

In Vivo Detection of Thalamic Gliosis

A Pathoradiologic Demonstration in Familial Fatal Insomnia

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Background: Increasing evidence supports the usefulness of brain magnetic resonance imaging (MRI) for the diagnosis of human prion diseases. From the neuroradiological point of view, fatal familial insomnia is probably the most challenging to diagnose because brain lesions are mostly confined to the thalamus.

Objective: To determine whether multisequence MRI of the brain can show thalamic alterations and establish pathoradiologic correlations in a patient with familial fatal insomnia.

Design: Radioclinical prospective study. We describe a patient with fatal familial insomnia and normal MRI images. Because the MRI study was performed only 4 days before the patient's death, we were able to compare radiological data with the lesions observed at the neuropathologic level.

Patient: A 55-year-old man with familial fatal insomnia.

Main Outcome Measure: Magnetic resonance spectroscopy combined with the measurement of apparent diffusion coefficient of water in different brain areas.

Results: The neuroradiological study showed, in the thalamus but not in the other brain regions studied, an increase of apparent diffusion coefficient of water and a metabolic pattern indicating gliosis. These alterations closely correlated with neuropathologic data showing an almost pure gliosis that was restricted to the thalamus.

Conclusion: Considering fatal familial insomnia as a model of thalamic-restricted gliosis, this case demonstrates that multisequences of magnetic resonance can detect prion-induced gliosis in vivo, as confirmed by a neuropathologic examination performed only a few days after radiological examination.

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SEVERAL REPORTS HAVE UNDERLINED the usefulness of brain magnetic resonance imaging (MRI) in the diagnosis of Creutzfeldt-Jakob disease.¹⁻⁴

The fluid-attenuated inversion recovery (FLAIR) sequence and diffusion-weighted imaging (DWI) are considered highly sensitive sequences to detect signal alteration of the cortex and deep gray matter. Advances in therapeutic approaches for patients with prion diseases have emphasized the need for earlier diagnostic markers that would authorize the onset of treatment before severe and irreversible lesions of the brain occur.⁵ We designed a radioclinical prospective study using a multimodality MRI standardized procedure that aimed to estimate the differential sensitivities of FLAIR, DWI, and magnetic resonance spectroscopy for the diagnosis of human prion diseases. As part of this study, we focused on a patient with fatal familial insomnia and the D178N-

129M mutation. From the neuroradiological point of view, fatal familial insomnia is probably the most challenging diagnosis among human prion diseases, because neuropathologic studies show very limited brain lesions⁶ compared with sporadic and variant Creutzfeldt-Jakob disease cases, which usually exhibit severe spongiform change and gliosis in the cortex and deep gray matter.

REPORT OF A CASE

A 55-year-old man was referred to a neurologic center for cognitive impairment. A familial history of dementia was reported in his mother (died with dementia at the age of 73 years), 1 of his brothers (died with ataxic encephalopathy and dysautonomic symptoms at the age of 55 years), and a maternal first cousin (died with dementia at age 44 years). The first symptoms were asthenia and insomnia

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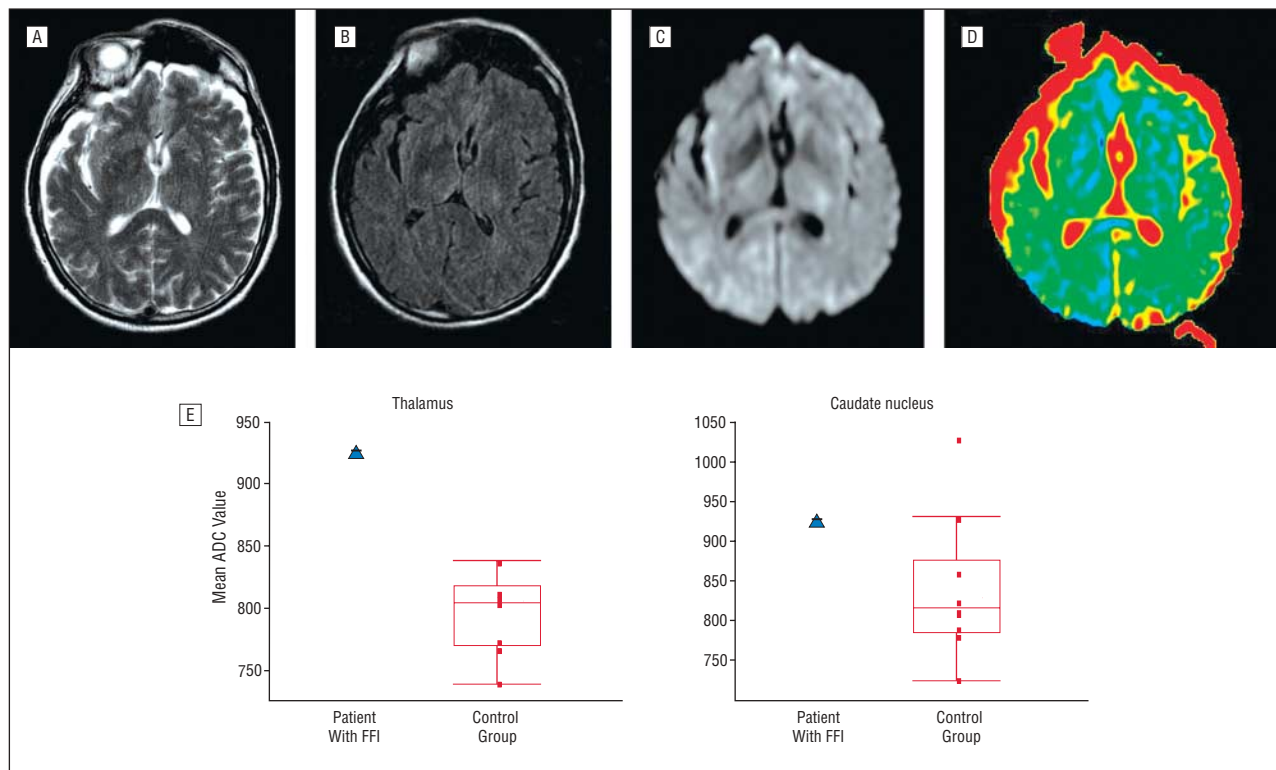


Figure 1. Magnetic resonance imaging findings in a patient with familial fatal insomnia (FFI) and the D178M mutation (A, T2-weighted imaging sequence; B, fluid-attenuated inversion recovery sequence; C, diffusion-weighted imaging; D, apparent diffusion coefficient of water [ADC] map). Both thalami showed normal signals. E, When compared with the control group, the mean ADC value was increased in the thalamus but not in other regions commonly involved in Creutzfeldt-Jakob disease, such as the caudate nucleus.

rapidly associated with hypersomnia behavior. Acute agitation, gait disturbance, cognitive impairment, and visual hallucinations occurred in a few months. Neurological examination showed pyramidal signs, cerebellar ataxia, myoclonus and dementia with memory impairment, constructional apraxia, temporospatial disorientation, and frontal symptoms. Routine biological examination results in blood and cerebrospinal fluid were normal. Cerebrospinal fluid was negative for 14-3-3 protein. No periodic sharp wave complexes were recorded by electroencephalography. Twenty-four-hour video polysomnography indicated a subtotal insomnia with only 23 minutes of stage 1 sleep. Circadian variations of cortisol secretion were abolished. Dysautonomic symptoms occurred, including bilateral myosis with negative light reflex, unexplained hyperthermia (39°C), and tachycardia without any change in blood pressure. An MRI examination of the brain was performed 4 days before death, which occurred 6 months after clinical onset. The patient's relatives gave informed consent for genetic study and autopsy. Study of the prion protein-coding sequence (PRNP) showed the D178N mutation and methionine homozygosity at codon 129.

MRI STUDY

The patient's relatives and the control volunteers gave informed consent for the MRI study, which was approved by the ethics committee of our institution. Examination was performed on a 1.5-T MRI system (Signa; General Electric, Milwaukee, Wisconsin) and included

sagittal and axial T1-weighted sequences (repetition time/echo time=644/15 milliseconds), FLAIR (repetition time/inversion time/echo time=8000/180/110 milliseconds), DWI (single-shot echo planar imaging sequence gradient=0, 500, and 1000 seconds/mm² applied in the x, y, and z planes, respectively; matrix=128×128, field of view=256×256 mm²). Apparent diffusion coefficient (ADC) maps were constructed as previously described.⁷ The ADC values were calculated using the software provided by the manufacturer. Three magnetic resonance spectroscopy acquisitions (stimulated-echo acquisition mode single-voxel spectroscopy, repetition time/echo time=1500/20 milliseconds) were performed on the cerebellar vermis (voxel size, 20×20×15 mm); the thalamus, including the dorsomedial nucleus (voxel size, 30×15×15 mm); and the frontal cortex (voxel size, 40×20×15 mm). Magnetic resonance spectroscopic data were analyzed using dedicated software and resonances were assigned according to those previously reported.⁸ Spectra were processed as described⁸ and the following metabolites were integrated: N-acetyl aspartate, creatine, choline, myo-inositol, glutamine-glutamate-γ-aminobutyric acid, and scyllo-inositol/taurine. Each metabolite estimation was divided by the sum of all metabolites. The myo-inositol to sum ratio was considered a marker of gliosis.⁹ Control cases for the MRI study were 11 healthy volunteers (5 men, 6 women) free of any neurological disease with a mean age of 51 years (range, 25-64 [SEM 3.3] years). The myo-inositol to sum ratio showed no correlation with age.

NEUROPATHOLOGIC STUDY

Brain areas were sampled from 1-cm-thick coronal sections fixed in formalin, 10%, as previously described.¹⁰ Prion protein immunohistochemistry was performed using 12F10 monoclonal antibody (Commissariat à l'Énergie Atomique, Saclay, France). Immunostaining of glial fibrillary acidic protein and CD68 (DAKO, Trappes, France) were performed to detect astrogliosis and microglial proliferation, respectively.

RESULTS

The patient's brain appeared normal on T2-weighted, FLAIR, and DWI sequences (**Figure 1**). Neither atrophy nor signal alterations could be observed notably in the thalamus. When ADC of water was calculated from DWI data, we observed an increased ADC value in the thalamus compared with those in the control group, suggesting gliosis (Figure 1). The spectroscopic study showed a striking increase in the resonance of myo-inositol and of the myo-inositol to sum ratio in the thalamus of the patient when compared with control cases, which strongly indicates gliosis (**Figure 2**). The other studied brain regions of the patient were normal. Because the MRI study was performed only 4 days before death, we were able to correlate the spectroscopic/ADC pattern with the neuropathologic lesions.

Macroscopic examination of the brain did not show thalamic atrophy. At the microscopic level, no prion-associated spongiform change was observed in isocortical areas, the striatum, or the cerebellum. In contrast, there was a marked gliosis of the thalamus characterized by abundant glial fibrillary acidic protein–positive astrocytes and numerous CD68-positive microglial cells (Figure 2). Gliosis predominated in the anterior, dorso-medial, and centromedial nuclei of the thalamus, while the pulvinar was relatively spared. Minor spongiform change was seen in the pulvinar and the subthalamic nucleus. Immunostaining of the prion protein yielded negative results in all the studied brain regions, including the thalamus. In contrast, the proteinase K-resistant form of the prion protein was detected by Western blot in the thalamus, isocortical areas, the striatum, and the hippocampus, the lower band that corresponds to the unglycosylated form of the protein migrating at 19 kDa on the Western blot (**Figure 3**). As we previously reported in this case and other patients with the D178N mutation,¹¹ some regional variability of the glycoform pattern was observed.

COMMENT

The familial history and the clinical features of our patient, including prominent insomnia and dysautonomic symptoms associated with negative 14-3-3 detection in the cerebrospinal fluid, suggested the diagnosis of fatal familial insomnia, which was confirmed by brain study and PRNP sequencing showing a D178N mutation with a methionine at codon 129 of the mutated allele.⁶ Interestingly, results of the neuropathologic study strictly cor-

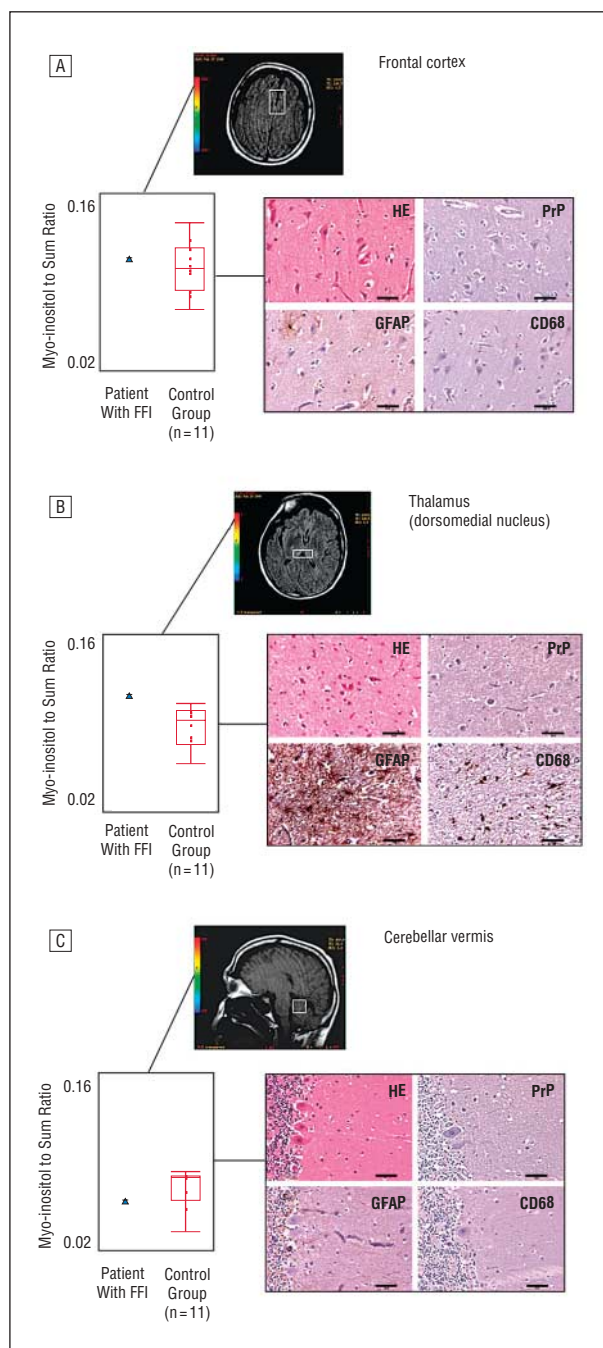


Figure 2. Correlation between spectroscopic study and neuropathologic findings in the frontal isocortex (A), thalamus (B), and cerebellar vermis (C) in a patient with familial fatal insomnia (FFI). Myo-inositol to sum ratio is myo-inositol divided by the sum of all metabolites estimated in the same voxel (voxel position is indicated for each region on the magnetic resonance imaging picture of the patient with FFI). CD68 indicates immunostaining of CD68 expressed by microglial cells; GFAP, immunostaining of the glial fibrillary acidic protein expressed by astrocytes; HE, hematoxylin-eosin; PrP, immunostaining of the prion protein. All bars equal 200 μ m.

related with the metabolite and ADC patterns, indicating gliosis in the thalamus without detectable alteration of other regions of the central nervous system. Consistently, we observed a selective involvement of the thalamus with a very low level of spongiform change, severe gliosis, and very low pathological prion protein load as detected by immunohistochemistry.⁶ This lesion profile

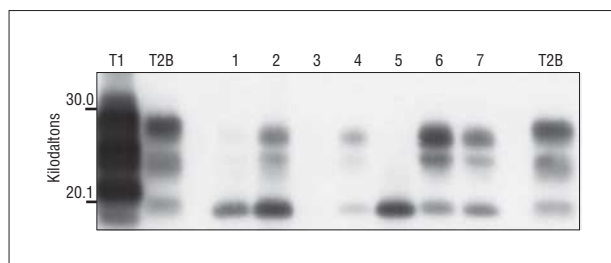


Figure 3. Detection of the proteinase K-resistant form of the prion protein (PrP) by Western blot. Lanes 1 through 7, respectively, are the frontal isocortex, thalamus, cerebellum, pulvinar, occipital isocortex, hippocampus, and caudate nucleus from the patient with familial fatal insomnia. T1 indicates control sample with PrP type 1; T2B, control sample with PrP type 2B.

in a patient with a short duration of the disease is consistent with previous postmortem studies, reports that used [18F]2-fluoro-2-deoxy-D-glucose positron emission tomography that showed a more widespread cerebral involvement in patients with fatal familial insomnia with prolonged disease only (> 18 months).^{6,12-14} In correlative studies, while brain hypometabolism was more widespread than histopathologic changes, all areas that showed detectable lesions (neuronal loss and astrogliosis) were also hypometabolic.^{6,14}

In other forms of human prion diseases, such as sporadic or variant Creutzfeldt-Jakob disease, the lesions responsible for MRI signal abnormalities regularly observed on DWI and FLAIR sequence continue to be debated.¹⁵⁻¹⁷ In general, the interpretation of radiopathologic correlation in humans suffers notably from the time interval between MRI and autopsy. In the case reported here, this interval was only 4 days. Fluid-attenuated inversion recovery alterations observed in Creutzfeldt-Jakob disease could be explained by astrogliosis, as suggested by MRI studies in scrapie-infected mice (139A strain) or hamsters (263K strain).^{18,19} However, in our case, we observed no alterations of the signal in either thalami, while an intense gliosis was detected in this structure. This suggests that prion-induced gliosis does not induce signal alteration in FLAIR sequence by itself. Our results—no or minor spongiosis without detectable pathological prion protein deposits and no signal abnormalities—are consistent with the role of spongiosis and amyloid prion protein load in signal alterations frequently observed on FLAIR imaging and DWI in other prion diseases.

From a clinical point of view, it is remarkable that magnetic resonance spectroscopy could detect thalamic gliosis while FLAIR and DWI showed no signal alteration. Both sequences are regarded as the more sensitive for the diagnosis of human prion diseases.^{3,20} This suggests that magnetic resonance spectroscopy may be a helpful and sensitive tool for the diagnosis of the disease before the occurrence of signal modifications. Our results are consistent with those of a recent experimental study in a mouse model of scrapie and in mice infected with a bovine strain that showed that histologically proven astrogliosis can be detected by magnetic resonance spectroscopy while no signal alteration is seen in T2-weighted imaging or DWI even at the late stages of the disease.²¹ In conclusion, considering fatal familial insomnia as a model of thalamic-restricted gliosis in humans, this case

demonstrates that multisequences of MRI can detect prion-induced gliosis in vivo, as confirmed by our neuropathologic examination performed just a few days after radiological examination.

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