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Characteristics of Chinese patients with genetic CJD who have E196A or E196K mutation in *PRNP*: comparative analysis of patients identified in the Chinese National CJD Surveillance System

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ABSTRACT

Objective Two different mutations at codon 196, namely E196A and E196K, have been reported to be related to genetic Creutzfeldt-Jakob disease (CJD). We aimed to comparatively analyse the features of Chinese patients with these two mutations from the CJD surveillance system in China.

Design and setting Comparative analysis of patients identified via the Chinese National CJD Surveillance System during the period 2006–2020.

Participants 16 Chinese patients with genetic CJD with E196A mutation and 5 with E196K mutation.

Methods Neurological examination, EEG and MRI, western blot, gene sequence, and RT-QuIC.

Results The age of onset of E196K genetic CJD cases (median of 61 years) was older than the E196A cases (median of 67 years). Generally, these two subtypes of genetic CJD were more like sporadic Creutzfeldt-Jakob disease (sCJD) clinically. The E196A cases showed more major symptoms, while those of E196K cases were restricted to dementia and mental problems. During progression, more sCJD-associated symptoms and signs gradually appeared, but none of the E196K cases showed cerebellum and visual disturbances. Typical periodic sharp wave complexes on MRI were recorded in 25% of E196A cases but not in E196K cases. sCJD-associated abnormalities on MRI, positive cerebrospinal fluid (CSF) 14-3-3 and increased CSF total tau were observed frequently, ranging from two out of three cases to four out of five cases, without a difference. Positive CSF RT-QuIC was detected in 37.5% (6 of 16) of E196A cases and 60% (3 of 5) of E196K cases. The duration of survival of E196K cases (median of 4.5 months) was shorter than the E196A cases (median of 6.5 months). Moreover, female cases and cases with young age of onset (<60 years) in E196A displayed longer survival time than male patients and cases with older age of onset (≥60 years).

Conclusions This is the largest comprehensive report of genetic CJD with mutations at codon 196 to date, describing the similarity and diversity in clinical and laboratory tests between patients with E196A and with E196K mutations.

Strengths and limitations of this study

- This is the largest comprehensive report of genetic Creutzfeldt-Jakob disease (gCJD) with mutations at codon 196 to date.
- The study provides clinical, genetic and laboratory data on E196A and E196K gCJD, which is a rare disease.
- Mutations in E196K have been described in Caucasians and Asians, while mutations in E196A seem to be reported only in Chinese.
- There was a limited number of enrolled patients (N=21) in the data set, especially of cases with E196K mutation, which may have resulted in observational bias.
- The pathogenic prion protein (PrP^{Sc}) and neuropathological features of Chinese patients with E196A and E196K gCJD remain unclear due to lack of brain specimens from patients.

INTRODUCTION

Genetic human prion diseases, caused by different mutations in the prion protein (PrP)-encoding gene *PRNP*, account for approximately 10%–15% of human prion diseases. Genetic human prion diseases have various medical terms based on their clinical and neuropathological phenotypes, that is, genetic Creutzfeldt-Jakob disease (gCJD), Gerstmann-Straussler-Scheinker syndrome and fatal familial insomnia, which are closely related to different mutations within *PRNP*.[1 2] In the context of one same disease term, such as gCJD, the clinical, neuropathological and laboratory features may vary according to mutations at different positions.[3 4] Different mutations at the same position lead to substitution of different amino acids, subsequently displaying different phenotypical features, for example, mutations at codons 105, 188 and 196.[4]

Two different mutations at codon 196 have been reported to be related to gCJD: E196A and E196K.[4–8] In a surveillance activity conducted by the Chinese National Surveillance for CJD (CNS-CJD) in Chinese mainland, 16 Chinese gCJD cases with E196A mutation and 5 cases with E196K mutation have been identified in the past 10 years.[9 10] Among them, E196A gCJD was the fourth most frequently observed genetic prion disease in China (Shi *et al*, unpublished data, 2020). In this study we comparatively analysed the clinical and laboratory characteristics of Chinese gCJD cases with E196A or E196K mutation. Both types of E196 gCJD revealed sporadic Creutzfeldt-Jakob disease (sCJD) like phenotype in general, while showing differences in some clinical and laboratory features.

MATERIALS AND METHODS

Data collection

As described previously,[10 11] clinical data of patients suspected of CJD were collected by neurologists at the local hospitals while epidemiological data were collected by staff from the local provincial Chinese Centers for Disease Control and Prevention (CDCs) from 2006 to 2020. The final diagnosis was given by an expert team consisting of neurologists, epidemiologists and laboratory staff based on the diagnostic criteria for CJD issued by the Chinese National Health Commission in 2017. Follow-up surveys of patients were conducted by the staff at the centre of CNS-CJD via telephone and/or WeChat.

Western blot for 14-3-3 protein in CSF

Cerebrospinal fluid (CSF) samples were mixed with 5× loading buffer and boiled for 8 min. Proteins were separated in 15% sodium dodecyl sulfonate (SDS)-polyacrylamide gel electrophoresis (PAGE) and electronically transferred onto nitrocellulose membranes (Whatman, Pittsburgh, Pennsylvania, USA) by semidry method in a transfer buffer and immunoblotted with anti-14-3-3 polyclonal antibody (1:1000 dilution; Santa Cruz Biological). Reactive signals were visualised using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA).

PCR and sequencing assays for the *PRNP* gene

Genomic DNA was extracted from peripheral blood leucocytes using a commercial kit (QIAGEN, Germany). One hundred nanograms of the extracted DNA were amplified by PCR using specific *PRNP* primers (forward primer: 5′-GGC AAA CCT TGG ATG CTG G-3′ and reverse primer: 5′-CCC ACT ATC AGG AAG ATG AGG3′).[10] Sequencing analysis of the *PRNP* gene was conducted according to standard operating procedure.[10]

ELISA for total tau in CSF

The amount of protein tau in the CSF samples was quantitatively measured by a commercial ELISA kit (81572; Innotest hTau-Ag, Belgium). Briefly,[12] 25 µL of the CSF sample were diluted with the buffer supplied by the manufacturer and added to the wells of the antibody-coated plate in duplicate. The plate was incubated at room temperature (RT) overnight. After washing five times, 100 µL of peroxidase, horseradish (HRP)-conjugated detection antibodies were added to each well and incubated at RT for 30 min. Absorbance at 450 nm in each well was measured by a microplate reader (Perkin Elmer, USA) after developing with 100 µL substrate working solution for 30 min in dark and terminated with 2M H_2SO_4. Tau concentrations in the tested CSF samples were calculated based on a tau standard curve.

RT-QuIC assays

Real-time quaking induced conversion (RT-QuIC) assay was performed according to the working procedures described previously.[13] Briefly, each reaction contained 10 µg of a recombinant hamster protein (rHaPrP90-231), 1X phosphate buffer saline (PBS), 170 mM NaCl, 1 mM EDTA, 0.01 mM thioflavin T (ThT) and 0.001% SDS, together with 15 µL of the CSF sample, in a final volume of 100 µL. The assay was conducted in a black 96-well, optical-bottomed plate (Nunc, 265301) on a BMG FLUOstar Plate Reader (BMG LABTECH). The following were the working conditions: temperature, 55°C; vibration speed, 700 revolution per minute (rpm); vibration/incubation time, 60/60 s; and total reaction time, 60 hours. The ThT fluorescence value (excitation wavelength, 450 nm; emission wavelength, 480 nm) of each reaction was automatically counted every 45 min and further presented as relative fluorescence units. Each sample was tested in quadruplicate simultaneously. The cut-off value was set as the mean value of the negative controls plus 10 times the SD. A sample was considered to be positive when ≥2 wells revealed positive reaction curves. A 10^{-5} diluted brain homogenate of the scrapie agent 263K-infected hamster was used as the positive control, while 10^{-5} diluted brain homogenate of the normal hamster was used as the negative control.

Statistical assays

Statistical analyses were performed using the SPSS V.11.5 statistical software program.

Patient and public involvement

No patients were involved.

RESULTS

General information

Since 2006, more than 200 cases of genetic human prion diseases have been identified and diagnosed via CNS-CJD, which consisted of 19 different subtypes of mutations in *PRNP*.[9 10] Among them, 16 patients with E196A gCJD and 5 patients with E196K gCJD were identified via the Chinese National CJD Surveillance System during the period 2006–2020. The first E196A and E196K gCJD cases were reported in 2011 and 2009, respectively (figure 1A).

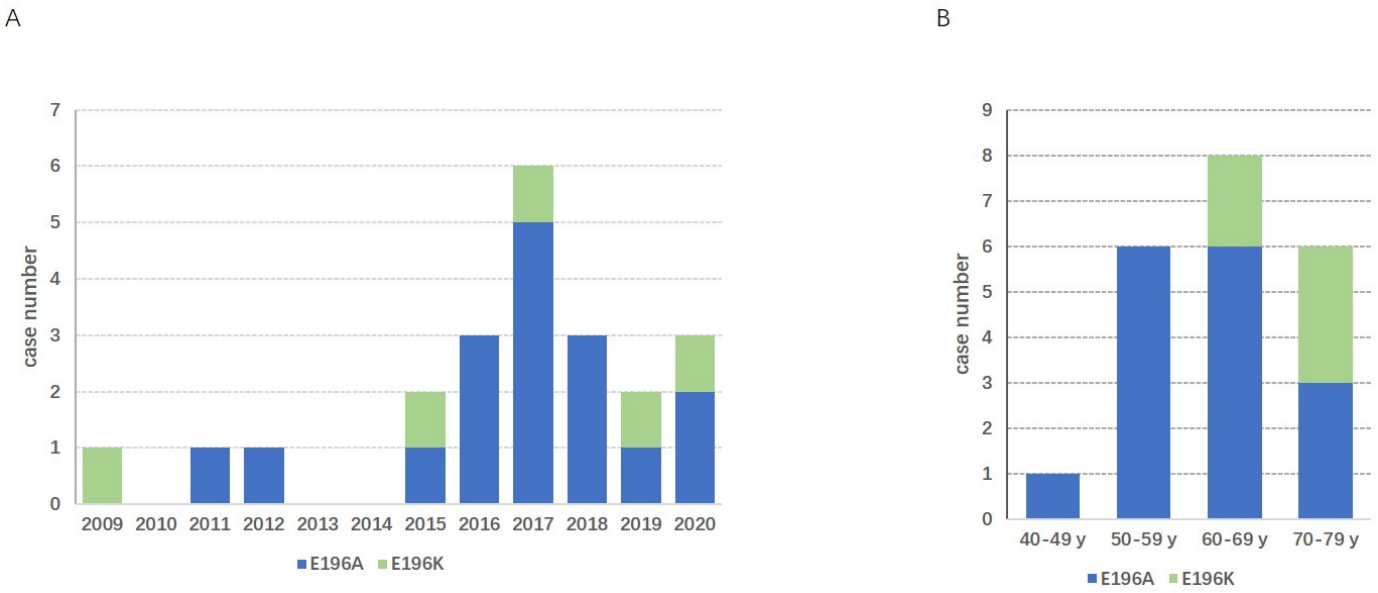

Figure 1 Distribution of Chinese patients with E196A and E196K genetic Creutzfeldt-Jakob disease based on (A) year of diagnosis and (B) age of onset.

Afterwards, more cases of E196A gCJD were diagnosed, particularly since 2015, and peaked in 2017. E196K gCJD cases were markedly less frequent, with one case reported in 2015, 2017, 2019 and 2020, respectively. The gender (male:female) distribution of E196A and E196K cases was 1:0.78 (9:7) and 1:1.5 (2:3). The age of onset of E196A cases varied from 43 to 76 years old, with a median of 61 years, while that of E196K cases varied from 61 to 77 years old, with a median of 67 years. The peak age of onset in E196A patients was 50–59 years and 60–69 years, which looked younger than that of E196K patients (figure 1B). Analysis of age of onset of patients based on gender found that the median age of onset among E196A male patients was older than of female patients (65 years vs 56 years), while the median age of onset among E196K male patients was younger than of female patients (62.5 years vs 73.5 years). No significant geographical-associated and occupational-associated phenomenon was observed.

Clinical features

The clinical, genetic and laboratory data of 16 cases of E196A gCJD and 5 cases of E196K gCJD are summarised in table 1. The intervals from onset to diagnosis varied largely, ranging from 1 to 13 months. Majority of the patients (18 of 21) were diagnosed within 6 months of onset, without notable differences between the E196A and E196K groups. Most data on clinical manifestations, examinations and laboratory tests of these patients were obtained during the period of hospitalisation and were referred to CNS-CJD. Some information was collected via follow-up surveys after discharge. Patients with E196A mutation displayed two to four major symptoms (table 2). Dementia (cognitive decline and memory loss) was the complaint in 68.8% (11 of 16) of cases, followed by mental problems (emotional lability and anxiety) in 62.5% (10 of 14), extrapyramidal dysfunction (unsteady gait, drooling and shaking of limbs) in 43.8% (7 of 16) and cerebellum disorder (ataxia, speech dysgraphia and dysmetria) in 43.8% (7 of 16). Three patients described cortical blindness and one complained of paraesthesia. A slight difference in major symptoms was observed between gender and between young (<60 years) and elderly (>60 years) patients, but without statistical significance. In contrast, five patients with E196K mutation showed fewer initial disorders, limited to dementia (4 of 5) and mental problems (2 of 5). Other symptoms were rarely recorded.

Along with progression, rapid progressive dementia was reported in all patients regardless of mutation, E196A or E196K. Other sCJD-associated symptoms and signs were also observed gradually. In the group of patients with E196A mutation, five patients were recorded to have four major sCJD-associated symptoms, seven cases with three symptoms and three cases with two symptoms. Only one case (case 9) did not show the four major symptoms, although the patient had clear mental problems (table 1). The rates of detection of myoclonus, cerebellum and visual disorders, pyramidal and extrapyramidal symptoms, and mutism were 68.8% (11 of 16), 81.3 (13 of 16), 87.5% (14 of 16) and 62.5% (10 of 16), respectively (figure 2A). In the group of patients with E196K mutation, all five patients showed myoclonic movement, while none reported cerebellum and visual disorders. Four cases displayed pyramidal and extrapyramidal symptoms and two had mutism (table 1 and figure 2B).

EEG and MRI features

All patients received electroencephalogram (EEG) and MRI examinations at least one time. In the group of patients with E196A mutation, four patients recorded typical periodic sharp wave complexes (PSWCs) on EEG, seven showed different abnormalities but without PSWCs, and another five cases had uncertain PSWCs (table 1 and

Table 1 Main features of Chinese patients with E196A and E196K gCJD

Type	Case	Gender, age of onset, province	Initial disorders	Dementia*	Other major CJD-associated problems†				EEG	MRI		CSF			Polymorphism		Duration (months)
					I	II	III	IV	PSWC	Ribbon-like signal	High signals in caudate/putamen	14-3-3	Total tau	RT-QuIC	Codon 129	Codon 219	
E196A	1	M, 70s, Jilin	Mental problem, dementia	+	+	+	+	–	+	NR	–	+	+	+	M/M	E/E	5
	2	F, 50s, Heilongjiang	Dementia, cerebellum disorder	+	–	+	+	–	NC	NR	+	+	–	+	M/M	E/E	24
	3	F, 50s, Zhejiang	Cerebellum disorder	+	+	+	+	+	–	+	+	+	+	–	M/M	E/E	17
	4	F, 60s, Shanghai	Paraesthesia, cerebellum disorder	+	+	+	+	+	NC	–	–	+	+	+	M/M	E/E	4
	5	M, 50s, Guangdong	Dementia, mental problem, extrapyramidal dysfunction	+	+	–	+	+	–	–	+	+	+	–	M/M	E/E	10
	6	M, 60s, Guangdong	Cerebellum disorder, alalia	+	+	+	+	–	–	+	+	+	+	–	M/M	E/E	3
	7	M, 72 years, Jilin	Dementia, mental problem, extrapyramidal dysfunction	+	+	–	+	+	NC	–	–	–	+	+	M/M	E/E	2
	8	M, 60s, Chongqing	Dementia, cerebellum disorder, extrapyramidal dysfunction	+	+	+	+	–	+	+	–	+	+	–	M/M	E/E	2
	9	M, 60s, Guangdong	Dementia, mental problem	+	–	–	–	–	–	+	+	–	–	–	M/M	E/E	Alive
	10	M, 60s, Chongqing	Dementia, cerebellum disorder, extrapyramidal dysfunction, mental problem	+	+	+	+	+	–	+	–	–	+	–	M/M	E/E	Lost
	11	F, 50s, Yunnan	Dementia, mental problem, extrapyramidal dysfunction	+	+	+	+	+	+	+	–	+	ND	–	M/M	E/E	28
	12	F, 70s, Fujian	Dementia, mental problem, extrapyramidal dysfunction	+	–	–	+	+	–	+	–	+	ND	+	M/M	E/K	8

Continued

Table 1 Continued

Type	Case	Gender, age of onset, province	Initial disorders	Dementia*	Other major CJD-associated problems†				EEG	MRI		CSF			Polymorphism		Duration (months)
					I	II	III	IV	PSWC	Ribbon-like signal	High signals in caudate/putamen	14-3-3	Total tau	RT-QuIC	Codon 129	Codon 219	
	13	F, 50s, Jilin	Dementia, mental problem, cortical blindness, extrapyramidal dysfunction	+	–	+	+	+	NC	–	–	+	ND	–	M/M	E/E	13
	14	M, 60s, Fujian	Dementia, cortical blindness	+	+	+	–	+	+	–	–	+	ND	–	M/M	E/E	5
	15	F, 40s, Zhejiang	Mental problem, cortical blindness	+	+	+	+	+	–	+	+	–	ND	+	M/M	E/E	Alive
	16	M, 50s, Sichuan	Dementia, cerebellum disorder, mental problem	+	–	+	+	–	NC	+	–	+	ND	–	M/M	E/E	Alive
E196K	1	F, 70s, Jilin	Dementia	+	+	–	+	+	–	–	–	–	+	+	M/M	E/E	5
	2	F, 70s, Beijing	Dementia	+	+	–	+	–	–	+	–	+	–	–	M/M	E/E	Lost
	3	F, 70s, Shanghai	Mental problem, dementia	+	+	–	+	+	–	+	+	+	+	–	M/M	E/E	3
	4	M, 60s, Hebei	Mental problem	+	+	–	–	–	–	+	+	–	ND	+	M/M	E/E	2
	5	M, 60s, Fujian	Dementia	+	+	–	+	+	–	+	–	+	ND	+	M/M	E/E	2

*Rapid progressive dementia.
†I: myoclonic movement; II: cerebellum and visual disturbances; III: pyramidal or extrapyramidal dysfunction; IV: akinetic mutism.
CJD, Creutzfeldt-Jakob disease; CSF, cerebrospinal fluid; EEG
, electroencephalogram; F, female; gCJD, genetic Creutzfeldt-Jakob disease; M, male; NC, uncertain; ND, not done; NR, not recorded; PSWC, periodic sharp wave complex; RT-QuIC, Real-time quaking induced conversion.

Table 2 Major symptoms of patients with genetic Creutzfeldt-Jakob disease with E196A or E196K mutation

Major symptoms	E196A					E196K		
	Total (n=16) n (%)	Male (n=9) n (%)	Female (n=7) n (%)	<60 years (n=7) n (%)	>60 years (n=9) n (%)	Total (n=5) n (%)	Male (n=2) n (%)	Female (n=3) n (%)
Dementia	11 (68.8)	7 (77.8)	4 (57.1)	5 (71.4)	6 (66.7)	4 (80.0)	1 (50)	3 (100.0)
Mental problem	10 (62.5)	6 (66.7)	4 (57.1)	5 (71.4)	5 (55.6)	2 (40.0)	1 (50)	1 (33.3)
Extrapyramidal dysfunction	7 (43.8)	4 (44.4)	3 (42.9)	3 (42.9)	4 (44.4)	0	0	0
Cerebellum disorder	7 (43.8)	4 (44.4)	3 (42.9)	3 (42.9)	3 (33.3)	0	0	0
Cortical blindness	3 (18.8)	1 (11.1)	2 (28.6)	2 (28.6)	1 (11.1)	0	0	0

figure 3A). The positivity rate of definite PSWC on EEG in E196A cases was 25%. In the group of patients with E196K, none of the five patients showed typical PSWCs (figure 3A). The sCJD-associated MRI abnormalities (ribbon-like signal on diffusion weighted imaging (DWI) and/or high signals in the caudate/putamen) were observed in 68.8% (11 of 16) of E196A cases and 80% (4 of 5) of E196K cases (figure 3B). Ribbon-like signals (9 out of 14 cases with E196A and 4 with E196K) were more frequently detected than high signals in the caudate/putamen (6 cases with E196A and 2 cases with E196K) (table 1).

PRNP gene sequencing

Mutations at codon 196 in one *PRNP* allele of the patients were verified by direct sequencing of the PCR products, which was routinely repeated at least two times with newly extracted DNAs. Sixteen suspected CJD cases contained a missense mutation at codon 196 of the *PRNP* gene, leading to a substitution of glutamic acid by alanine, while five cases had a mutation causing substitution of glutamic acid by lysine. No additional nucleotide exchanges were found in other regions of the *PRNP* sequences of those cases. All patients had methionine homozygosity at codon 129 (M129M). Out of 16 E196A cases, 15 had glutamic acid homozygosity at codon 219 (E219E) and 1 case (case 12) had glutamic acid/lysine heterozygosity (E219K). All five E196K cases had the sequencing data of codon 219, revealing E219E.

CSF protein 14-3-3 and tau

Lumbar puncture was conducted in all patients. Routine items (cells, proteins, glucose, electrolytes, etc) on CSF biochemistry were all in the normal ranges. Western blots for CSF 14-3-3 were positive in 75% (12 of 16) of E196A cases and 60% (3 of 5) of E196K cases (figure 3C). The total tau level in the CSF samples from 10 cases with E196A and 3 cases with E196K (table 1) was measured with a commercial ELISA kit and a tau level higher than 1400 pg/mL was considered positive based on previous studies.[12 14] Eight (out of ten) cases with E196A mutation and two (out of three) cases with E196K mutation were positive (figure 3D).

RT-QuIC features

All cases with E196A and E196K mutations in this study were subjected to RT-QuIC tests with 15 µL CSF sample each, using a recombinant truncated hamster PrP protein amino acid 90-231 (rHaPrP90-231) as the substrate. Under our experimental condition, 37.5% (6 of 16) of E196A gCJD cases and 60% (3 of 5) of E196K gCJD cases were positive on CSF RT-QuIC (figure 4 and table 1). There was

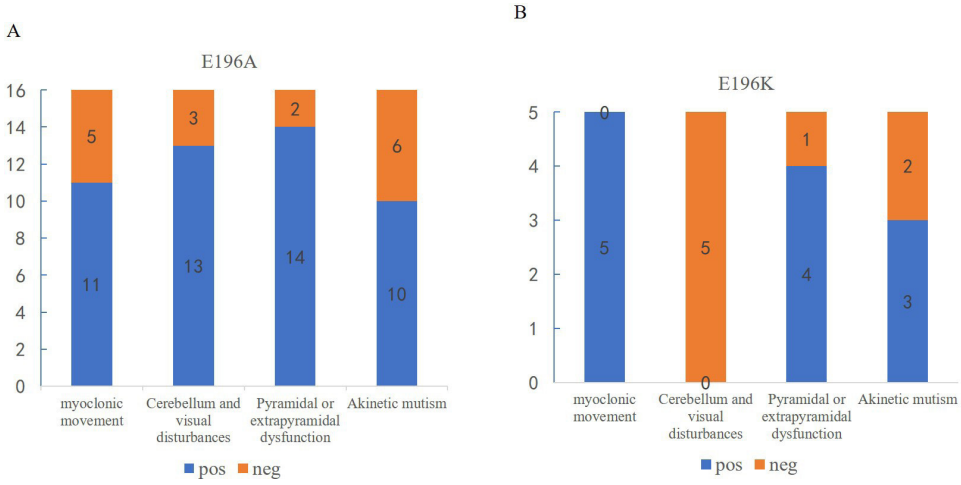

Figure 2 Positive rates and case numbers of four major sCJD-associated symptoms in Chinese patients with (A) E196A and (B) E196K genetic Creutzfeldt-Jakob disease. neg, negative; pos, positive; sCJD, sporadic Creutzfeldt-Jakob disease.

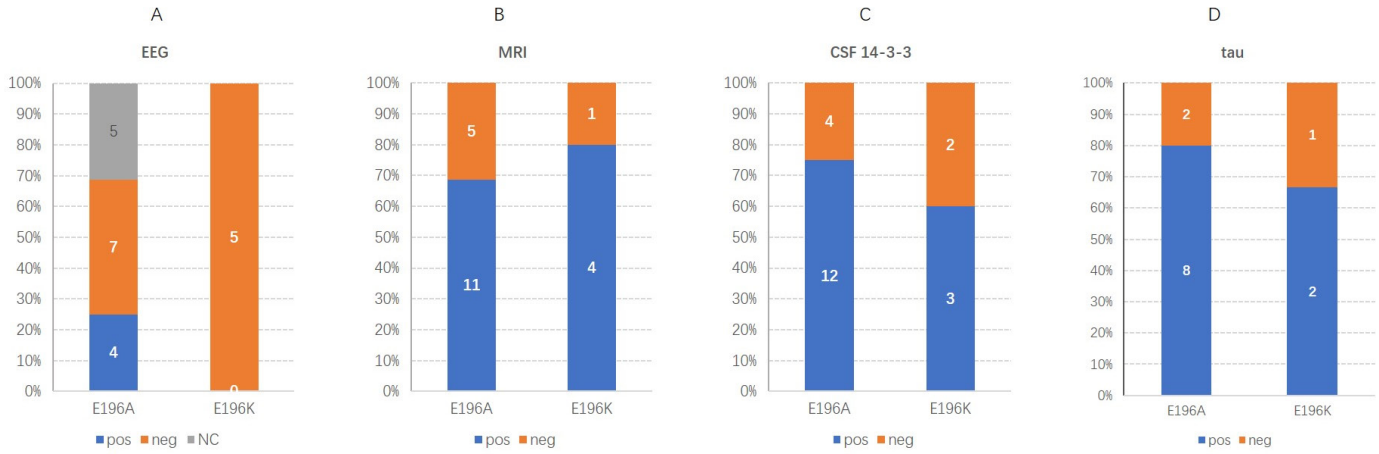

Figure 3 Positive rates and case numbers of various clinical examinations and CSF laboratory tests in Chinese patients with E196A and E196K genetic Creutzfeldt-Jakob disease: (A) PSWC on EEG (B) sCJD-associated abnormality on MRI, (C) CSF 14-3-3 and (D) CSF total tau. CSF, cerebrospinal fluid; EEG, electroencephalogram; neg, negative; pos, positive; PSWC, periodic sharp wave complex; sCJD, sporadic Creutzfeldt-Jakob disease; NC, uncertain.

no marked difference in the positive conversion time and in the peak of the reactive curves in RT-QuIC between the two groups.

Survival time

By the end of July 2020, 12 cases with E196A had died, 3 cases were alive and 1 was lost, while 4 cases with E196K had died and 1 was lost (table 1). As shown in figure 5A, the duration of survival of patients with E196A gCJD varied from 2 to 28 months, with a median survival of 6.5 months. Half of the dead cases died within 5 months of onset. All four dead cases of E196K gCJD died within 5 months of onset, with a median of 4.5 months (range 2–5 months). Analysis of median survival between the two types of gCJD revealed statistical difference (p=0.018). Three E196A cases were alive, with a clinical duration of 36, 20 and 3 months, respectively (table 1). Further, the survival time of 12 dead cases with E196A was analysed based on gender and age of onset. Male patients (n=6) showed much shorter survival time (median: 4 months, range 2–10 months) than female patients (n=6) (median: 15 months, range 4–28 months) (figure 5B), with significant difference (p=0.009). Senior cases (≥60 years, n=7) had much shorter survival time (median: 4 months, range 2–8 months) than the younger ones (<60 years, n=5) (figure 5C), showing significant difference (p=0.001).

DISCUSSION

Human genetic prion diseases display apparent diversity in clinical and laboratory features. In this study, we have comparatively analysed the features of 16 Chinese patients with E196A gCJD and 5 patients with E196K gCJD in terms of demographics, clinical, EEG and MRI, and CSF laboratory tests. Unfortunately, we did not have any brain specimens, either postmortem or biopsy, from the patients so the neuropathological and pathogenic

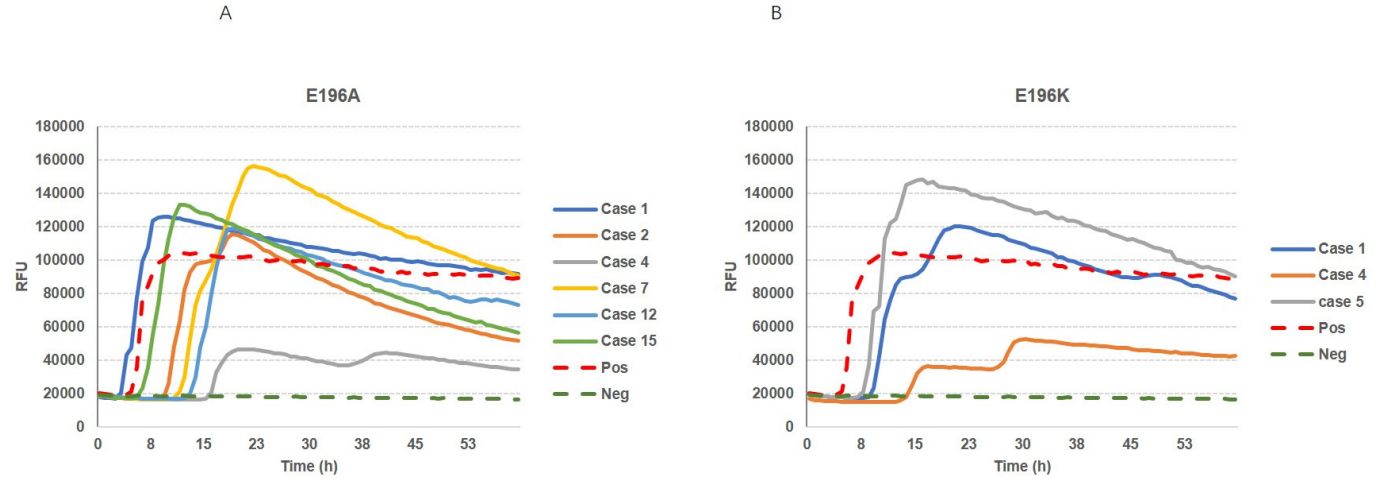

Figure 4 Positive reactive curves of CSF RT-QuIC of (A) six E196A patients and (B) three E196K patients with genetic Creutzfeldt-Jakob disease. 10^{-5} diluted brain homogenate of scrapie agent 263K-infected hamster was used as the positive control and that of normal hamster was used as the negative control. ThT value is shown on the y-axis and the time post reaction is indicated on the x-axis. CSF, cerebrospinal fluid; neg, negative; pos, positive; RFU, relative fluorescence units.

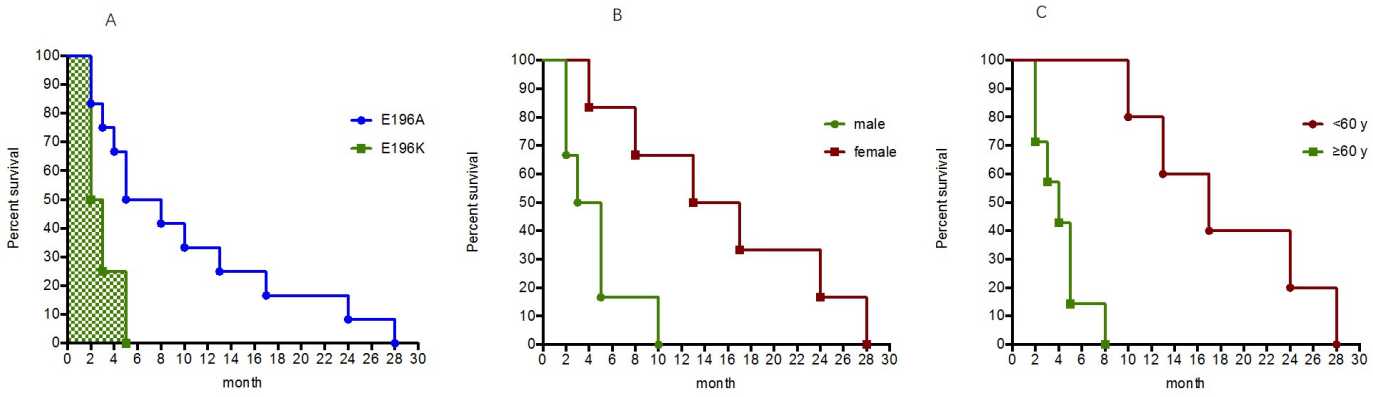

Figure 5 Survival time of Chinese patients with E196A and E196K gCJD. (A) Survival graph of E196A and E196K gCJD cases. (B) Survival graph of E196A cases based on gender. (C) Survival graph of E196A cases based on age of onset. Median survival is indicated in the graphs. gCJD, genetic Creutzfeldt-Jakob disease.

prion protein (PrP^{Sc}) features of Chinese patients with E196A and E196K gCJD remain unclear. Also, there was a limited number of enrolled patients, especially patients with E196K mutation, which may have resulted in observational bias. Generally, the clinical features of both E196A and E196K gCJD cases are like sCJD, for example, displaying rapid progressive dementia and other major manifestations, and high positive rates of MRI abnormalities, CSF 14-3-3 and total tau. However, the E196A and E196K cases in this study also show some degree of differences in some aspects. The age of onset of E196A patients is similar to Chinese patients with sCJD[10]; however, the five E196K cases were relatively older. E196A cases showed more diverse major symptoms, while symptoms in E196K cases were confined to dementia and mental problems. Cerebellum and visual disturbances were frequent in E196A cases, but not observed in E196K patients. PSWCs on EEG were not observable in all E196K cases but were recordable in a small portion of E196A cases. Additionally, E196K cases seem to have higher ratios of positive CSF RT-QuIC than E196A cases. These observations may be biased due to the small cohort (N=21). In fact, E196K gCJD has been reported in many European countries. Unlike the five Chinese cases, the phenotypes in European patients are more diverse, such as having cerebellum problems and PSWC on EEG in a portion of patients.[15–18] We have to say that majority of the data and specimens of the patients in this study were collected and tested during their last hospitalisation so we cannot exclude the possibility of other neurological signs appearing afterwards. As the number of E196K cases in this study is small, more cases are needed in order to define the difference between Chinese and European E196K gCJD cases.

Four dead cases with E196K displayed much shorter clinical duration than 12 dead E196A cases in this study. However, the survival time of published Caucasian E196K gCJD differs considerably, varying from 2 to 18 months, with a median of 7.5 months.[6 16–19] The cases with E196A in this study showed a wide range of durations, similar to sCJD cases in China.[10 20] Notably, Chinese female patients and senior patients with E196A gCJD seem to have longer duration of survival than male and young patients in general. More cases are needed to determine the exact feature of survival time in E196 gCJD.

Our data show about three times more E196A cases than E196K cases in the past 10 years. As we do not know the frequency of these two genotypes in general Han Chinese, such diversity in case numbers reflects only the difference in disease occurrence and identification. None of the family members from the 21 patients undertook assays for *PRNP* sequencing. Thereby, we are also unable to speculate the exact penetrance of these two mutations. The penetrance of mutations in *PRNP* has been evaluated in several studies.[4 21 22] However, the exact pathogenicity of many rare mutations remains poorly understood. The penetrance of *PRNP* mutations is associated with family history. Low-penetrance mutations seem to have low rates of positive family history.[21] None of the cases in this study recorded family history, which might highlight the low penetrance of E196A and E196K mutations. The penetrance of *PRNP* mutations is also influenced by age. One example is the Sephardic E200K mutation carrier, the penetrance of which is 70% at age 70 and close to 100% at age 85.[23] Screening *PRNP* mutations in senior patients with neurological problems will be beneficial in identifying untypical gCJD. On the other hand, relatively late age of onset, such as the Chinese E196K gCJD cases who had a short duration of survival in this study, may also increase the probability of case loss due to death or misdiagnosis of other diseases. Genomic assays, such as whole exome analysis, on patients with E196 mutations in the future may be beneficial in exploring possible modifiable risk factors associated with the disease process.

E196K gCJD was first reported in 2000 and was described in six German patients,[16] while E196A gCJD was reported late in China.[24] Based on the literature, dozens of E196K gCJD cases have been described in European countries, such as in Germany, Italy, France and UK.[2 4 6 16–18 25] In contrast, E196K mutation is rarely reported in East Asians, besides the Chinese cases described previously[8] and in this study. On the other hand, E196A mutation seems to be confined to Chinese and to be extremely rare in other

countries and ethnicities to date. Again, it shows differences in *PRNP* mutations and polymorphisms between ethnicities. Nevertheless, E196A gCJD has become the second predominant subtype in Han Chinese after T188K gCJD,[9 10 26] which differs not only from Caucasians but also from other East Asians, for example, Japanese and Korean.

CONCLUSION

E196A gCJD is now the fourth most frequently observed genetic prion disease in China. This is the largest report to date of gCJD with mutations at codon 196. The diversity in clinical and laboratory tests between patients with E196A and E196K mutations indicates that substitutions of different amino acids at the same position with PrP may associate with different clinical phenotypes.

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REFERENCES

1 Chen C, Dong X-P. Epidemiological characteristics of human prion diseases. *Infect Dis Poverty* 2016;5:47.
2 Kovács GG, Puopolo M, Ladogana A, *et al*. Genetic prion disease: the EUROCJD experience. *Hum Genet* 2005;118:166–74.
3 Baldwin KJ, Correll CM. Prion disease. *Semin Neurol* 2019;39:428–39.
4 Kim M-O, Takada LT, Wong K, *et al*. Genetic PrP prion diseases. *Cold Spring Harb Perspect Biol* 2018;10:a033134.
5 Jeong B-H, Kim Y-S. Genetic studies in human prion diseases. *J Korean Med Sci* 2014;29:623–32.
6 Schelzke G, Eigenbrod S, Romero C, *et al*. Genetic prion disease with codon 196 PRNP mutation: clinical and pathological findings. *Neurobiol Aging* 2011;32:756.e1–756.e9.
7 Shi Q, Zhou W, Chen C, *et al*. Rare E196A mutation in PRNP gene of 3 Chinese patients with Creutzfeldt-Jacob disease. *Prion* 2016;10:331–7.
8 Shi Q, Chen C, Song X-N, *et al*. A Chinese Creutzfeldt-Jakob disease patient with E196K mutation in PRNP. *Prion* 2011;5:117–20.
9 Shi Q, Zhou W, Chen C, *et al*. The features of genetic prion diseases based on Chinese surveillance program. *PLoS One* 2015;10:e0139552.
10 Shi Q, Chen C, Zhou W, *et al*. The characteristics of Chinese prion diseases based on 10 years surveillance data from 2006 to 2015. *Neuropsychiatry* 2018;8:739–44.
11 Shi Q, Zhang X-C, Zhou W, *et al*. Analysis of the advantage features of Beijing surveillance network for Creutzfeldt-Jakob disease. *Prion* 2015;9:304–14.
12 Chen C, Hu C, Shi Q, *et al*. Profiles of 14-3-3 and total tau in CSF samples of Chinese patients of different genetic prion diseases. *Front Neurosci* 2019;13:934.
13 Xiao K, Shi Q, Zhou W, *et al*. T188K-Familial Creutzfeldt-Jacob disease, predominant among Chinese, has a reactive pattern in CSF RT-QuIC different from D178N-Fatal familial insomnia and E200K-Familial CJD. *Neurosci Bull* 2019;35:519–21.
14 Wang G-R, Gao C, Shi Q, *et al*. Elevated levels of tau protein in cerebrospinal fluid of patients with probable Creutzfeldt-Jakob disease. *Am J Med Sci* 2010;340:291–5.
15 Eigenbrod S, Frick P, Giese A, *et al*. Comprehensive neuropathologic analysis of genetic prion disease associated with the E196K mutation in PRNP reveals phenotypic heterogeneity. *J Neuropathol Exp Neurol* 2011;70:192–200.
16 Peoc'h K, Manivet P, Beaudry P, *et al*. Identification of three novel mutations (E196K, V203I, E211Q) in the prion protein gene (PRNP) in inherited prion diseases with Creutzfeldt-Jakob disease phenotype. *Hum Mutat* 2000;15:482.
17 Béjot Y, Osseby G-V, Caillier M, *et al*. Rare E196K mutation in the PRNP gene of a patient exhibiting behavioral abnormalities. *Clin Neurol Neurosurg* 2010;112:244–7.
18 Clerici F, Elia A, Girotti F, *et al*. Atypical presentation of Creutzfeldt-Jakob disease: the first Italian case associated with E196K mutation in the PRNP gene. *J Neurol Sci* 2008;275:145–7.
19 Tumani H, Windl O, Kretzschmar HA, *et al*. [Clinically atypical CJD: diagnostic relevance of cerebrospinal fluid markers and molecular genetic analysis?]. *Dtsch Med Wochenschr* 2002;127:318–20.
20 Chen C, Wang J-C, Shi Q, *et al*. Analyses of the survival time and the influencing factors of Chinese patients with prion diseases based on the surveillance data from 2008-2011. *PLoS One* 2013;8:e62553.
21 Minikel EV, Vallabh SM, Lek M, *et al*. Quantifying prion disease penetrance using large population control cohorts. *Sci Transl Med* 2016;8:322ra9.
22 Takada LT, Kim M-O, Cleveland RW, *et al*. Genetic prion disease: experience of a rapidly progressive dementia center in the United States and a review of the literature. *Am J Med Genet B Neuropsychiatr Genet* 2017;174:36–69.
23 Spudich S, Mastrianni JA, Wrensch M, *et al*. Complete penetrance of Creutzfeldt-Jakob disease in Libyan Jews carrying the E200K mutation in the prion protein gene. *Mol Med* 1995;1:607–13.
24 Zhang H, Wang M, Wu L, *et al*. Novel prion protein gene mutation at codon 196 (E196A) in a septuagenarian with Creutzfeldt-Jakob disease. *J Clin Neurosci* 2014;21:175–8.
25 Unit, T.N.C.R.S. 27Th annual report 2018, Creutzfeldt-Jakob disease surveillance in the UK. 2018.
26 Shi Q, Zhou W, Chen C, *et al*. Rare genetic Creutzfeldt-Jakob disease with T188K mutation: analysis of clinical, genetic and laboratory features of30 Chinese patients. *J Neurol Neurosurg Psychiatry* 2017;88:889–90.