16 age-matched healthy subjects were obtained [occipital lateral cortex (OLC): controls: 6.92 ± 1.18 mg 100 g−1 min−1] and used to evaluate the 18FDG-PET data during the follow-up of carriers 5, 6, 8 after 1994 when a new PET scanner became available. ANOVA statistics program was used to test for significant differences between controls, carriers and asymptomatic carriers of the D178N PRNP mutation on rCMRglc values for all cerebral regions evaluated at the beginning of the study. Whenever ANOVA revealed significant differences, a multiple comparison of means was done [Tukey Honest Significance Difference (HSD) for unequal sample size] and significant differences (P < 0.05) between group means were determined.

The individual 18FDG-PET follow-up studies of subjects 5, 6, 8, who became clinically affected during the follow-up were analysed by comparing the normalized value of each brain region considered to the average of rCMRglc of the same brain area obtained in the control group. In addition, in subject 6 who had five PET scans from 1993 to her death in 2000, each brain region examined in the serial follow-up PET scans was expressed as percentage change of the glucose metabolism normalized to OLC since the latter revealed a stable metabolic consumption rate (study 1: 5.66 mg 100 g−1 min−1; study 2: 5.44 mg 100 g−1 min−1; study 3: 5.49 mg 100 g−1 min−1; study 4: 5.75 mg 100 g−1 min−1; study 5: 5.26 mg 100 g−1 min−1).

Results

Subject populations and clinical tests

The mean age of both the ten non-carriers and nine carriers of the N178D-129M haplotype, all belonging to the same family, was 43 years (Table 1). It was not significantly different from the mean age of 49 years of the 19 subjects used as normal controls (range 31–69 years). All laboratory examinations including EEG, polysomnography and EEG spectral analysis in wakefulness and during sleep (Ferrillo et al., 2001), physiological and biochemical autonomic evaluations as well as neuropsychological tests were within normal ranges in all subjects in each group. Therefore, clinical examination and all tests were within normal limits in the carriers and non-carriers at the time of the first PET scan.
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Table 2 Clinical findings of the four subjects of group 2 (carrier D178N) who became affected by FFI during the follow-up

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Codon</th>
<th>Age at onset (months)</th>
<th>Disease duration (months)</th>
<th>Symptoms at onset</th>
<th>Dysautonomia</th>
<th>Ataxia</th>
<th>Myoclonus</th>
<th>Deficit attention and vigilance</th>
<th>Deficit verbal and working memory</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>M/V</td>
<td>129</td>
<td>49</td>
<td>24</td>
<td>Inability to sleep and apathy</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>M/V</td>
<td>129</td>
<td>52</td>
<td>26</td>
<td>Inability to sleep and apathy</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>M/M</td>
<td>58</td>
<td>14</td>
<td>14</td>
<td>Inability to sleep and diplopia</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>8</td>
<td>M/V</td>
<td>64</td>
<td>24</td>
<td>24</td>
<td>Inability to sleep and apathy</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

Dysautonomia: + mild = BP ≥ 150/90 mmHg ≤ 160/100 mmHg; HR ≥ 90 bps/min ≤ 100 bps/min; BcT = ≥ 37°C ≤ 38°C. ++ moderate = BP > 160/100 mmHg ≤ 180/110 mmHg; HR > 100 bps/min < 120 bps/min; BcT = > 38°C < 39°C. +++ severe = BP > 180/100 mmHg; HR = ≥120 bps/min; BcT = ≥ 39°C. Ataxia: + mild = disequilibrium; ++ moderate = stand and walk only with support; +++ severe = inability to stand and walk. Myoclonus: + mild = evoked; ++ moderate = evoked and spontaneous multifocal; +++ severe = spontaneous generalized. Deficit attention and vigilance: + mild = 1-2/6 impaired tasks; ++ moderate = 3-4/6 impaired tasks; +++ severe >4/6 impaired tasks. Deficit verbal and working memory: + mild = 1/5 impaired tasks; ++ moderate = 2-3/5 impaired tasks; +++ severe >3/5 impaired tasks.

Table 3 Regional cerebral metabolic rate of glucose consumption (rCMRglc) at the beginning of the study (1993) shown as [mg/100 g -1 × min -1] for each brain region considered in subjects of the FFI family

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Non-carriers (n = 10)</th>
<th>Carriers (n = 9)</th>
<th>Controls (n = 19)</th>
<th>ANOVA (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limbic cortex</td>
<td>5.99 ± 0.80</td>
<td>5.79 ± 0.70</td>
<td>6.04 ± 0.7</td>
<td>0.69</td>
</tr>
<tr>
<td>Neocortex</td>
<td>6.18 ± 0.83</td>
<td>6.57 ± 1.13</td>
<td>6.75 ± 0.8</td>
<td>0.32</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>6.52 ± 0.64</td>
<td>6.99 ± 1.24</td>
<td>6.85 ± 0.9</td>
<td>0.49</td>
</tr>
<tr>
<td>Cerebellar cortex</td>
<td>5.03 ± 0.78</td>
<td>5.49 ± 0.74</td>
<td>5.36 ± 0.7</td>
<td>0.34</td>
</tr>
<tr>
<td>Thalamus</td>
<td>5.96 ± 0.60</td>
<td>6.40 ± 1.37</td>
<td>6.15 ± 0.8</td>
<td>0.74</td>
</tr>
</tbody>
</table>

No significant differences were found between non-carriers and carriers of the D178N mutation of the PRNP gene and controls (statistics were computed on value normalized to the occipital lateral cortex).

Clinical follow-up

As of January 2005, all non-carriers interviewed and examined by the same neurologist were in good health and, as expected, had not developed any neurological disease. In contrast, four carriers (cases 5–8) became symptomatic and had died of the disease by January 2005. They presented with the typical clinical features of FFI (Table 2). The presence of insomnia was confirmed in all subjects by a 24 h polygraphic recording, which typically showed a disappearance of slow wave sleep, brief episodes of REM sleep without atonia and the lack of circadian rhythms of blood pressure, heart rate and body core temperature. An MRI scan performed at disease onset was normal in all subjects. Patients lost weight and died from sudden cardiorespiratory arrest. Autopsy including PrP analyses confirmed the diagnosis of FFI in patients 5, 6 and 8. Permission to perform autopsy was not given for case 7.

The other carriers (subjects 1–4 and 9), who currently are still asymptomatic, except for the mean age, which was more advanced in the affected individuals (51.5 ± 5 years versus 38 ± 16 years). During the follow-up none of the carriers developed any chronic disease or was treated with drugs for a sustained amount of time.

Baseline ¹⁸FDG-PET scanning (1993)

No difference in the global CMRglc was found between non-carriers and carriers, or between each of the two groups and the normal controls. Glucose utilization was also comparable in the three groups when the rCMRglc consumption or rCMRglc was examined in the individual brain regions, including limbic cortex, neocortex, basal ganglia, cerebellar cortex and thalamus (Table 3). Similarly, no difference in rCMRglc was found when each carrier of the D178N-129M haplotype was individually compared with the non-carriers analysed as a group.

Follow-up ¹⁸FDG-PET scanning

Subject 7, who did not agree to undergo follow-up ¹⁸FDG-PET scans, became affected 63 months after the baseline ¹⁸FDG-PET examination which had shown a normal global and rCMRglc (see above). Subjects 5 and 8, in whom we obtained one follow-up ¹⁸FDG-PET study 21 and 56 months, respectively, before clinical disease onset, showed no...
abnormalities in any of the $^{18}$FDG-PET areas examined. Subject 6 was examined by $^{18}$FDG-PET twice after 1993, 32 and 13 months before the clinical onset of the disease. While at 32 months rCMRglc values were within normal range, statistically significant hypometabolism with 16% reduction of rCMRglc was detected in the thalamus at 13 months before the disease became symptomatic (Fig. 1).

At this time, none of the clinical, neurophysiological and neuropsychological tests performed, including neurological examination, autonomic and neuropsychological evaluations, standard EEG, brain MR and two night polysomnographic recordings (Oxford-Medilog 9200 polygraph) revealed abnormalities. However, while the macro-structure of sleep was normal, spectral analysis with calculation of the absolute and relative EEG power of the four main bands [delta (SWA 0.5–4.0 s$^{-1}$), theta (TB 4.5–8 s$^{-1}$), alpha (AB 8.5–12 s$^{-1}$), sigma (SA 12.5–16 s$^{-1}$)] demonstrated a significant reduction of the sigma band with respect to the same examination performed 19 months earlier (Table 4).

$^{18}$FDG-PET after the disease onset

The $^{18}$FDG-PET was performed twice in subject 6, at 7 and 10 months after disease onset; once each in subject 8 and 5, at 7 and 12 months, respectively, while subject 7 refused any instrumental follow-up. The rCMRglc progressively decreased and affected several brain regions in all patients. The thalamic metabolism had further decreased to $-40$ and $-45\%$ at 7 and 10 months after clinical onset in subject 6, who showed a 16% metabolic decrease limited to the thalamus 13 months before disease onset (Fig. 2). The same patient had metabolic reductions of 28 and 31% in the limbic cortex and 25 and 29% in the basal ganglia. No significant reduction was seen in the neocortex or cerebellar cortex (Fig. 2). Subjects 8 and 5 examined by $^{18}$FDG-PET at 7 and 12 months after clinical onset respectively, showed a 30 and 31% metabolic reduction in the thalamus, while the rCMRglc was normal in the limbic cortex, basal ganglia and neocortex, as well as in the cerebellar cortex.

Discussion

Two major unanswered questions concerning familial neurodegenerative diseases are when and where the degenerative process starts. This issue is raised by the common observation that familial neurodegenerative diseases usually become symptomatic at mature or advanced age although the mutated protein thought to trigger the disease is present from the early stages of brain development. However, prion diseases may differ from other neurodegenerative diseases in that the mutated protein probably maintains a normal conformation until some time in adulthood, when it changes conformation converting adjacent PrPs and ultimately leading to neuronal injury and loss.

Knowing the time and anatomical location of the initial degenerative and dysfunctional process is important not only for understanding the pathogenic mechanisms, but also for...
timing the therapeutical interventions that might prevent the disease, if and when these interventions become available. The present study addresses these issues in FFI using longitudinal 18FDG-PET and electrophysiological recordings to assess metabolism and function of neuronal populations during the pre-symptomatic and symptomatic stages of the disease in subjects carrying the FFI genetic mutation.

FFI is particularly suitable for this kind of study for several reasons. FFI has a rapid course and high penetrance, therefore, it is easier to follow the disease progression, and virtually all mutation carriers become symptomatic. The disease presents with impairment of sleep and autonomic functions that can be tested fairly accurately. The pathology is relatively simple, at least in cases with short duration, because it is mostly limited to the thalamus and essentially consists of neuronal loss and gliosis. Furthermore, the central event that triggers the pathogenesis of FFI and other familial prion diseases is believed to be the conversion of the mutated PrP into an abnormal isoform through a conformational change that renders the PrP unfit to perform its normal function and makes it pathogenic. Both these PrP changes may result in the impairment of rCMRglc in FFI patients that can be assessed by 18FDG-PET (Perani et al., 1993; Cortelli et al., 1997). Tests of autonomic system function, neuropsychological assessments and analysis of the macro-structure of sleep in a 24 h polysomnogram revealed no difference between the members of the FFI affected family with and without the mutation and between these two populations and a control group until the disease became symptomatic in the mutation carriers. This indicates that, at least in this FFI pedigree, the presence of the D178N-129M haplotype has no effect that can be detected even with extensive clinical examination before the disease becomes clearly symptomatic. Assessment of the baseline regional glucose metabolism in the brain (rCMRglc) with 18FDG-PET also failed to disclose any difference among pre-symptomatic carriers, non-carriers and the control group. PET studies and the EEG spectral analyses also were normal 63, 56, 32 and 21 months before clinical disease onset in four carriers, respectively. One of these four, subject 6, who had been assessed and found normal at 32 months, was tested again 13 months before the disease onset. At 13 months both 18FDG-PET and spectral analysis of sleep recording were significantly different from those of the controls. Even if spectral EEG analysis is a less consistent method than PET,
it is noteworthy that the $^{18}$FDG-PET revealed a metabolic reduction confined to the thalamus while the spectral EEG analysis demonstrated a reduction of the sigma band EEG power which is related to the mechanisms of cortical thalamic synchronization and involved in sleep spindles formation (Nunez et al., 1992; Steriade et al., 1993). The concomitant occurrence of these two changes suggests a relationship between the thalamic dysfunction demonstrated with PET and the reduction of spindle frequency activity, a typical polysomnographic presenting sign of FFI (Sforza et al., 1995). Furthermore, it indicates that thalamic dysfunction resulting in the lack of efficiency of the mechanisms involved in cortical thalamic synchronization is the first detectable change in FFI, before the symptomatic onset of the disease. Although our data have been obtained from a limited number cases due to the complexity and time requirements of the tests, when combined they argue that the D178N-129M haplotype might trigger the disease process associated with FFI as little as 13 months before the appearance of the clinical signs. These findings together with our previous data also are consistent with the notion that FFI arises in the thalamus and subsequently spreads to other areas of the brain by an as yet unknown mechanism (Perani et al., 1993; Cortelli et al., 1997).

In the context of the commonly accepted pathogenesis of familial prion diseases, the present findings of decreased metabolism of the neuronal cells of the thalamus 13 months before clinical onset in case 6 might be explained by postulating that at that time the mutated PrP begins to convert into or to accumulate in sufficient amount as a pathogenic isoform impairing neuronal metabolism and subsequently leading to the neuronal loss found at post-mortem examination. The $^{18}$FDG-PET findings in the follow-up study of this patient showed a further metabolic reduction from 16% (when the patient was asymptomatic) to 40% seven months after clinical disease onset. Hence, an impairment or loss of about twice as many neurons is likely to be needed for the disease to become symptomatic, although these findings await confirmation in additional FFI patients also from other pedigrees.

The scenario of initial impairment of neuronal metabolism followed by neuronal death is supported by the PET data from other neurodegenerative diseases showing that glucose utilization may be significantly reduced with no significant neuronal loss (Meltzer et al., 1996; Ibanez et al., 1998). Few serial $^{18}$FDG-PET studies carried out through the presymptomatic and symptomatic disease stages have been performed in other neurodegenerative diseases. In Alzheimer’s disease, $^{18}$FDG-PET studies or MRI scans (to determine the volume of the hippocampal formation) have been performed in individuals at risk for Alzheimer’s disease because they were carriers of either an Alzheimer’s disease pathogenic mutation or of the Alzheimer’s disease risk factor APO E4 (Kennedy et al., 1993; Small et al., 1995; Fox et al., 1996; Reiman et al., 1996; Perani et al., 1997; Small et al., 2000). Significant changes in glucose metabolism or hippocampal volume were observed when the carriers were still asymptomatic. In one study, subjects at risk for Alzheimer’s disease had an abnormal $^{18}$FDG-PET 12 years before the age at which they were expected to become symptomatic according to the mean historical age at disease onset of affected members in their families (Small et al., 1995). Another 2 year follow-up of ten at risk, asymptomatic subjects with an already abnormal baseline $^{18}$FDG-PET showed a significant decline of the glucose metabolism without any of the subjects becoming symptomatic (Small et al., 2000). Similar observations have been made in Huntington’s disease and frontotemporal dementia (Janssen et al., 2005; Kipps and Hodges, 2005).

Combined these findings indicate that tissue impairment, as revealed by impaired metabolism or atrophy, occurs longer before the symptomatic onset in these neurodegenerative diseases than in FFI. This discrepancy probably reflects the rapid course of FFI and other familial prion diseases (Kong et al., 2004) or the normal functioning of the mutated prion until it changes conformation to become PrPSc.

Nonetheless, the findings in our FFI pedigree suggest that there may be a time interval of between 13 and 21 months before clinical disease onset which may allow preventive treatment. Studies that can detect the initial damage to the nervous system before the disease impairs patient performance will be essential for accurate timing of preventive treatment in familial prion diseases.

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References


