



Fatal Familial Insomnia: Insight of the Most Common Genetic Prion Disease in China Based On the Analysis of 40 Patients

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ABSTRACT

Fatal familial insomnia (FFI) is one of the subtypes of human genetic prion diseases and is the most frequently identified genetic prion disease in China. In this study, the epidemiological, clinical, laboratory and genetic features of 40 Chinese FFI patients were systematically analyzed. Despite of the distribution in 12 provinces, apparently more cases located in Henan and Guangdong provinces. The median onset age was 51 year-old (from 19-70 y) and the gender ratio was 1:1.5 (M:F). 45% cases displayed clinical symptoms less than 50 y and 32.5% cases did not have definitely disease family history. Sleeping disturbances, mental problems and recognition disorders were most common foremost symptoms, in which sleeping disturbances and recognition disorders were significantly frequent in the patients with positive family history and those with the onset age ≥ 50 y, respectively. Sympathetic symptoms, such as excessive sweating, salivation and minor evening pyrexia, were commonly noticed. 85% patients showed hypertension and 57.5% had obvious weight loss. 37.9% cases were cerebrospinal fluid (CSF) 14-3-3 positive and 25% were CSF RT-QuIC positive. Sporadic Creutzfeldt-Jakob disease (sCJD) associated abnormalities on MRI were recorded only in 6 patients with the onset-age ≥ 50 y. 126 individual from 14 families conducted prion gene (*PRNP*) sequencing and 36 asymptomatic carriers of D178N mutation and M129M homozygous were identified, among them 7 mutation carriers from 5 families were in the parent-generation and 5 from 3 families were elder siblings of the probands. The median survival was 10 months varying from 5 to 30 months. This is the largest study on FFI patients in China, as well as in Asia, which is helpful to enrich the knowledge of FFI.

Key words

Fatal familial insomnia, Genetic prion disease, *PRNP* sequencing, D178N mutation

Introduction

Fatal familial insomnia (FFI) is a special subtype of human genetic prion disease which prominent pathology is occurs mainly in the thalamus, showing severe astrogliosis and loss of neurons. Genetically, FFI is linked to a GAC to AAC point mutation (substitution from aspartic

acid to asparagines) at codon 178 of the prion protein gene (*PRNP*) (D178N) [1,2]. Same as other human genetic prion diseases, including genetic or familial Creutzfeldt-Jakob disease (gCJD or fCJD) and Gerstmann-Sträussler-Scheinker diseases (GSS), FFI is an autosomal dominant heredopathy. However, clinically

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FFI displays completely different phenotype, characterized by a disordered sleep-wake cycle, dysautonomia and motor signs. Pathologically, instead of spongiform degeneration and PrP^{Sc} deposits that are frequently observable in the brains of sporadic CJD (sCJD), which is the predominant type of human prion disease, and majority of human genetic prion disease, FFI is characterized by severe neuron loss and active astrogliosis in some brain regions, especially thalamus [3,4].

FFI was firstly described in an Italian family in 1986 [5]. Afterwards, FFI cases have been reported in many countries and regions, as well as in different races. FFI may occur without family history, or with definite clinical family history and/or genetic evidence [6-9]. Based on the surveillance data for human prion disease in China, FFI is most frequently detected genetic prion disease [10,11]. Up to June 2017, 40 FFI cases were diagnosed by the center of CJD surveillance system under the framework of China CDC, making up about 33% of all diagnosed genetic prion diseases. In this study, the epidemiological, clinical, genetic and laboratory characteristics of these 40 FFI cases were comparatively investigated based on the disease family history, gender and the onset-age. The RT-QuIC reactive profiles of the CSF samples from 39 patients and those of the brain samples from 3 patients were also proposed.

Materials and Methods

■ Study population and data collection

40 diagnosed D178N/M129M FFI cases via China CJD surveillance program were enrolled into this study. The general information, the clinical data, the results of MRI and EEG, and the results of CSF 14-3-3 and *PRNP* sequencing were obtained from the database of national CJD surveillance program. According to the protocols of the national CJD surveillance, all referred cases were conducted with *PRNP* sequencing. The geography distributions of the patients were determined on the places they registered as permanent addresses. EEG abnormality was recorded only with the presences of periodic sharp wave complexes (PSWC). MRI abnormality was recorded with the presences of high signal in caudate/putamen and/or symmetrical or dissymmetrical cortical ribbon syndrome on diffusion-weighted imaging (DWI). The survival time was evaluated from disease onset to death.

■ Laboratory tests

The specimens of blood, CSF and postmortem brain tissues of the suspected CJD patients were collected by physicians or pathologists in the local hospitals. CSF samples were obtained by routine lumbar puncture and employed into Western blot for protein 14-3-3. The peripheral blood leukocytes were isolated and the whole DNAs were extracted for sequencing analysis of *PRNP* and polymorphism of codon 129, using an automatic genetic analyzer (ABI3130XL). To avoid of DNA contamination in PCR and misreading in sequencing, all identified mutations were repeated at least one time using new blood samples. The standard operation procedure (SOP) of each assay was documented in the national CJD surveillance program described previously [10,12].

■ RT-QuIC

The detailed operating protocol was described elsewhere [13]. Briefly, 10 µl CSF sample was subjected into RT-QuIC reaction containing 10 µg purified recombinant hamster PrP protein (aa23-230), 10 mM PBS, 170 mM NaCl, 10 µM EDTA, 1 mM Thioflavin T (ThT), 0.001% SDS in final concentration, with a final volume of 100 µl. RT-QuIC was performed on FLUOStar Omega (BMG). The working conditions were temperature: 50°C; vibration speed: 700 rpm; vibration/incubation time: 90/30 sec; total reaction time: 90 h. 10⁻⁸ diluted brain homogenate of the hamsters infected with scrapie strain 263K were used as positive control and the same diluted brain homogenate of normal hamster was used as negative control. The cut-off value was set as the average value of the negative controls plus 3 times of SD. Each sample was duplicated in parallel. The sample was considered as positive when at least two parallel wells revealed positive reactive curves.

■ Statistical assays

The statistical analyses were performed using the *SPSS 11.5* statistical software program. Categorical variables were compared using the Chi-Square test. Probabilities (p value) less than 0.05 were considered to be statistical difference and less than 0.01 were considered to be significant difference.

■ Ethics Statement

Usage of the stored patients' information and samples in China CJD Surveillance Center has been approved by the Research Ethics

Committee of National Institute for Viral Disease Control and Prevention, China CDC. The written informed content of each case had been asked and signed by the family member or the relative of the patient according to the requirement of CJD surveillance.

Results

■ General information

Up to June 2017, 40 FFI cases were recognized and diagnosed in China CJD Surveillance Center. All 40 FFI cases were Han-Chinese. According to the data of China CJD surveillance started officially since 2006, D178N FFI was the most commonly identified human gPrD in Chinese [10,11], making about 33% of all diagnosed

gPrD. All 40 FFI were M129M homozygous. No polymorphisms of M129V or V129V were identified. Analysis of the permanent addresses of the patients revealed regional-associated tendency. 15 cases were from Henan (HN) province, 9 from Guangdong (GD), 4 from Hebei (HB), 2 from Shandong (SD), Jiangsu (JS) and Anhui (AH), 1 from Beijing (BJ), Zhejiang (ZJ), Jiangxi (JX), Guizhou (GZ), Qinghai (QH) and Hainan (HI), respectively (Figure 1A). The gender ratio (M: F) was 1:1.5 (16:24 cases). The onset ages varied from 19 to 70 year-old (y), with the median of 51 y. Majority of the patients (14 cases) was in the group of 50-59 y, followed by the groups of 40-49 y (7 cases) and 20-29 y (7 cases) (Figure 1B).

27 out of 40 FFI cases (67.5%) had positive family histories obtained by careful interview and/or

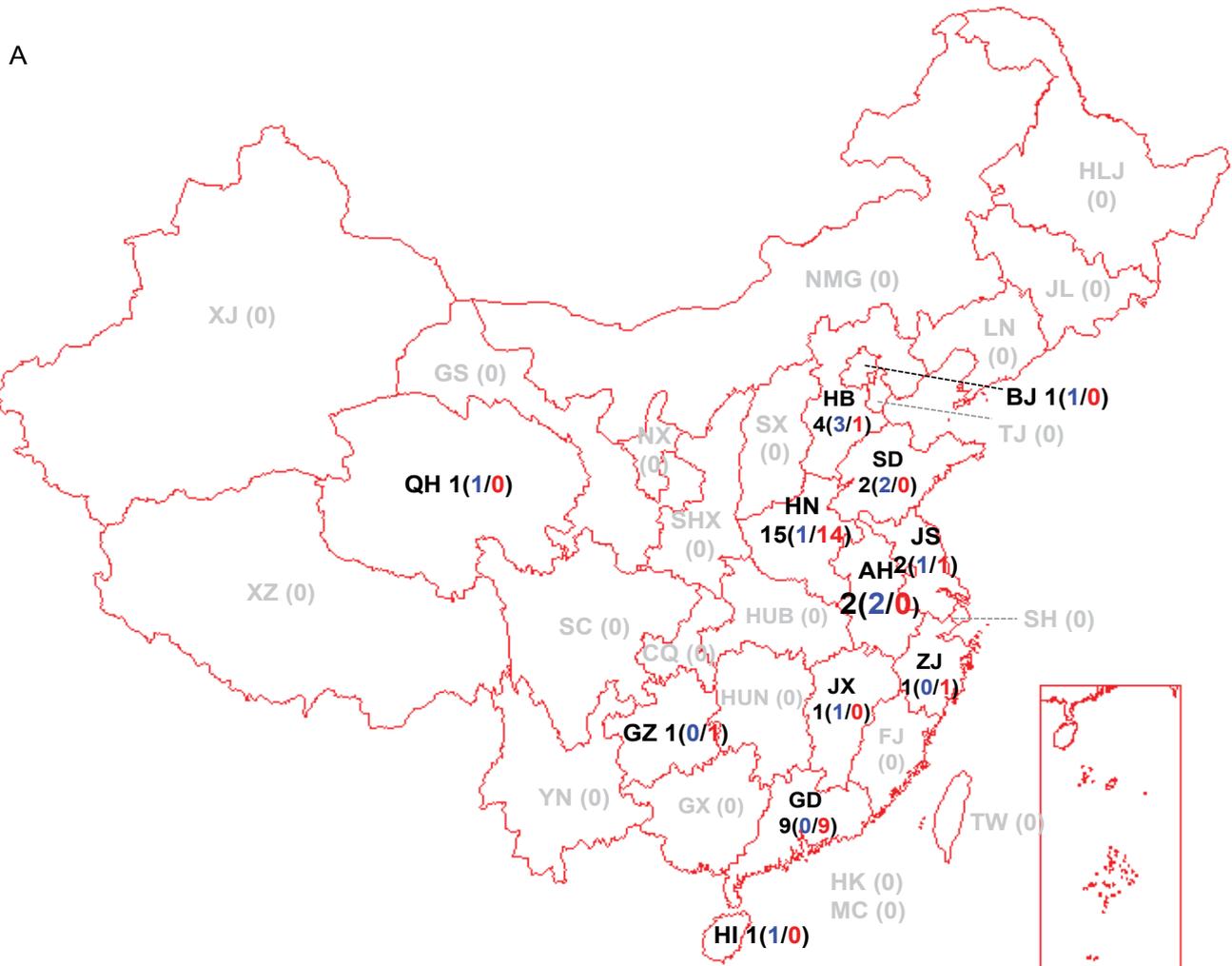


Figure 1: Geographic and onset-age distributions of 40 Chinese D178N/M129M FFI patients. A. Geographic distribution based on the permanent addresses. The case number each province is shown inside, with the blue number as the patients without disease family history and red number as those with positive family history. B. Onset age distribution. The medians of the onset age in total and in different groups are shown in the top right.

PRNP sequencing, while 13 (32.5%) cases did not (Table 1). Male patients distributed equally in the groups with and without family history (8:8 cases), while more female patients were in the group with family history (19:5 cases) but without statistical difference (P=0.0536). The onset ages of the cases without family history (median 42 y, ranging from 24-57 y) seemed to be younger than those with family history (median 53 y, ranging from 19-70 y). No FFI case without family history over 60 y was found (Figure 1B). Analysis of the onset ages between the groups with and without family history and between that of gender did not revealed statistical difference.

■ Clinical features

The foremost symptoms of FFI cases varied largely, most patients appeared more than one symptom at onset. As shown in Table 1, mental problems (such as anxiety, dystropy, irritability and emotional lability) were reported in 50% (20/40) cases. Higher ratio of the cases without family history (61.5%, 8/13) described mental symptoms than those with family history (44.4%, 12/27). Sleep disturbances (such as insomnia and hypersomnia) were reported in 40% (16/40) cases, with more ratio in the cases with family history (51.6%, 13/27) than those without family history (15.4%, 2/13), showing statistical difference (P=0.0274). Also, 40% (16/40) of FFI recorded cognition disorders (such as memory decline, progressive dementia), showing 53.8% (7/13) in the cases without family history and 33.3% (9/27) in those with family history. 25% (10/40) cases

described movement disturbances (such as gait instability), which distributing in 30.7% (4/13) cases without family history and 22.2% (6/27) cases with family history. Additionally, vision problems were reported in 4 cases, dizzy in 3 cases and fever in 3 cases. Cognition disorders were more frequently reported in the patients over 50 y (59.1%, 13/22) than those younger than 50 y (16.7%, 3/18), with statistical difference (P=0.0064). The positive ratios of mental problems, sleep disturbances, and movement disorders were 44.4%, 44.4% and 22.2% in the group of younger patients (<50 y), as well as 54.5%, 36.4% and 27.3% in that of older ones (≥ 50 y), respectively. No statistical differences in foremost symptoms were addressed between male and female cases.

Along with the disease progression, more neurological symptoms and signs appeared. sCJD-associated symptoms (dementia, pyramidal and extrapyramidal symptoms, myoclonus, visual or cerebellar disturbance and mutism) were noticed in most of the patients during the clinical courses. Sympathetic symptoms were also reported frequently. Excessive sweating and minor evening pyrexia were observed in 55% (22/40) and 47.5% (19/40) patients, respectively. Eight cases (20%) recorded salivation clearly. Comparison of the sympathetic symptoms in the groups between the patients with and without family history, between the cases <50 and ≥ 50 y at onset, and between male and female patients did not reveal significant different (Table 1). Obvious weight loss was reported in 57.5% (23/40) cases, without statistical difference between various

Table 1: The main clinical and laboratory features of Chinese FFI patients.

Clinical manifestations	Total (n=40)	Family history			Age of onset			Gender		
		With (n=27)	Without (n=13)	P value	<50 (n=18)	≥50 (n=22)	P value	Male (n=16)	Female (n=24)	P value
Age of onset (median)	51 y (19-70)	53 y (19-70)	42 y (24-57)	0.3403	-	-	-	50 y (24-62)	51.5 y (19-70)	0.6410
Foremost symptoms										
Mental problems	20 (50.0%)	12 (44.4%)	8 (61.5%)	0.3111	8 (44.4%)	12 (54.5%)	0.5250	9 (56.3%)	11 (45.8%)	0.5186
Sleeping disturbances	16 (40.0%)	14 (51.6%)	2 (15.4%)	0.0274	8 (44.4%)	8 (36.4%)	0.8084	7 (43.8%)	9 (37.5%)	0.6926
Recognition disorders	16 (40.0%)	9 (33.3%)	7 (53.8%)	0.2148	3 (16.7%)	13 (59.1%)	0.0064	6 (37.5%)	10 (41.7%)	0.7921
Movement symptoms	10 (25.0%)	6 (22.2%)	4 (30.7%)	0.5587	4 (22.2%)	6 (27.3%)	0.7136	3 (18.8%)	7 (29.2%)	0.4560
Sympathetic symptoms										
Excessive sweating	22 (55.0%)	14 (51.9%)	8 (61.5%)	0.5640	11 (61.1%)	11 (50.0%)	0.4822	9 (56.3%)	13 (54.2%)	0.8967
Salivation	8 (20.0%)	5 (18.5%)	3 (23.1%)	0.7356	4 (22.2%)	4 (18.2%)	0.7506	3 (18.8%)	5 (20.8%)	0.8717
Minor evening pyrexia	19 (47.5%)	11 (40.7%)	8 (61.5%)	0.2173	10 (55.6%)	9 (40.9%)	0.3560	8 (50.0%)	11 (45.8%)	0.7960
Weight loss	23 (57.5%)	17 (63.0%)	6 (46.2%)	0.9305	11 (61.1%)	12 (54.5%)	0.6760	10 (62.5%)	13 (54.2%)	0.6014
High blood pressure	34 (85.0%)	23 (85.2%)	11 (84.6%)	0.9622	17 (94.4%)	17 (77.3%)	0.1302	14 (87.5%)	20 (83.3%)	0.7176
CSF 14-3-3	14 (35.9%) ^a	9 (34.6%) ^a	5 (38.5%)	0.8134	7 (41.2%) ^a	7 (31.8%)	0.5457	8 (50.0%)	6 (26.1%) ^a	0.1256
CSF RT-QuIC	10 (25.0%)	7 (25.9%)	3 (23.1%)	0.8454	7 (38.9%)	3 (13.6%)	0.0665	5 (31.3%)	5 (20.8%)	0.4560

^a: One case in the group did not received CSF-14-3-3 test.

groups (Table 1). Hypertension was frequently noticed. Based on the normal values of blood pressure for various ages of Chinese, 85.0% FFI cases (34/40) showed high blood pressure (systolic and/or diastolic pressure) during the clinical courses. The younger patients looked to have higher ratio (94.4%, 17/18) of high blood pressure than the old ones (77.3%, 17/27), but without statistical difference (Table 1, Figure 2).

■ Clinical examination and laboratory tests

39 patients received EEG examination at least one time during hospitalization. None of them revealed typical PSWC. All 40 cases took MRI scanning and five of them showed the sCJD-associated abnormalities. Two cases showed high signal in caudate/putamen, one with high signal in right thalamus, one with high signals in caudate/putamen and bilateral thalamus, one with dissymmetrical cortical ribbon syndrome on DWI. Notably, all five cases with MRI abnormality were in the group of older patient (two 57 y, two 61 and one 65 at onset), showing statistical difference ($P=0.0305$). Western blot for CSF 14-3-3 was performed in 39 patients and the positive bands were detected in 35.9% (14/39) tested samples. Male patients in this study seemed to have higher positive rates (50%, 8/16) than the female (26.1%, 6/23), but without statistical significance ($P=0.1256$, Table 1).

The CSF samples of 40 FFI patients were subjected into RT-QuIC assays using full-length recombinant hamster PrP (23-231) as substrate. Each sample underwent three times of RT-QuIC. The patient showing positive reactions at least in two independent tests was considered to be positive in CSF RT-QuIC. Out of 40 cases, 10 (25%) were positive in CSF RT-QuIC. The converting times of the positive samples varied from 18 to 39 h post-reaction and the maximal ThT values were from 29500 to 110000 rfu (Figure 3). Analysis of the distribution of the cases with positive CSF RT-QuIC in the patients with and without family history, <50 and ≥ 50 y, and male and female did not Figure out any statistical difference (Table 1).

■ Survival time

All FFI patients were carefully followed-up. Up to the end of June 2017, 33 cases have died with definite date, 4 were still alive and 3 were lost contact. The clinical durations of 33 dead cases varied largely from 5 months to 30 months,

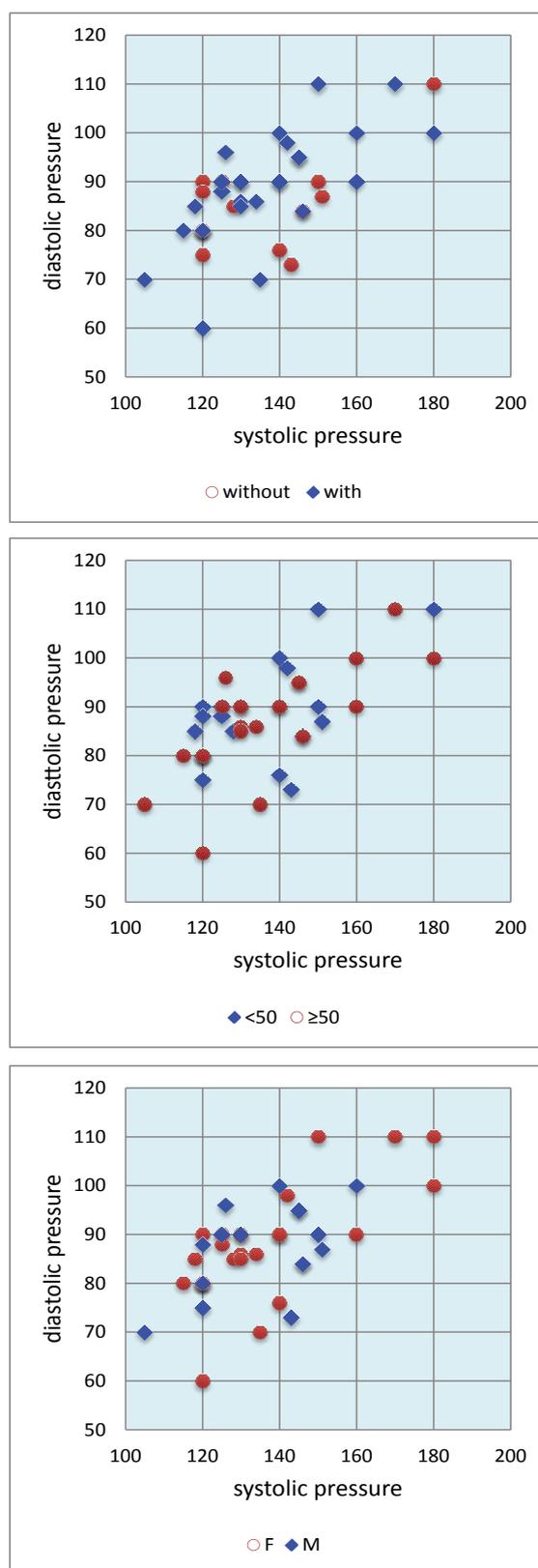


Figure 2: The blood pressures of 40 FFI patients based on different groups. Left panel: with or without disease family history; Middle panel: <50 or ≥ 50 y at onset; Right panel: male and female patients. The highest recorded blood pressures of 40 FFI cases during the clinical courses are taken. X-axis: systolic pressure; Y-axis: diastolic pressure.

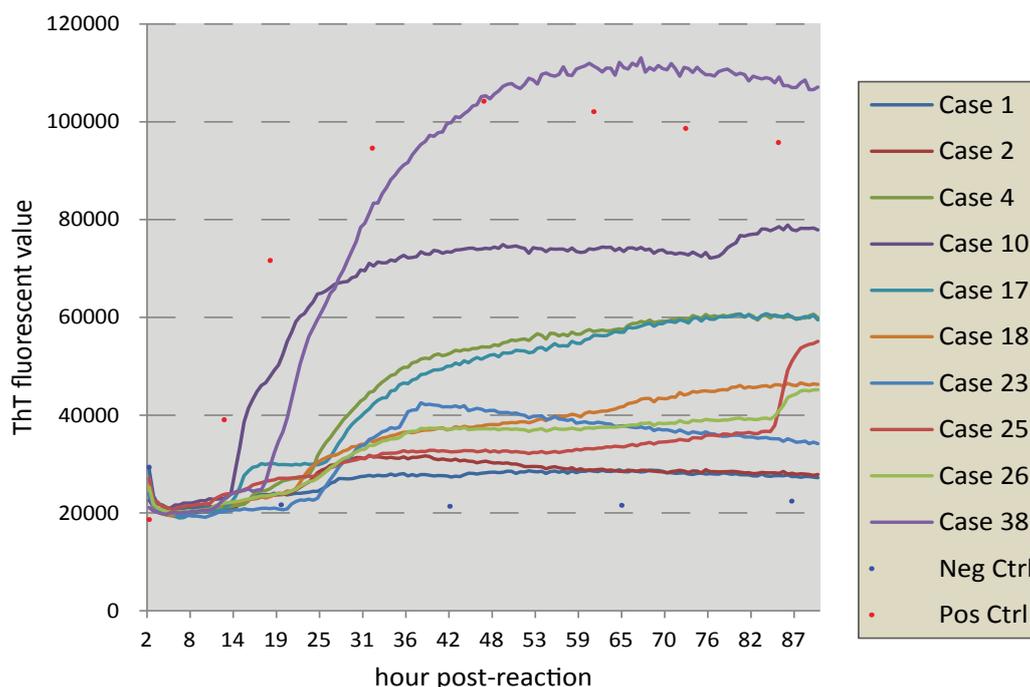


Figure 3: The positive reactive curves of 10 FFI cases in CSF RT-QulC. The positive control (red dot line) is 10^{-5} diluted brain homogenate of the hamster infected with scrapie agent 263K and the negative control (blue dot line) is 10^{-5} diluted brain homogenate of normal hamster.

with the median of 10 months. 75.6% of the cases (25/33) died within one year after onset, while only one case lived longer than two years. Although the patients with family history, the patients <50 y at onset and female patients had relatively long median of survival, statistical assays did not show significance (Figure 4).

■ **PRNP sequencing and family history**

All 40 FFI cases were confirmed to have a D178N mutation in one PRNP allele genetically, while methionine homozygous genotype at codon 129 (M129M). Clinical interviews a part of cases came from the same blood ties, but was reported and diagnosed independently. As described previously, Case 1 and Case 2 were uncle and niece [14]. Case 14 was half-blood aunt of Case 7, who appeared symptoms 3 years later at the age of 61. Case 24 (onset age: 27 y) was the niece of Case 11 (onset age: 42), who had clinical symptoms 3 years late.

Besides of the probands, totally 126 family members across 3 to 5 generations from 14 families also donated the blood samples for PRNP sequencing. The blood samples of all tested healthy individuals from one family were usually collected in the health section and transferred to our laboratory for sequencing assays simultaneously. Therefore, the tested samples

contained the individuals whose parents (usually were proband’s siblings or uncle/aunt) were wild-type PRNP genotype verified by subsequent sequencing assays. Totally, 36 individuals were confirmed to be D178N carriers with M129M homozygous. Further, the tested family members and the mutation carriers were divided into different generations according to the proband’s blood ties, including the generations of grandparent, parent (including the parent and parent’s sibling), proband (proband’s sibling), son (proband’s own children) and grandson (proband’s own grandchildren). Only two grandparents (Case 6 and Case 33) conducted PRNP sequencing with wild-type genotype. In parent-generation, 15 individual received the tests and 8 (53.3%) contained the D178N mutation. In proband-generation, 17 proband’s sibling (besides of proband) were tested and 8 (47.8%) were D178N positive. In son- and grandson-generations, 18 and 15 individuals were tested and 9 (50.0%) and 6 (40.0%) were confirmed to have the D178N mutation, respectively. The rest five D178N carriers were the cousins or nephews of probands [14,15].

Except for one mutation carrier (the elder brother of Case 7) who appeared typical clinical symptoms and died more recently (not included in this study), the rest of the mutation carriers

maintained healthy till the last follow-up (varied according to each case). 7 individuals from 5 families were in the parent-generation, including the mothers of Case 2, Case 5, Case 6 and Case 24, the father and two uncles and one aunt of Case 33. Five elder siblings of the probands of 3 families were also asymptomatic mutation carriers, including one elder sister of Case 1, two sisters of Case 2 and two brothers of Case 33.

Discussion

In this study 40 Chinese patients with D178N FFI are systematically analyzed for the features of epidemiological, clinical, laboratory and examination, genetic and family history, as well as survival time. To our knowledge, it is largest study of Chinese FFI patients. Based on our surveillance data for human prion diseases, more than 120 cases of genetic prion diseases have been identified, covering 15 different subtypes of *PRNP* mutations. D178N FFI is the most frequently observed genetic prion disease among Chinese [11]. In general, the epidemiological, clinical, laboratory characteristics of Chinese FFI patients are similar as Caucasian patients [4,6,16], with relatively early onset age, obvious sympathetic symptoms and relatively long clinical duration compared with the features of sCJD. High blood pressure and weight loss are also frequently observed.

Among the 40 Chinese FFI cases in this study, there are more female patients than male ones. No special gender-associated difference in clinical and examination items has been found. 45% FFI cases appeared the clinical manifestations below 50 y, showing significant less chance to have recognition problems as the foremost symptoms. Despite of less frequent, sCJD-associated abnormalities on MRI are observed only in the group of old patients (elder than 50 y). Roughly 1/3 of the FFI patients deny disease-related family history during interview in hospitalization and follow-up survey. Compared with the patients with positive family history, they have obviously fewer proportions to have sleeping disturbances as the foremost symptoms. The clinical durations of the patients with positive family history, the patients <50 y at onset and female patients are slightly longer, but do not display significance in statistical analysis.

Like the FFI cases reported worldwide [4,6,17,18], none of 40 Chinese patients recorded PSWC on EEG and a few of them appeared sCJD-associated abnormalities on

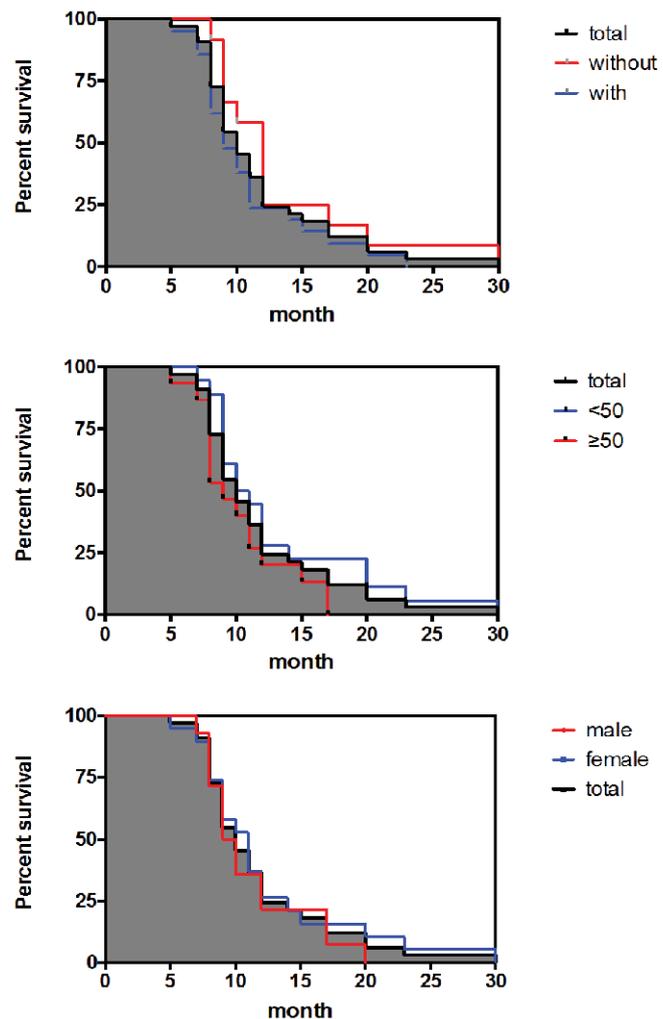


Figure 4: The survival times of 40 FFI patients based on different groups. Upper panel: with or without disease family history; Middle panel: <50 or ≥ 50 y at onset; Lower panel: male and female patients. The median of survival is indicated in the top right.

MRI. More recently, Grau-Rivera et al have screened 15 patients with CJD and 5 patients with fatal insomnia (FI) using a so-called “3-Tesla magnetic resonance imaging scanner” and proposed the possibility of quantitative MRI in FI cases [19]. It might be an interesting topic for further observation. About 1/3 FFI cases were CSF 14-3-3 positive and 25% were CSF RT-QuIC positive, whose positive ratios are remarkably lower than that of another two most frequent Chinese genetic prion diseases, T188K gCJD and E200K gCJD (unpublished data). Compared with the data of the patients with E200K- and V201I-gCJD, apparently lower positive rate of D178N FFI cases in CSF RT-QuIC are also described elsewhere [20]. No correlation has been addressed between CSF 14-3-3 positive and CSF RT-QuIC positive.

Three FFI cases have been confirmed to have relative weak but definite proteinase K (PK)-resistant PrP^{Sc} in the postmortem brain tissues [14,15,21]. The RT-QuIC assays with the brain homogenates from temporal lobes of those three cases at the same experimental condition also show positive, even in the high dilution (10⁻⁷ diluted brain homogenates) (data not shown). However, only two of these three patients show the positive reaction in CSF RT-QuIC. The correlation between presence of brain PrP^{Sc} and reactivity in CSF RT-QuIC in FFI patients needs further study with more paired brain/CSF samples.

These 40 FFI cases distribute in 12 provinces based on their permanent addresses. However, there are marked more cases in Henan and Guangdong provinces, highlighting a geographic-associated tendency. Interestingly, most (14 out of 15) of the cases from Henan province and all 9 cases from Guangdong province recorded positive family history. Even in a county of Henan province, 6 FFI cases with family history have been identified. Our further investigation have traced definite blood ties for six patients that belong to three large family with different surnames, despite of onset in different places and reported from different hospitals at different times. Such geographic and family clusters may highlight the possibility that the genotype of D178N/M129M in Han-Chinese may have common origins of the chromosome carrying this mutation. Such regional-associated high incidences of FFI are also observed in some European countries, such as Italy and Spain [22,23]. However, since there were several large waves of migrations from Northern to Southern over last two thousands year in China history, it is extremely hard to Figure out such possibility, especially the patients between Henan and Guangdong provinces that are two thousands kilometers apart.

Asymptomatic carriers with D178N mutation are commonly found among the FFI families [14, 24]. In this study, 33 mutation carriers have been identified out of 114 tested individuals from 14 families. Except one person who appeared symptoms and died more recently, the rest mutation carries do not report to have the typical clinical manifestations even for more than 10 years after *PRNP* sequencing. Particularly, many asymptomatic carriers are the parents or the individuals of the last generation of the probands, or elder brothers and sisters of the

probands. D178N mutation is described to be extremely strong pathogenicity, being consistent with up to 100% penetrance, together with other three mutations including P102L, A117V and E200K based on large population control cohorts [25]. Although D178N mutation causes spontaneous disease in mouse models [26-28], many studies of multiple multigenerational families have revealed asymptomatic carriers [9,24]. It is obvious that besides of special D178N mutation, other unknown factor(s) may also influence the onset of the disease.

FFI is one of the genetic prion diseases that show high ratio of positive family history. Based on the EURO-CJD collaborative surveillance project from 1993 to 2002, 88% of FFI patients have positive family history [6]. A German study covering 41 FFI patients illustrates 65% positive family history [29]. Coincidental with the previous studies on Caucasian population, the about 2/3 (67.5%) Chinese FFI cases show definite positive family history. In contrast, FFI is not frequently reported in Japan [30] and all 4 Japanese FFI cases enrolled in the study finished by Minikel and the colleagues do not have family history [25]. FFI is also less frequent in Korea that only one case has been reported [31,32]. Certainly, determination of disease-associated family history depends largely on the careful inspection and communication clinically and good follow-up survey. Whether there are some unknown reasons that may affect the family history among different countries and races is not clear. Lacking of positive family history may increase the difficulty in the clinical diagnosis for FFI. Although some other clinical examinations, such as polysomnography and PET, seem to be helpful for FFI diagnosis clinically [18,29,33], they are not routinely accessible in a number of hospitals. Therefore, *PRNP* sequencing is still indispensable tool for the diagnosis of FFI.

Acknowledgements

We appreciate all staffs in China CJD surveillance system for their great work. We also thank all referring patients and their family members for supplying the required information and donating specimens. This work was supported by Chinese National Natural Science Foundation Grants (81630062, 81572048), National Key R&D Program of China (2016YFC1202700, 2017YFC1200500) and SKLID Development Grant (2015SKLID503 and 2016SKLID603).

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