

## Original Article

# Clinical, histopathological and genetic studies in a case of fatal familial insomnia with review of the literature

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Received April 4, 2015; Accepted May 19, 2015; Epub September 1, 2015; Published September 15, 2015

**Abstract:** To explore clinical, histopathological and genetic features of a case with fatal familial insomnia (FFI) and review the related literatures. A middle-aged woman who complained of "insomnia for 9 months and psychosis for 3 months" was suspicious of FFI. The clinical features of the patient were analyzed, and the dead patient was examined by autopsy and the brain tissues were obtained for histopathological studies, and the blood samples from the patient and some of her familial members were collected for the sequencing of prion protein gene (PRNP). The main clinical features included intractable insomnia, psychiatric symptoms and abnormal night sleep behavior, unsteady gait, difficulty swallowing, sudden death, and positive family history. The pathological studies showed neuronal loss and gliosis of multiple brain tissues in the proband, predominated with thalamus; and analysis of PRNP revealed gene D178N mutation, and linkage with 129 methionine (Met) allele in the proband and a relative. FFI patients may manifest as sudden death, and may have prominent psychiatric symptoms; the corresponding gene mutation could occur in the asymptomatic carriers; the data of autopsy and brain tissue pathology is helpful for further understanding of this disease.

**Keywords:** Fatal familial insomnia, sudden unexpected natural death, PRNP, prion disease, histopathology

## Introduction

Fatal familial insomnia (FFI) is a rare autosomal dominant human prion disease characterized clinically by a disordered sleep/wake cycle (progressive untreatable insomnia), dysautonomia, and motor signs and pathologically by predominant thalamic degeneration [1]. The typical neuropathological changes in FFI are predominantly in the thalamus where there is severe neuronal loss and astroglia in the anterior medial thalamus and inferior olives [2]. There were several previous reports of Chinese FFI kindreds, but their pathology and genetic findings were rarely reported together. We collected information about a Chinese patient with FFI who characterized by psychiatric symptoms. And present the native Chinese patient with clinical, pathological, genetic findings and autopsy report.

## Material and methods

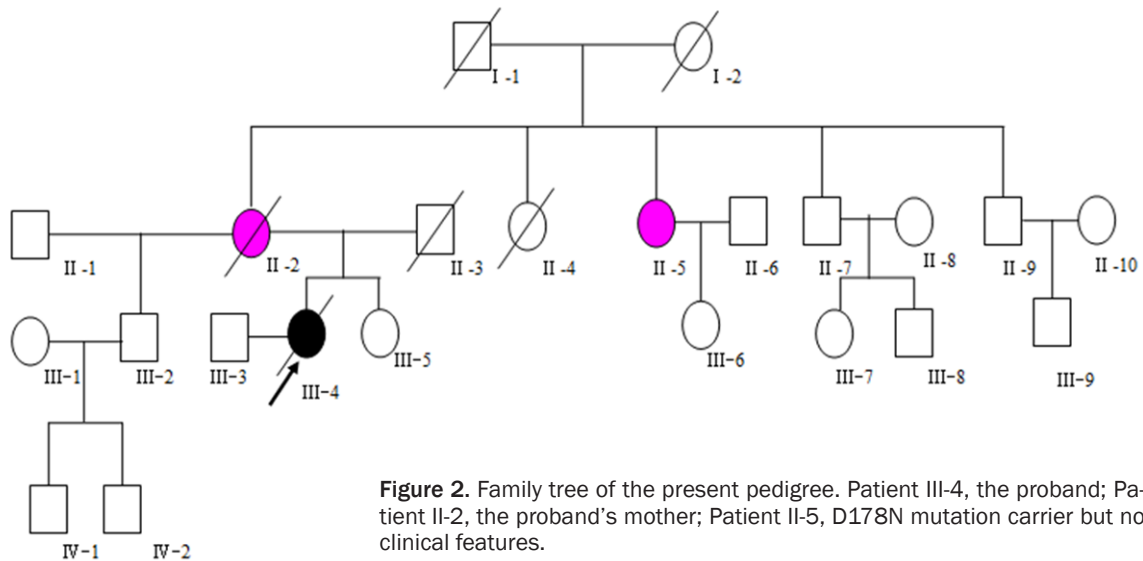
### Patient

The proband, was a 42-year-old woman who was referred to the Department of Neurology,

Hubei Province People Hospital, China in 2010 with a 9-month history of progressive sleep loss and a 3-month history of hallucination. The patient complained initially of dizziness, headache, dim eyesight, memory loss, wasting, constipation, emotional instability, and insomnia with total sleep time decreasing from 7 to 4 h per night after her mother died in march 2010. Five months before admission, she developed nocturnal psychotic mood disturbances with murmur to herself. Two months later, she had depressive symptoms with worsening unsteady gait. One week before admission, she developed dysphagia. She was first treated as an outpatient with flupentixol, risperidone, alprazolam, olanzapine. But she felt worse and the general condition worsened progressively. Body weight dropped rapidly from 55 to 45 kg. Her mother died within a few months after developing similar clinical features in March 2010. On admission, physical examination showed psychotic symptoms, stupor and disorientation. The patient was mental confusion and an attention deficit. Muscle tone was normal. Before admission, routine laboratory tests, thyroid function, Tumor markers, CSF and EEG were



**Figure 1.** The imaging of the patient.



**Figure 2.** Family tree of the present pedigree. Patient III-4, the proband; Patient II-2, the proband's mother; Patient II-5, D178N mutation carrier but no clinical features.

normal. Brain MRI showed the cerebral ganglion was normal (**Figure 1**). Five hours after admission, she had a fever of 38°C. Nine hours later, she died and total clinical duration was about 9 months. The family history indicated that her mother and aunt had been affected by a similar disorder (**Figure 2**).

#### Autopsy

Permission was obtained for post mortem examination and tissues were retained for diagnosis and research. The study was approved by ethics committees. The autopsy was performed after informed consent had been obtained. Sections were taken from all areas of the cerebral cortex, the caudate nucleus, putamen, pallidum, hippocampal formation, including the transentorhinal and entorhinal regions, amygdala, thalamus, brainstem and cerebellum.

Haematoxylin and eosin stains were performed using standard techniques.

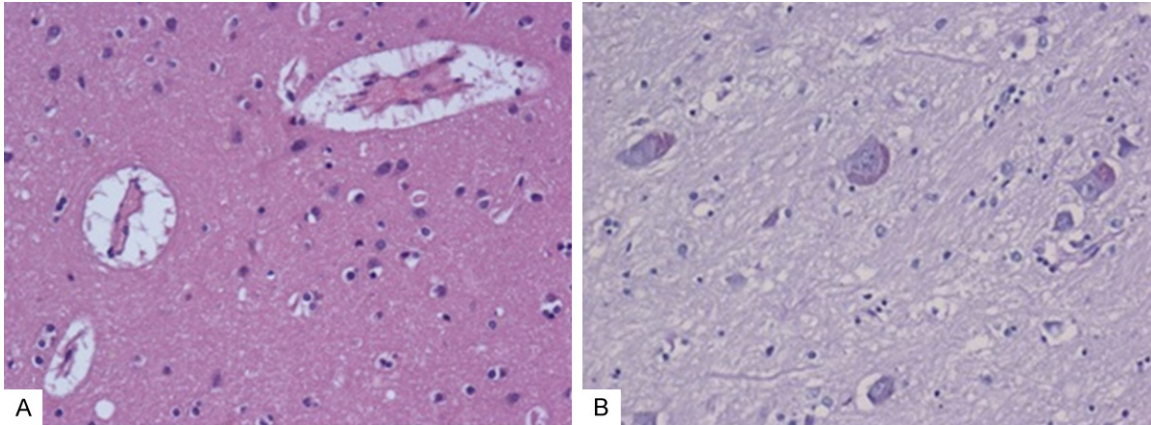
#### PRNP analyses

The blood samples of the patient (blood of heart), her aunt and 2 health family members (peripheral blood) across three generations were taken with informed consent. Then the extracted DNA was amplified for the coding region of PRNP. The genotype at codon 129 of PRNP was analyzed.

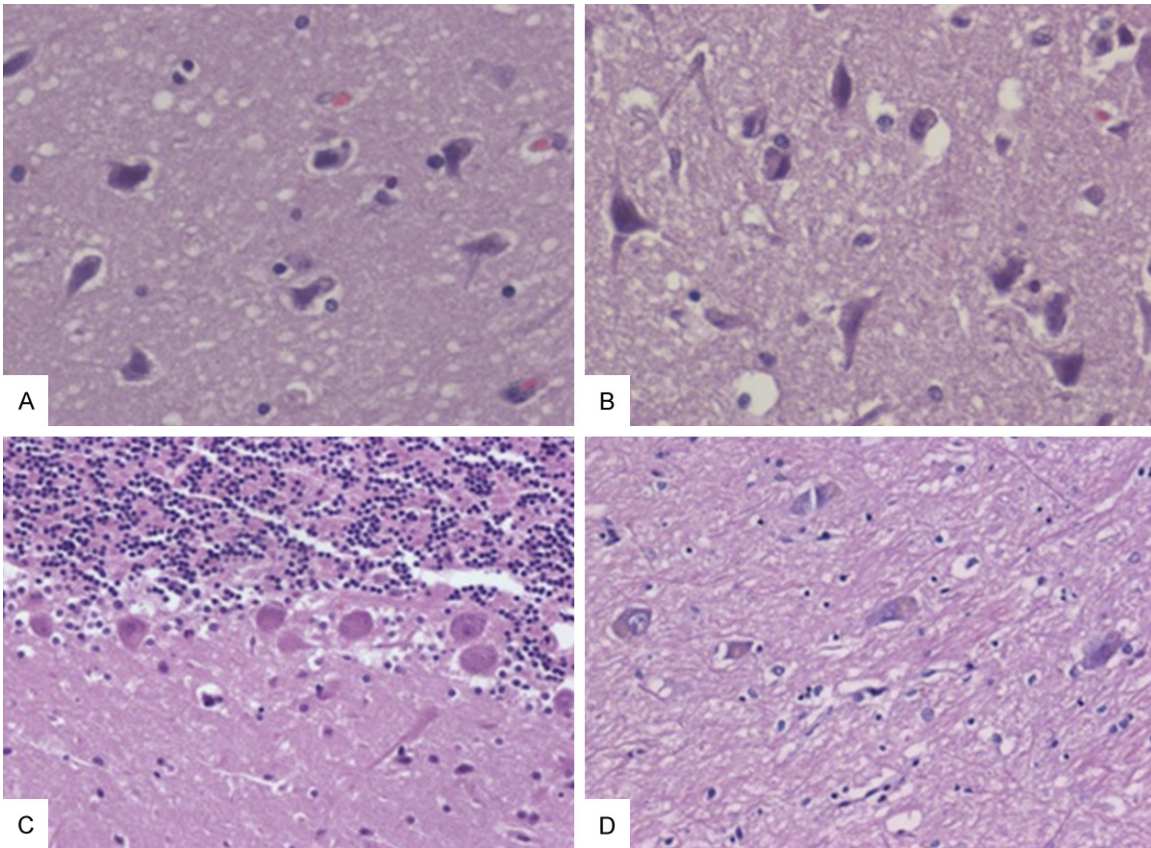
#### Results

##### Neuropathological assays

At autopsy the brain (weighed 1400 g) showed diffuse edema and congestion, more obvious in the cerebrum. Microscopic examination



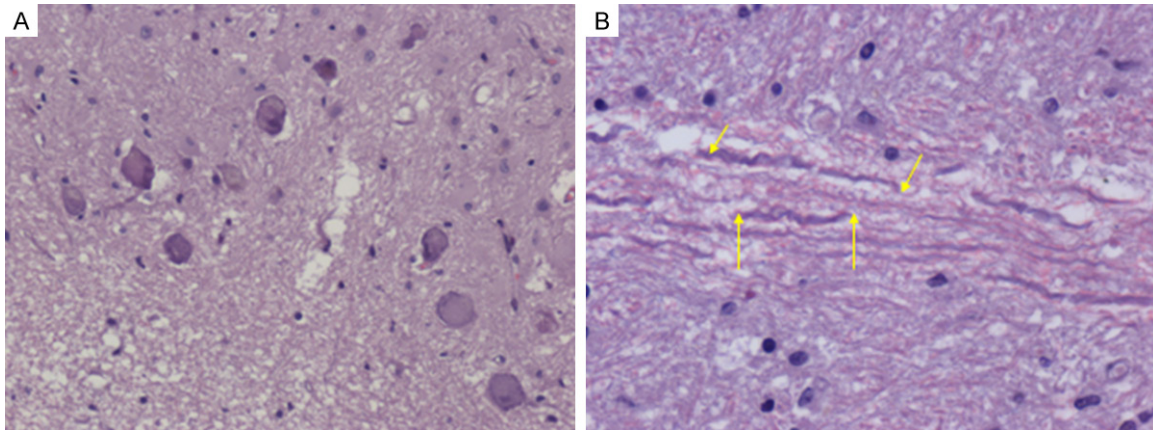
**Figure 3.** Severe spongiosis in the cerebrum (A) (HE  $\times$  200); Neurons of thalamus PAS (+) (B) ( $\times$  200).



**Figure 4.** Neurons karyopycnosis in frontal lobe (A), hippocampus (B) [HE  $\times$  400]; In the cerebellum, there was mild Purkinje cell loss (C) [HE  $\times$  400]; lipofuscin deposition in thalamus (D) [HE  $\times$  200].

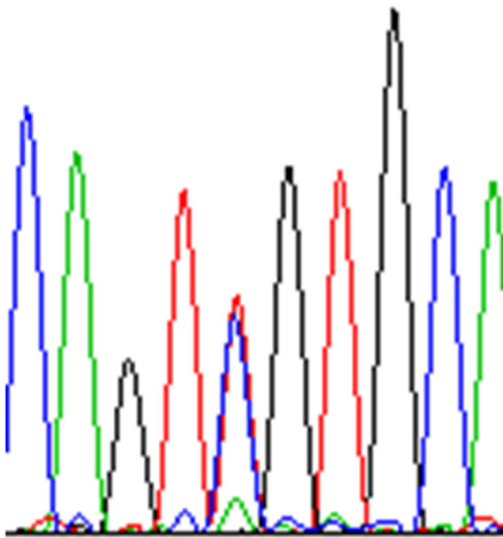
revealed severe spongiosis in the cerebrum (**Figure 3A**). Most conspicuously, part neuronal karyopycnosis neuronal degeneration occurred in frontal lobe (**Figure 4A**), temporal lobe, hippocamp (**Figure 4B**) and thalamus, and lipofuscin deposition was observed (**Figure 4C**). In the

cerebellum, there was mild Purkinje cell loss (**Figure 4D**). In addition, there was neuronal loss and reactive gliosis obviously in thalamus. Neuronal degeneration and necrosis were observed in olivary nucleus (**Figure 5A**). In pons, the axon was swelling and degeneration,



**Figure 5.** Neuronal degeneration and necrosis in olivary nucleus (A); In pons, the axon was swelling and degeneration, torpedo-like change was remarkable (B) [HE × 400].

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 C A G T T G T G C A



**Figure 6.** Gene sequence diagram (the D178N mutation). A missense mutation (G to A) at the position of nt 532 in one PRNP allele.

torpedo-like change was remarkable (**Figure 5B**).

Immunohistochemical analyses were performed by a standard indirect method. Patho-

logical examination of the frontal pole and thalamus showed negative Congo red stain. Parts of neuronal lipofuscin granules in the thalamus and lenticular nucleus showed PAS (+) (**Figure 3B**), MBP (-). In some parts of the hippocampus shows CD43 (+), the individual parts showed CD68 (+).

#### PRNP analyses

Sequencing of PRNP demonstrated the c.532G>A mutation which is predicted to change aspartic acid to asparagine at codon 178 (p.Asp178Asn) of the protein. The D178N mutation is always associated with methionine at the polymorphic position 129 of the mutant allele, consistent with FFI (**Figure 6**).

One sample of proband's three relatives present D178N mutation, consistent with the genetic characteristics of FFI. The other two did not detect the mutant gene.

#### Pedigree investigation

The pedigree is presented in **Figure 2**. A total of 23 family members were retrospectively or directly investigated. The age and cause of death in the proband's grandfather and grandmother were uncertain. The proband's mother died aged 62 years within 8 months of developing insomnia associated with psychiatric symptoms. The proband's aunt was confirmed to have the mutation at codon 178 (D178N). She developed insomnia for 5 months, and had poor response to hypnotic, but did not develop dementia, psychiatric symptoms, myoclonus,

and other manifestations. None of other family members have neurological symptoms to date.

### Discussion

Fatal Familial Insomnia was first described in 1986 in an Italian family [1]. In humans, inherited prion diseases are associated with three major phenotypes: Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) disease and Fatal Familial Insomnia (FFI). Most people who develop a prion disease have no definable cause and a few acquire the disease through an identified source of infection. About 10-15% of patients are affected by a genetic form and carry either a point mutation or an insertion of octapeptide repeats in the prion protein gene [3]. Unlike other genetic disorders, FFI is experimentally transmissible in suitable animal model [4]. Mean-while, unlike classical infectious diseases, FFI and other subtypes of genetic CJD are dominantly inherited. Iatrogenic transmission of CJD agent has been reported in over 400 patients worldwide [5]. During the incubation period, prion agent can be detected in the blood of infected patients sometimes [6]. Control and prevention of this type of disease needs more particular attention.

Genetically, FFI is linked to a GAC to AAC point mutation at codon 178 of PRNP that leads to a substitution of asparagine for aspartic acid (D178N) [7]. In FFI the D178N mutation is always associated with methionine at the polymorphic position 129 of the mutant allele. More than 30 pathogenic mutations of the prion protein gene (PRNP) have been reported to date worldwide [8]. This geographic spread fits the recurrent mutational event hypothesis [9]. The exact influence of potential new genetic loci and the molecular mechanisms that might control susceptibility or the phenotype must be investigated further. There is a big difference between PRNP genes in 129 amino acids polymorphisms of different races. The 129 Met allele frequencies of three main ethnic in Chinese (Han, Hui and Uygur) are much higher than European populations, suggesting that Chinese people may be more susceptible to prion disease than Europeans [10].

The D178N mutation has been detected in a relative of the proband, but the patient has no typical performance currently. One data indicate that the neurodegenerative process asso-

ciated with FFI begins in the thalamus between 13 and 21 months before the clinical presentation of the disease [11]. On the genetic side it is increasingly clear that the mutations per se are not always sufficient to cause the disease and that other environmental or genetic factors also play a role in triggering the disease onset [3]. The exact influence of potential new genetic loci and the molecular mechanisms that might control susceptibility or the phenotype must be investigated further.

Indeed, severe thalamic neuronal loss and gliosis are characteristically seen in postmortem studies of FFI, usually without concomitant spongiform change, except in very advanced stages of the disease. The most seriously affected thalamic nuclei are the anteroventral, mediodorsal nuclei, and the pulvinar [12]. Morphometric investigations have shown a 90% loss of neurons in association nuclei and in motor nuclei and a 60% loss in limbic-paralimbic, intralaminar, and reticular nuclei. Atrophy of the inferior olive with neuronal loss and gliosis is also commonly observed. In this study, the pathological results were consistent with these changes. The immunohistochemical staining of brain tissue could exclude inflammatory disease and vascular disease. Tauopathy, which is sometimes seen in prion diseases with amyloidosis, has thus far not been reported in patients with FFI.

Usually, onset of FFI is between ages 32 and 62 (mean 51 years) [13]. However, several cases of sporadic CJD have been reported at ages younger than 30, with the youngest patient being a 18-year-old man. The first Chinese FFI was reported in 2004 by Spacey, etc [14]. Eight patients have been reported in China. The clinical course was 6, 8, 8, 8, 8, 9, 11 and 16 months, with an average of 9.25 months. In this study, the patient died nine months after the onset, consistent with the data in China, but had shorter duration than abroad.

The main neurologic changes in FFI include progressive disturbance of attention and vigilance associated with defective memory and difficulties with the temporal sequencing of events. In most cases, there are no clinically evident personality changes, and psychiatric symptoms are relatively uncommon. Furthermore, unlike CJD, social behavior is preserved in early stages, although patients may look apathetic [15].

Our patient is distinct from other cases due to prominent psychiatric symptoms and mood disorders, but did not develop dementia, pyramidal signs, myoclonus. The patient developed sympathetic and autonomic dysfunction, such as high blood pressure, fever, weight loss, constipation. She also developed an unsteady gait, followed by dysphagia. These symptoms should not be ignored. In addition, like two additional cases reported in China [16], the patient developed insomnia after a major psychological blow (her mother's death); It suggest mental stimulation may induce the onset of FFI. The mutations per se are not always sufficient to cause the disease and that other environmental or genetic factors also play a role in triggering the disease onset. In addition, different with the observations of some FFI cases described previously, the sudden death of the patient was another important feature. Perhaps for FFI, we should think of the possibility of sudden death.

The current report highlights that catatonia and psychotic symptoms may belong to the clinical phenotype of early-stage FFI [15]. Therefore, FFI should be considered in a patient without apparent sleep disturbances if a combination of cognitive or psychiatric deficits with ataxia, visual disturbances, or myoclonus is accompanied by weight loss, even when insomnia is mild and familial history is negative. In China, although diagnostic criteria for FFI have been proposed, a number of family had been reported, due to constraints of various conditions, lack of appropriate diagnostic tools and experience, the diagnosis might still be missed.

Because the disease duration is mostly prolonged and signs typical of CJD are either absent or seen only late in the disease. Findings of routine laboratory tests and technical investigations are usually normal or nonspecifically changed and do not serve as further clues for diagnosis. A study demonstrates that multi-sequences of magnetic resonance can detect prion-induced gliosis in vivo, as confirmed by a neuropathologic examination performed only a few days after radiological examination [17]. Positron emission tomography (PET) studies can help support FFI diagnosis but are rarely performed.

The diagnosis of FFI, a hereditary disease, is of high relevance for other family members, especially when no family history is given or when family history is negative. Failure to diagnose

prion disease may be of crucial importance because hygienic measures must be taken to prevent prion transmission. FFI should be considered in a patient without apparent sleep disturbances if a combination of cognitive or psychiatric deficits with ataxia, visual disturbances, or myoclonus is accompanied by weight loss and vegetative signs. Polysomnography and genetic testing after comprehensive genetic counseling should be performed. A certain level of clinical diagnostic accuracy regarding results expected from genetic testing is of benefit to patients. A targeted screening for the D178N mutation of PRNP may help establish an early FFI diagnosis.

Neuroleptics and benzodiazepines may contribute to the clinical worsening as well. In particular, neuroleptics may aggravate catatonia, leading to a malignant form that some authors relate to neuroleptic malignant syndrome [15]. However, because neuroleptics, antidepressants, and benzodiazepines may induce transient benefit, such aggravation was not described in FFI cases or in vCJD. In our patient, she was first treated as an outpatient with flupentixol, risperidone, alprazolam, olanzapine. But she felt worse and the general condition worsened progressively. Therefore, benzodiazepines, neuroleptics, and electroconvulsive therapy may worsen catatonia and clinical course in some neurologic diseases.

For our patient, in addition to the typical clinical manifestations, it is special in that the incentive of mental stimulation, prominent psychiatric symptoms and sudden death; The relative present D178N mutation as an asymptomatic carrier, and autopsy and brain pathology information were important for a better understanding of this disease.

### Acknowledgements

The work reported here was supported by the Natural Science Foundation Key Project of Hubei Province (Grant No. 2009CDA070), and WuHan Science and Technology Bureau (Grant No. 201161038342-03), China.

### Disclosure of conflict of interest

None.

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