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ORIGINAL ARTICLE

Genetic Prion Disease: Insight from the Features and Experience of China National Surveillance for Creutzfeldt-Jakob Disease

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Abstract Human genetic prion diseases (gPrDs) are directly associated with mutations and insertions in the *PRNP* (Prion Protein) gene. We collected and analyzed the data of 218 Chinese gPrD patients identified between Jan 2006 and June 2020. Nineteen different subtypes were identified and gPrDs accounted for 10.9% of all diagnosed PrDs within the same period. Some subtypes of gPrDs showed a degree of geographic association. The age at onset of Chinese gPrDs peaked in the 50–59 year group. Gerstmann–Sträussler–Scheinker syndrome (GSS) and fatal familial insomnia (FFI) cases usually displayed clinical symptoms earlier than genetic Creutzfeldt–Jakob disease (gCJD) patients with point mutations. A family history was more frequently recalled in P105L GSS and D178N FFI patients than T188K and E200K patients. None of the E196A gCJD patients reported a family history. The gCJD cases with point mutations always developed clinical manifestations typical of sporadic CJD (sCJD). EEG examination was not sensitive for gPrDs. sCJD-associated abnormalities on MRI were found in high proportions of GSS and gCJD patients. CSF 14-3-3 positivity was frequently detected in gCJD patients. Increased CSF tau was found in more than half of FFI and T188K gCJD cases, and an even higher proportion of E196A and E200K gCJD patients. 63.6% of P105L GSS cases showed a positive reaction in cerebrospinal fluid RT-QuIC. GSS and FFI cases had longer durations than most subtypes of gCJD. This is one of the largest studies of gPrDs in East Asians, and the illness profile of Chinese gPrDs is clearly distinct. Extremely high proportions of T188K and E196A occur among Chinese gPrDs; these mutations are rarely reported in Caucasians and Japanese.

Keywords Genetic prion disease · Mutation · Surveillance · Creutzfeldt–Jakob disease · Gerstmann–Sträussler–Scheinker syndrome · Fatal familial insomnia

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Introduction

Human prion diseases are a group of fatal and transmissible spongiform encephalopathies that are classified into sporadic, genetic, and acquired forms. The etiologic agent for these diseases is the prion, a pathological protein (PrP^{Sc}) with an amino-acid (aa) sequence identical to a normal cell surface protein (PrP^{C}) but having a different conformational structure [1]. Genetic prion diseases (gPrDs) are a group of inherited diseases involving point mutations and insertions in the gene encoding the PrP protein. Generally, gPrDs account for approximately 10%–15% of all human

prion diseases worldwide [2–4]. Clinically, genetic Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome (GSS), and fatal familial insomnia (FFI) are gPrDs.

Full-length human PrP is 253 aa long encoded by the *PRNP* gene that is located on chromosome 20 [2, 4]. Currently, >55 mutations in the *PRNP* gene have been described as being directly associated with gPrDs or considered to be polymorphisms [5]. Uniquely, different mutations at different positions in *PRNP* result in different phenotypes of gPrDs, which vary greatly in their clinical, neuropathological, and laboratory characteristics [5, 6]. Even different mutations at the same position may have different phenotypical features, e.g., the mutations at codons 105, 188, and 196 [5]. In addition, polymorphisms at codons 129 and 219 have great impact not only on the disease susceptibility, e.g., sporadic CJD (sCJD) and variant CJD, but also on the disease phenotype, e.g., D178N-129MM FFI and D178N-129MV gCJD [4–6].

Although the proportions of gPrDs among all prion diseases do not differ markedly in most countries, some genotypes of mutations show ethno-correlations. V210I gCJD and G117V GSS are common subtypes of gPrDs in Europe and the USA [6–10], but are rare in East Asia. On the contrary, some mutations are frequent in East Asians but rare in Caucasians, such as V180I, M232R and P105L in Japanese [4, 11], and T188K and E196A in Chinese [12, 13]. Even in East Asia, the profiles of gPrDs are remarkably different in China and Japan.

Fifteen years ago, a surveillance network, The China National Surveillance for CJD (CNS-CJD), was created in Mainland China [12, 14]. By June 2020, 218 cases with 19 different *PRNP* mutations, involving gCJD, GSS, and FFI, were identified by the CNS-CJD. In this study, we present the demographic, epidemiological, clinical, and laboratory features of the 218 patients. All of the provinces in Mainland China, except for Xizang (Tibet), reported gPrD cases. T188K was the most frequent mutation, followed by D178N, E200K, E196A, and P102L.

Materials and Methods

Case Definition

Suspected CJD cases referred to the CNS-CJD were diagnosed and subtyped based on the diagnostic criteria issued by the Chinese National Health Commission, based on the diagnostic criteria for CJD issued by the World Health Organization [15]. The clinical and epidemiological data of the referred patients were collected with designed questionnaires [12]. The results of clinical examinations (MRI, EEG, and routine CSF biochemistry) and laboratory tests (CSF 14-3-3, CSF tau, CSF RT-QuIC, and *PRNP* PCR and sequencing) were obtained. The final diagnosis was made by an expert board of neurologists, neuropathologists, epidemiologists, and laboratory staff.

Study Population and Data Collection

From Jan 2006 to June 2020, a total of 218 diagnosed gPrD cases, including gCJD, FFI, and GSS, were enrolled in this study *via* the CNS-CJD. The general information, clinical data, MRI and EEG data, and results of CSF 14-3-3, CSF tau, CSF RT-QuIC, and *PRNP* sequencing were extracted from the CNS-CJD database and carefully reviewed. The geographical distribution of the patients was considered based on the provinces where they permanently lived. EEG abnormality was recorded only as the presence of typical periodic sharp wave complexes (PSWCs). MRI abnormalities were recorded as the presence of symmetrical or asymmetrical cortical "ribbon" signs on diffusion-weighted imaging (DWI), a high signal in the caudate/putamen, or a high signal in the bilateral posterior tuberosity of the thalamus in the proton the density phase. The interval from onset to diagnosis was calculated from disease onset to disease diagnosis issued by the CNS-CJD. Survival time was defined as the period from disease onset to death.

Specimen Collection

Specimens of blood and CSF from referred patients were collected by staff in the local hospital. CSF samples were obtained by routine lumbar puncture. All samples were transported to the National Reference Laboratory of the CNS-CJD in the Chinese Center for Disease Control and Prevention (CDC).

Western Blot for CSF Protein 14-3-3

A total of 20 μL of CSF was separated in 12% SDS-PAGE [14]. The fractionated proteins were electronically transferred to a nitrocellulose membrane (Whatman, USA). After blocking, the membrane was incubated with 14-3-3 polyclonal antibody (1:1000; Santa Cruz Biological) at room temperature for 2 h. The membrane was subsequently incubated with goat anti-rabbit HRP-conjugated secondary antibody and reactive signals were visualized using an enhanced chemiluminescence kit (Amersham-Pharmacia Biotech, USA).

ELISA for CSF Total Tau

The levels of total tau in CSF samples were measured using an ELISA kit (81572, Innotest hTau-Ag, Belgium). Briefly

[16], 25 μL of CSF was added to wells of the antibody-coated plate in duplicate and incubated at room temperature overnight. After washing, 100 μL of HRP-conjugated detection antibodies was added and incubated at room temperature for 30 min. The reaction was developed with 100 μL substrate working solution for 30 min in the dark. Absorbance at 450 nm was automatically measured using a microplate reader (Perkin Elmer, USA) after terminating the reaction by addition of 2 mol/L H_2SO_4. CSF tau concentrations were calculated based on a standard tau curve.

RT-QuIC (Real-Time Quaking-Induced Conversion) Assays

Briefly [17], each reaction contained 10 μg of rHaPrP90-231, 1× PBS, 170 mmol/L NaCl, 1 mmol/L EDTA, 0.01 mmol/L Thioflavin T (ThT), and 0.001% SDS, together with 15 μL of CSF 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, Ortenberg, Germany). The working conditions were: temperature, 55°C; vibration speed, 700 rpm; vibration/incubation time, 60/60 s; total reaction time, 60 h. The ThT fluorescence value (excitation wavelength, 450 nm; emission wavelength, 480 nm) in each reaction was automatically recorded every 45 min and is presented as relative fluorescence units (rfu). Each sample was tested simultaneously in quadruplicate. The cutoff value was set as the mean value of the negative controls plus 10 times the standard deviation. A positive sample was accepted when ≥2 wells revealed positive curves. Brain homogenates diluted 10^{-5} from hamsters infected with the scrapie agent 263K and normal hamsters were used as positive and negative controls, respectively.

Prion Protein (PRNP) PCR and Sequencing

Whole DNAs were extracted from the blood samples using a commercial kit (Qiagen 51104). The *PRNP* gene was amplified using a PCR protocol with the specific primers forward: 5-GGCAAACCTTGGATGCTGG-3, and reverse: 5-CCCACTATCAGGAAGATGAGG-3. Sequencing analysis of *PRNP* and polymorphisms of codons 129 and 219 were conducted using an automatic genetic analyzer (ABI3130XL). To avoid of DNA contamination in the PCR and misreading in the sequencing, all identified mutations were repeated at least once using new blood samples.

Statistical Analysis

The data were processed with SPSS 17 software, and descriptive data are expressed as the median (range) for continuous variables and as a percentage for categorical variables.

Results

Profiles and Spatiotemporal Features of Genetic Prion Diseases (gPrDs) in China

By June 2020, 218 cases with various *PRNP* mutations were identified by *PRNP* sequencing in the cases referred from the CNS-CJD (Fig 1A). Of these, 214 were Han Chinese, and 4 were members of minorities (one Miao-, one Hui-, one Tong-, and one Man-Chinese). The most common mutation was T188K (n = 65), which accounted for 29.8% of all gPrD cases. The other mutated *PRNP* genotypes more than 10 cases were D178N (n = 56, 25.7%), E200K (n = 41, 18.8%), E196A (n = 16, 7.3%). and P102L (n = 14, 6.4%). Other mutants were E196K (n = 5), V203I (n = 3), R208H (n = 3), V210I (n = 3), G114V (n = 2), R148H (n = 2), P105L (n = 1), V180I (n = 1), T183A (n = 1), and E200G (n = 1). Four cases were confirmed to contain mutations in the octapeptide repeat (OR) region: one with 7 extra ORs, one with 1 extra OR, one with 1 OR deletion, and one with 1 octarepeat deletion and a G114V point mutation in the same *PRNP* allele (Guo *et al,* in preparation). gPrDs accounted for 10.9% (218/2003) of all diagnosed PrDs (sCJD + gPrD) within the same period in Mainland China.

The numbers of gPrD cases identified per year are shown in Fig 1B. The case numbers increased along with the years of surveillance, which coincided with the increase of referred cases [12]. Cases of gPrD were identified in all provinces of Mainland China [except Xizang (Tibet)] based on their permanent addresses. As shown in Table 1, the top five provinces with more gPrD cases were Henan (n = 33), Shandong (n = 24), Hebei (n = 21), Guangdong (n = 14), and Beijing (n = 13). The ratios of gPrDs to total PrDs varied greatly among provinces, and they ranged from 10.7% to 15.1% in the top 5 provinces. Analysis of the distributions of the five most frequent gPrDs revealed more D178N cases in Henan and Guangdong. T188K cases were distributed more widely in 20 provinces, with more cases in Shandong. E200K cases were identified in 16 provinces were more common in northern China (north of the Yangtze river). E196A and P102L cases were dispersed in many provinces. Further analysis based on the provinces revealed some geography-associated phenomena: 71.4% of gPrD cases in Guangdong and 60.6% of gPrD cases in

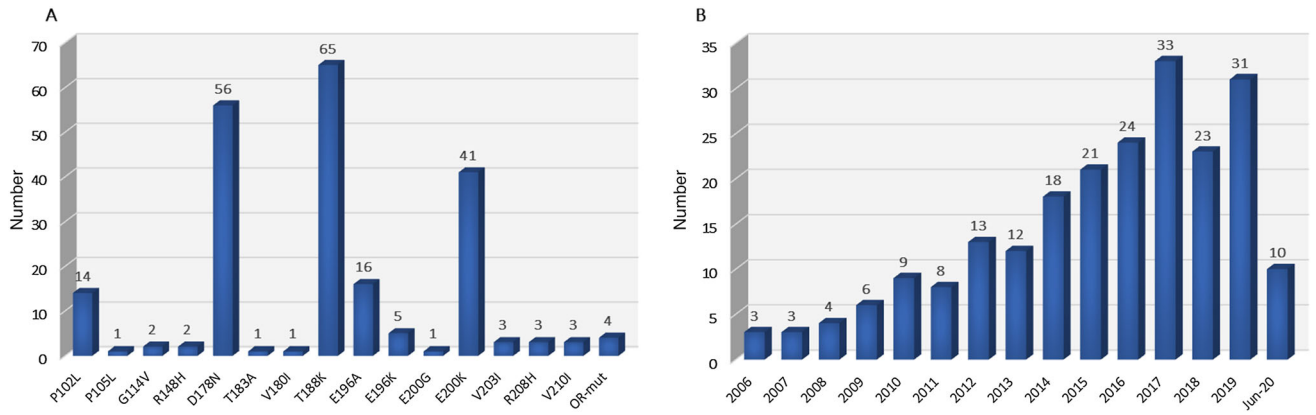

Fig. 1 Distributions of the subtypes of gPrDs. **A** Case numbers of various gPrDs. **B** Case numbers in surveillance years.

Henan were D178N FFI; and the predominant gPrD cases in the northern region (Beijing, Tianjin, Hebei, Shanxi, and Inner Mongolia) were T188K and E200K gCJD.

Age at Onset and Family History of Chinese gPrDs

The age at onset of the 218 gPrD cases ranged from 19 to 85 years, with a median of 58. As shown in Fig 2A, 33.3% of cases displayed clinical manifestations in the 50–59 year group and 30.1% were in the 60–69 year group Only a few cases were in the <20 and >80 year groups. The age at onset showed notable disease-associated patterns (Fig 2B, Table 2). The medians of the age at onset of cases with the mutations P102L (50 years), G114V (35 years), and D178N (51 years), and mutations in the octarepeat region (51 years) were relatively young, while those with the mutations T188K (61 years), E196A (61 years), E196K (70 years), V203I (63 years), and V210I (64 years) were older. The median age at onset of cases with the mutations E200K (57 years) and R208H (55 years) were slightly below that for all gPrDs.

As shown in Fig 2C, 50% of patients with the P102L mutation and 45% of those with the D178N mutation displayed neurological symptoms at <50 years. The age at onset for P105L ranged from 34 to 67 years with a peak (35.8%) in the 40–49 year group. The age at onset for D178N was distributed widely from 19 to 70 years with a peak (31.6%) in the 50–59 year group. Approximately 15% of patients with D178N showed symptoms at even <30 years. In contrast, only small proportions of the cases with E188K (6.2%) and E196A (6.3%) mutations displayed symptoms when younger than 50 years, while markedly more cases (20.0% of T188K and 18.8% of E196A) developed symptoms when older than 70 years. The age at onset of patients with the E200K mutation were in the middle between the above groups, with 19.5% younger than 20 years and 9.8% older than 70.

The family history of every patient was carefully and repeatedly reviewed by the local physician and the staff of CNS–CJD. The family members of some of the gPrD patients undertook *PRNP* sequencing. Only 28.4% of all 218 gPrDs cases recalled a clear family history (Table 2). Remarkably, different types of gPrDs displayed distinct patterns of family history. P102L cases showed the highest ratio (78.8%, 11/14) with a disease-associated family history, followed by D178N cases showing a 53.6% (30/56) positive family history. Fewer T188K (13.8%, 9/65) and E200K (14.6%, 6/41) cases reported a family history. None of the E196A and E196K patients described a definite disease family history. In addition, a family history was described in two G114V (2/2), one V203I (1/3), and one R208H (1/3) cases, as well as the case with 7 extra OR insertions and the case with one OR deletion plus G114V.

The sCJD-Associated Symptoms of Chinese gCJD Cases with Point Mutation

The clinical characteristics of 12 cases of P102L GSS and 40 cases D178N FFI have been described [18, 19]. The foremost symptoms of the 12 subtypes of gCJD with point mutation were multiple, mostly progressive dementia, motor symptoms, visual problems, and mental problems, without significant difference from sCJD. The appearance of dementia and four other sCJD-associated symptoms during hospitalization referred from the CNS-CJD were recorded and are summarized in Table 3. Generally, dementia was recorded in 95.8% gCJD cases, besides 4 patients with T188K and one with R148H. Myoclonic movements (68.5%), cerebellar and visual disturbances (69.9%), and pyramidal or extrapyramidal dysfunction (82.5%) were frequently noted. Akinetic mutism was relatively less common (37.6%) during the referral period. Unlike the data of the cases of E196A (62.5%) and E200K (53.7%), mutism was noted in fewer T188K cases (20.0%). Cerebellar and visual disturbances were not identified in all

Table 1 Distribution of the cases with various mutations within *PRNP* in the provinces of Mainland China

Regions	Province	P102L	P105L	G114V	R148H	D178N	V180I	T183A	T188K	E196A	E196K
Northeast	Heilongjiang								2	1	
	Jilin	1							3	3	1
	Liaoning								3		
North	Beijing					1			6		1
	Tianjin								4		
	Hebei	1			1	5			6		1
	Shanxi					1			2		
	Neimenggu								4		
East	Shandong	3	1			2			12		
	Anhui	1				3			4		
	Shanghai			2					2	1	1
	Jiangsu	1				3			1		
	Zhejiang	1				2			3	2	
	Jiangxi	1				4			1		
	Fujian									2	1
Middle	Hubei	1				1			2		
	Henan	1			1	20	1		1		
	Hunan										
South	Guangdong					10				3	
	Guangxi										
	Hainan					1					
Southwest	Sichuan								4	1	
	Guizhou	1				1				1	
	Yunnan								1	1	
	Xizang										
	Chongqing	1								1	
Northwest	Shaanxi	1				1			3		
	Gansu										
	Ningxia								1		
	Qinghai					1					
	Xinjiang							1			
Total		14	1	2	2	56	1	1	65	16	5

Regions	E200G	E200K	V203I	R208H	V210I	OR-mut	Total gPrDs	Total sCJD	% of PrDs	Resident (Mil.)
Northeast		1					4	45	8.2%	37.9
		1					9	51	15.0%	27.2
							3	44	6.4%	43.7
North		4			1		13	106	10.9%	21.7
		7					11	41	21.2%	15.6
		6			1		21	128	15.1%	75.2
							3	58	4.9%	37.0
		2					6	33	15.4%	25.3
East		3	1			2	24	143	14.4%	100.0
				1			9	64	12.3%	62.5
							6	81	6.9%	24.2
							5	69	6.8%	80.3
	1	2					11	82	12.0%	56.6
		3					9	36	25.0%	46.2
							3	61	4.7%	39.1

Table 1 continued

Regions	E200G	E200K	V203I	R208H	V210I	OR-mut	Total gPrDs	Total sCJD	% of PrDs	Resident (Mil.)
Middle							4	36	10.0%	59.0
		5	1	1		2	33	212	13.5%	95.6
		1					1	26	3.7%	68.6
South		1					14	117	10.7%	111.7
					1		1	12	7.7%	48.9
		1					2	6	25.0%	9.3
Southwest		2					7	77	8.3%	83.0
		1					4	40	9.1%	35.8
							2	24	7.7%	48.0
							0	0	/	3.4
			1				3	52	5.5%	30.5
Northwest							5	70	6.6%	38.4
				1			1	39	2.5%	26.3
							1	7	12.5%	6.8
							1	2	33.3%	6.0
		1					2	24	7.7%	24.4
Total	1	41	3	3	3	4	218	2003	10.9%	

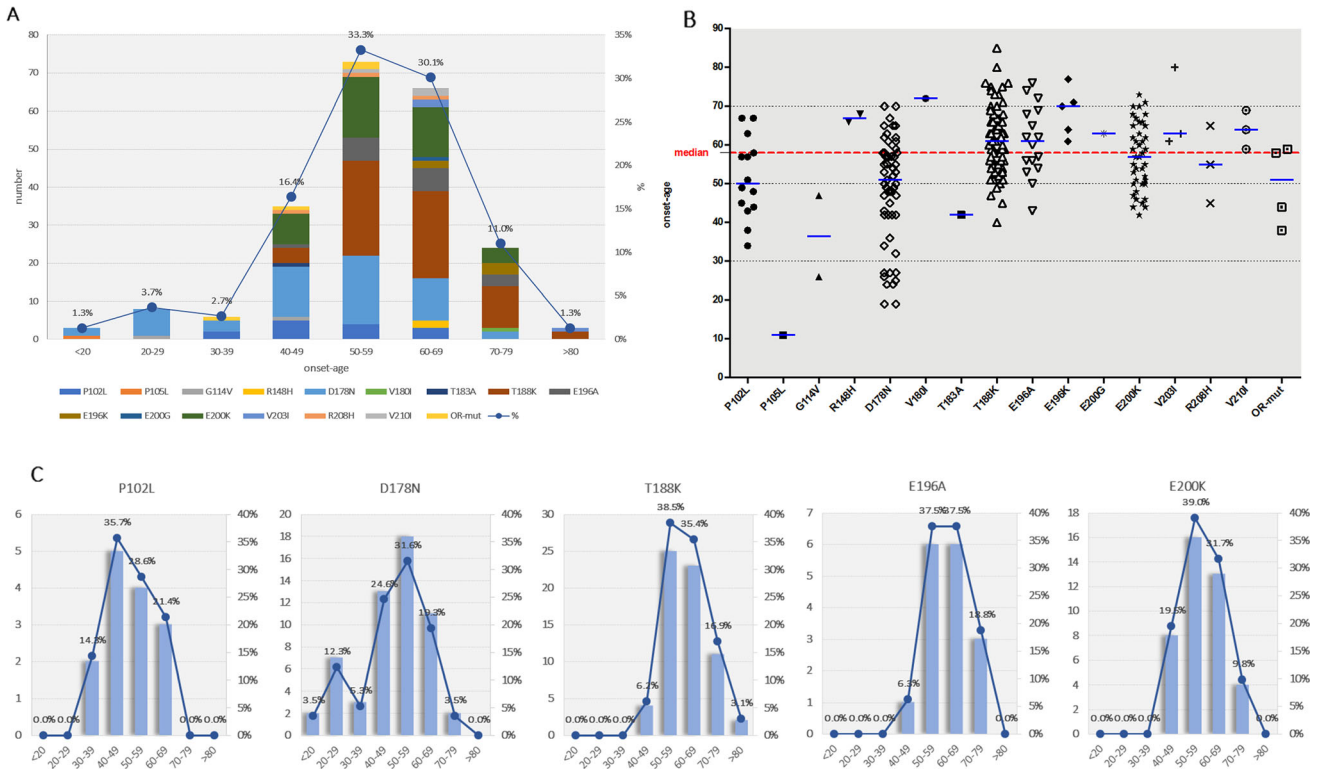

Fig. 2 Age at onset of patients with various gPrDs. **A** Distribution of the age at onset in different decades of life. **B** Distribution of the age at onset based on the type of gPrD (dashed red line, median for all 218 patients; blue bars, median for each gPrD). **C** Distributions of the age at onset of the top 5 most frequent gPrDs in different decades of life.

Table 2 General information of 218 gPrD cases with various mutants in *PRNP*

Mutation	Number	Gender		Race		Age at onset-		Family history (+)	Duration (months)		
		Male	Female	Han	Minority	Median (years)	Range (years)		Range	median	average
P102L	14	6 (42.9%)	8 (57.1%)	14	0	50	34-67	11 (78.8%)	7-44 (n = 10)	16	22.3
P105L	1	1	0	1	0	11	-	0	/	/	/
G114V	2	1	1	2	0	36.5	26, 44	2	25 (n = 1)		
R148H	2	1	1	2	0	67	66, 68	0	/	/	/
D178N	56	26 (46.4%)	30 (53.6%)	55	1 (Miao)	51	19-70	30 (53.6%)	4-44 (n = 44)	11	13.6
V180I	1	0	1	1	0	72	-	0	/	/	/
T183A	1	0	1	1	0	42	-	0	/	/	/
T188K	65	37 (56.9%)	28 (43.1%)	64	1 (Man)	61	40-85	9 (13.8%)	1-26 (n = 45)	4	5.9
E196A	16	9 (56.3%)	7 (43.7%)	16	0	61	43-76	0 (0.0%)	2-28 (n = 12)	6.5	10
E196K	5	2	3	5	0	70	61-77	0	2-5 (n = 4)	2.5	3
E200G	1	1	0	1	1	63	-	0	/	/	/
E200K	41	17 (41.5%)	24 (58.5%)	40	1 (Tong)	57	42-73	6 (14.6%)	1-40 (n = 30)	6	9.8
V203I	3	1	2	3	0	63	61-80	1	8, 9 (n = 2)	8.5	8.5
R208H	3	3	0	3	3	55	45-65	1	4 (n = 1)	/	/
V210I	3	3	0	2	1 (Hui)	64	59-69	0	2, 10 (n = 2)	6	6
OR-mut	4	1	3	4	0	51	38-59	2	50 (n = 1)	/	/
Total	218	109 (50.0%)	109 (50.0%)	214 (98.2%)	4 (1.6%)	58	19-85	62 (28.4%)	1-50 (n = 151)	8	10.2

Table 3 sCJD-associated symptoms and signs of the gCJD cases with different point mutations in *PRNP*

Mutation	Number	Dementia	Other major sCJD-associated symptoms and sighs*			
			I	II	III	IV
G114V	2	2	0	0	1	0
V180I	1	1	1	0	1	0
R148H	2	1	0	2	1	0
T183A	1	1	0	0	0	0
T188K	65	61 (93.8%)	45 (69.2%)	48 (73.8%)	54 (83.1%)	13 (20.0%)
E196A	16	16 (100%)	11 (68.8%)	12 (75.0%)	14 (87.5%)	10 (62.5%)
E196K	5	5	5	0	4	3
E200G	1	0	0	1	0	0
E200K	41	41 (100%)	31 (75.6%)	30 (73.1%)	35 (85.4%)	22 (53.7%)
V203I	3	3	3	2	1	1
R208H	3	3	1	2	2	1
V210I	3	3	1	3	3	3
Total	143	1367(95.8%)	98 (68.5%)	100 (69.9%)	118 (82.5%)	53 (37.1%)

*I: myoclonic movement; II: Cerebellar and visual disturbances; III: Pyramidal or extrapyramidal disfunction; VI: Akinetic mutism.

5 cases with E196K, but frequently in 75% of cases with E196A.

Clinical Examination of Chinese gPrDs

During the clinical course, 186 gPrD patients received at least one EEG examination. Despite the presence of different abnormalities, typical PSWCs were noted in 46 cases (24.7%) (Table 4). Only one out of 48 cases of D178N FFI showed PSWCs on the EEG. Nearly half of the patients with E200K (48.5%) revealed positive PSWCs on the EEG, higher than those of P102L (20.0%), T188K (27.9%), and E196A (25.0%) (Fig 3A). In 200 gPrD patients who received MRI scans, the general positivity rate was 66.0%. Among them, 92.3% (12/13) of P102L cases revealed at least one sCJD-associated abnormality on MRI, while only 25.5% (12/47) of D178N cases showed such an abnormality (Fig 3A, Table 4). The rates of abnormality on MRI did not differ considerably among E196A, T188K, and E200K cases, ranging from 64.3% to 77.8%. In additionally, sCJD-associated abnormalities on MRI were frequently detected in patients with E196K, V201I, and V210I (Table 4).

Further, the presence of three MRI abnormalities in the five most common gPrD cases were analyzed and are illustrated in Fig 3B. In the context of all gPrDs, the positivity rates of cortical "ribbon" signs on DWI, a high signal in the caudate/putamen, and a high signal in the bilateral posterior tuberosity of the thalamus in the proton density phase were 61.5%, 33.0%, and 13.3%, respectively. Cortical ribbon signs were the most frequently-identified abnormality in all five types of gPrD. A high signal in the caudate/putamen was recorded in a large proportion (>60%) of P102L and E200K cases, but relatively fewer in E196A (29.2%) and T188K (40.0%) patients. A high signal in the bilateral posterior tuberosity of the thalamus was least noted, particularly in T188K cases with a positivity rate of 6.3%. In addition, all three MRI abnormalities in D178N FFI patients were clearly low.

Table 4 Results of the patients with various mutants of *PRNP* in clinical examinations and laboratory tests

Mutation	Number	EEG PSWC[1]	MRI				CSF			*PRNP*			
			(+)[2]	Ribbon-like signal	High signal in caudate/ putamen	High signal in pulvinar	14-3-3 (+)	Tau (>1400 pg/ml)	RT-QuIC (+)	129 (MM)	129 (MV)	219 (EE)	219 (EK)
P102L	14	2/10 (20.0%)	12/13 (92.3%)	9/11 (81.8%)	8/13 (61.5%)	2/11 (18.2%)	6/13 (41.2%)	5/12 (23.8%)	7/11 (63.6%)	14	0	11	0
P105L	1	1/1	0/1	0/1	0/1	0/1	0/1	/	0/1	1	0	1	0
G114V	2	0/1	0/1	0/1	0/1	0/1	0/1	1/1	0/1	2	0	/	/
R148H	2	0/1	0/2	0/2	0/2	0/2	0/2	/	/	2	0	1	0
D178N	56	1/48 (2.1%)	12/47 (25.5%)	6/37 (16.2%)	5/46 (10.9%)	5/46 (10.9%)	21/54 (38.9%)	24/41 (58.5%)	6/37 (15.8%)	56	0	38	1
V180I	1	/	1/1	/	1/1	0/1	0/1	1/1	0/1	1	0	/	/
T183A	1	/	/	/	/	/	0/1	1/1	1/1	1	0	1	0
T188K	65	17/61 (27.9%)	50/64 (78.1%)	49/63 (77.8%)	19/65 (29.2%)	4/63 (6.3%)	38/61 (62.3%)	21/39 (53.8%)	9/35 (25.7%)	64	1	54	1
E196A	16	4/16 (25%)	11/15 (73.3%)	9/14 (64.3%)	6/15 (40.0%)	3/13 (23.1%)	12/16 (75.0%)	8/10 (80.0%)	6/16 (37.5%)	16	0	13	1
E196K	5	0/4	4/5	4/5	2/5	0/5	3/5	2/3	3/5	5	0	4	0
E200G	1	0/1	/	/	/	/	1/1	1/1	0/1	1	0	/	/
E200K	41	16/33 (48.5%)	35/41 (85.4%)	26/37 (70.3%)	27/41 (65.9%)	9/35 (25.7%)	27/38, (71.1%)	17/25 (68.0%)	11/25 (44.0%)	40	1	38	0
V203I	3	2/3	2/3	2/2	1/3	0/3	2/3	1/3	1/3	3	0	2	0
R208H	3	1/1	1/2	1/1	1/2	1/2	2/3	1/2	1/2	3	0	1	0
V210I	3	2/2	3/3	3/3	1/3	0/3	3/3	/	1/3	2	1	3	0
OR-mut	4	0/2	1/2	1/2	1/2	1/2	2/3	1/1	0/1	3	1	2	0
Total	218	46/186 (24.7%)	132/ 200 (66.0%)	110/179 (61.5%)	72/200 (36.0%)	25/188 (13.3%)	117/ 190 (61.6%)	84/140 (60.0%)	46/143 (32.3%)	214 (98.2%)	4 (1.8%)	169 (98.3%)	3 (1.7%)

[1]typical PSWC.

[2]one of three abnormalities considered positive in MRI.

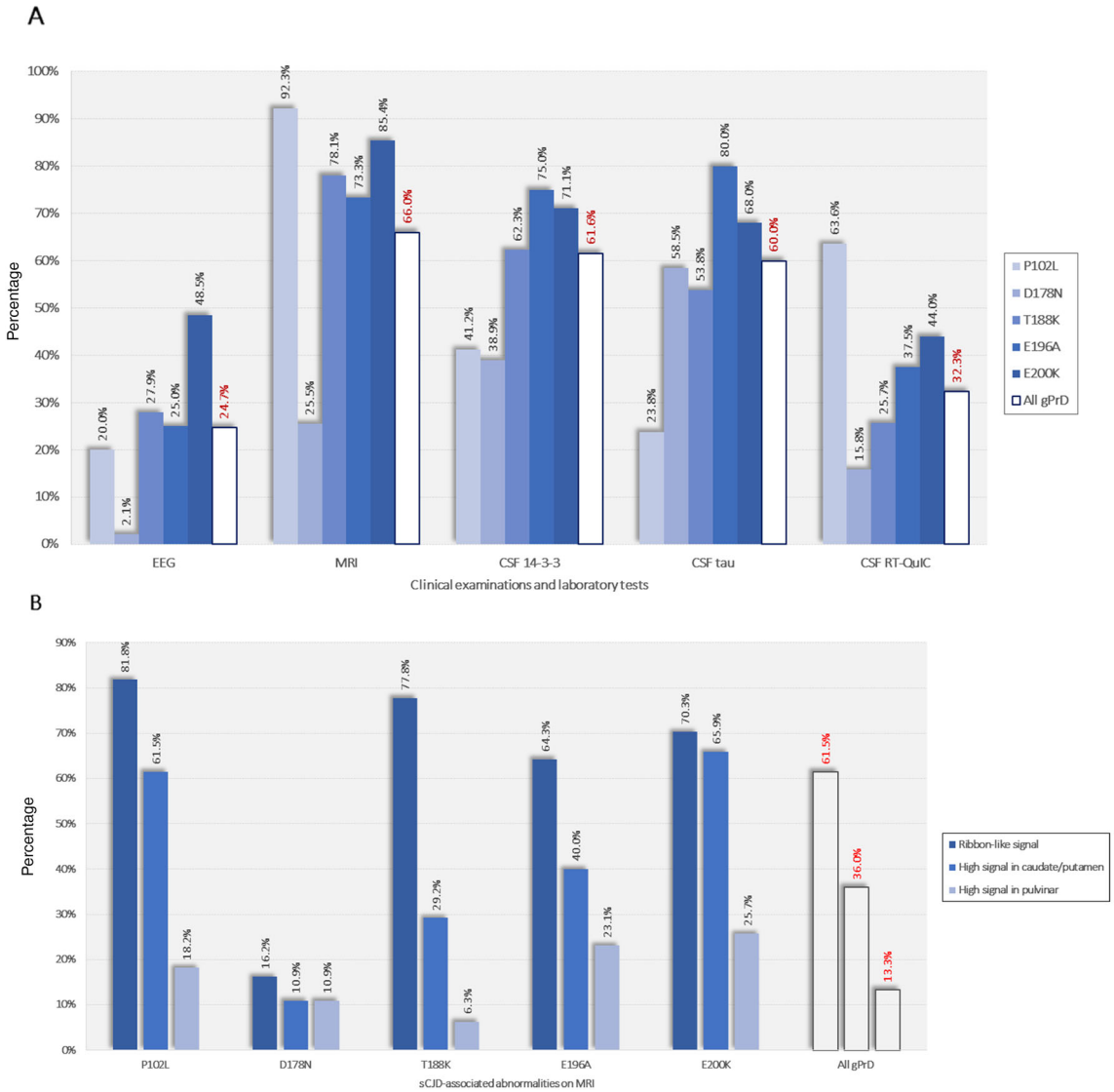

Fig. 3 Positivity rates of patients with the top 5 most frequent gPrDs in clinical examinations (EEG and MRI) and CSF laboratory tests (14-3-3, tau, and RT-QuIC). **A** Positivity rates in EEG, MRI, CSF 14-3-3, CSF tau, and CSF RT-QuIC. **B** Positivity rates of three sCJD-associated abnormalities on MRI in the top 5 most frequent gPrDs (empty columns with red numerals, rates for all gPrDs).

CSF Laboratory Tests and PRNP Polymorphisms of Chinese gPrDs

The results of different CSF tests of Chinese gPrDs are summarized in Table 4. Western blots for 14-3-3 were performed in 190 gPrD cases with a total positivity rate of 61.6%. Among the five commonest gPrDs, the positivity rates of T188K, E196A, and E200K were higher than those of P105L and D178N (Fig 3A). Meanwhile the CSF samples from cases with mutations in the C-terminus (i.e., after aa 188) showed a higher probability of 14-3-3 positivity. Total tau levels were measured in the CSF of 140 cases using a commercial ELISA kit and values >1400 pg/mL were considered to be positive as described elsewhere [16]. The general positivity rate of CSF tau was 60.0% (84/140). P102L cases had a markedly low positivity rate (23.8%), while E196A (80.0%) and E200K (68.8%) cases revealed relatively high positivity rates (Fig 3A). CSF RT-QuIC assays were conducted in 143 gPrD cases, showing a general positivity rate of 32.3%. The highest RT-QuIC positivity rate (63.6%) was in the P102L group and lowest (15.8%) in the D178N group, while the positivity rates of T188K, E196A, and E200K cases were 25.7%, 37.5%, and 44.0%, respectively (Fig 3A).

All 218 gPrD cases had data for codon 129, showing a predominance of the MM homozygote (98.2%) and a very low ratio of the MV heterozygote (4 cases, 1.8%) (Table 4). Those four heterozygous patients were one T188K, one E200K, one V210I, and one with 1 OR deletion plus G114V. 172 cases had data for codon 219 with 98.3% the

EE homozygote and 1.7% the EK heterozygote (3 cases) (Table 4). All heterozygotes in codons 129 and 219 were Han Chinese.

Duration of Chinese gPrDs

The duration of each gPrD case was carefully followed up by the CNS–CJD staff. By June 2020, 151 out of 218 gPrD patients had passed away with definite dates and the durations are summarized in Table 2. The rest were still alive or contact had been lost. The general durations ranged from 1 to 50 months (average, 10.2 months; median, 8 months). The duration varied considerably, not only between different types of gPrD, but also within the same type. The patients with mutations in the N-terminal of PrP, including P105L and D178N cases as well as one case of G114V and one case of 7 extra octarepeat insertion, had longer durations than those with mutations in the C-terminal (after aa 188). As shown in Fig 4, the survival time of P102L cases was notably longer than that of the others, with a median (50% percentile) of 16 months. Six P102L cases survived longer than 12 months and four longer than 24 months, the longest being 44 months. The median survival time of D178N cases was 11 months, the longest being 44 months as well. The median survival times of T188K (4 months), E196A (6.5 months), and E200K (6 months) were clearly shorter, the longest being 26, 28, and 40 months, respectively. Notably, the durations of the 4 cases of E196K were much shorter than the E196A cases, reflecting a diverse phenotype of the substitution of different aas at the same position within *PRNP*.

Furthermore, we calculated the distributions of the cases of the five top gPrDs in different periods: <3, 3–6, 6–12, 12–24, and >24 months. As shown in Table 5, no case of P102L died within 6 months after onset and 40% of the cases still survival 24 months after onset. The patients with D178N had the second-longest duration: none died with 3 months and approximately 40% survived longer than 12 months after onset. The duration of T188K cases was shortest: 44.4% were <3 months and the cumulative percentage within 12 months reached approximately 90%. The durations of E196A and E200K were similar: 33.3% and 26.7% of the patients died within 3 months and the cumulative percentages at 12 months were 75.0% and 73.4%, respectively. Apart from the P102L cases, >90% of the other four subtypes had durations <2 years.

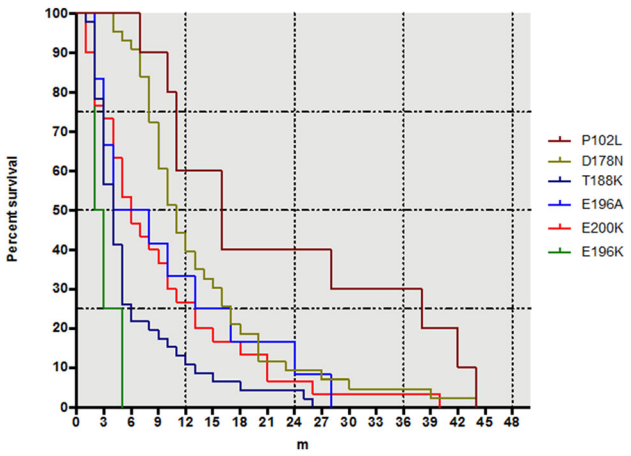

Fig. 4 Kaplan-Meier survival curves for patients with various gPrDs (X-axis, survival time in months; Y-axis, percent survival).

Discussion

In this study, we have systematically described the epidemiology, clinical examinations, and laboratory features of 218 Chinese gPrD cases identified by the CNS-CJD. Nineteen different subtypes of *PRNP* mutations were involved. Five types of mutation were frequently found in almost every surveillance year, i.e., T188K (29.8%), D178N (25.7%), E200K (18.8%), E196A (7.3%), and P102L (6.4%), accounting for 88% of all gPrD cases. The remaining mutations were very rare (no more than 5 cases each). The percentage of total gPrDs was estimated to be 10.9% of all diagnosed PrDs including sCJD and gPrDs, and this is consistent with the global findings [4, 5]. We believe that this is one of the largest studies of gPrDs in East Asians.

Among the 31 provinces, autonomous regions, and municipalities in Mainland China, 30 have reported gPrD cases according to the permanent addresses of the patients. The geographical distribution of the numbers of gPrD cases was markedly uneven, ranging from 1 to 33 cases per province. In addition to the populations, we believe that the knowledge of PrDs by local physicians and local surveillance contributed to this diversity, since the numbers of gPrD cases are consistent with that of sCJD generally. However, some types of gPrD and some provinces showed a certain geographical association. One significant example is D178N FFI, which was more concentrated in Henan and Guangdong provinces. Although several family clusters of D178N FFI have been identified [18, 20, 21], most of them had no blood lineage. T188K and E200K gCJD were widely distributed in many provinces, but more cases were identified in the northern provinces. In the provincial context, 71.4% (10/14) gPrDs in Guangdong and 60.6% (20/33) in Henan were D178N cases, while no T188K cases in Guangdong and only one in Henan were reported. On the contrary, T188K and E200K gCJD were the only gPrD types identified in Tianjin and Neimenggu (Inner Mongolia), and were predominant in many other northern provinces, such as Beijing, Shandong, and Hebei. The geographical feature of other types of gPrD is hard to

Table 5 Distribution of the top 5 commonest gPrDs in various periods

Mutation	<3 months		3-6 months		6-12 months		12-24 months		>24 months	
	% (*n*)	Cumulative % (*n*)	% (*n*)	Cumulative % (*n*)	% (*n*)	Cumulative % (*n*)	% (*n*)	Cumulative % (*n*)	% (*n*)	Cumulative % (*n*)
P102L	0% (0)	0% (0)	0.0% (0)	0% (0)	40.0% (4)	40.0% (4)	20.0% (2)	60.0% (6)	40.0% (4)	100% (10)
D178N	0% (0)	0% (0)	9.3% (4)	9.3% (4)	51.2% (22)	60.5% (26)	30.2% (13)	90.7% (39)	9.3% (4)	100% (43)
T188K	43.5% (20)	43.5% (20)	34.8% (16)	78.3% (36)	10.9% (5)	89.2% (41)	6.5% (3)	95.7% (44)	4.4% (2)	100% (46)
E196A	33.3% (4)	33.3% (4)	16.7% (2)	50.0% (6)	25.0% (3)	75.0% (9)	16.7% (2)	91.7% (11)	8.3% (1)	100% (12)
E200K	26.7% (8)	26.7% (8)	26.7% (8)	53.4% (16)	20.0% (6)	73.4% (22)	20.0% (6)	93.4% (28)	6.7% (2)	100% (30)

evaluate because of limited case numbers. Despite the several large migrations of Han Chinese in history and the frequent movement of populations in modern China, the geographical patterns of D178N, T188K and E200K mutations in Chinese may indicate a scenario similar to the "race"-associated distribution of gPrD reported in different countries or "races" in Europe [6, 7, 22, 23].

Here, we compared the similarity and diversity in the features of gender, age at onset, and family history in Chinese and European gPrDs. The gender distribution of 218 Chinese gPrDs was the same, but several subtypes of gPrD showed slight differences, more males having T188K and E196A and more females having P102L, D178N, and E200K. The profile of the age at onset of Chinese gPrDs was comparable with Europeans [6, 24], peaking in the 50–59 year group. Similarly, the age at onset of Chinese P105L GSS, and D178N FFI cases was young, while that of E200K gCJD cases was relatively old. Two subtypes of gCJD, T188K, and E196A that predominate in Chinese, display clinical symptoms at a relatively old age. Notably, the median age at onset of 5 cases of E196K gCJD was older than that of E196A cases. The general positivity ratio of family history for Chinese gPrDs was lower than for European cases [6]; however, they showed quite similar patterns of gPrD subtypes, such as high positivity rates in P102L GSS and D178N FFI cases, but low rates in E200K gCJD cases. Moreover, the positivity rate of family history in T188K cases was also low, similar to E200K, and no cases with the E196A mutation recalled a family history.

Clinically, Chinese patients with P102L and D178N showed features typical of GSS and FFI, respectively [18, 19], and the gCJD cases with various point mutations also showed considerable similarity to sCJD cases [12]. Progressive dementia and the other four symptoms were described in high proportions in gCJD cases during hospitalization. Compared with E200K [25] and E196A gCJD, the presence of mutism in T188K gCJD during hospitalization in this study and in our previous study [26] was less frequent. Cerebellar and visual problems were frequent in E196A cases but undetectable in E196K patients. EEG examination was less sensitive to most subtypes of Chinese gPrD. MRI scanning is helpful for most types of gPrD with positivity rates similar to sCJD, except for D178N FFI [12]. The cortical ribbon sign on DWI was the most common abnormality on MRI. CSF 14-3-3 showed high positivity rates, particularly in gCJD patients with a point mutation in the C-terminal (after aa 188), but not in P102L and D178N cases. Increased CSF tau was unusual in P102L GSS but was quite common in the other subtypes of gPrD, including D178N FFI. Among the top 5 frequent gPrDs, P102L and E200K cases had relatively high positivity rates in CSF RT-QuIC. Those similarities and differences in clinical and laboratory parameters not only between gPrD and sCJD but also between various subtypes of gPrDs supply useful information for further prion studies.

As incurable diseases, 151 (out of 218) gPrDs in this study had precise death dates. In line with the global data [6, 11], Chinese P102L GSS cases had a long duration. The only P105L GSS case in China also had a long duration, and was still alive in the latest follow-up survey 5 years after onset, although the clinical situation gradually worsened. Similarly, the mean survival time of D178N FFI cases is longer than that of Chinese sCJD cases [12]. Most types of gCJD with a point mutation share survival times similar to sCJD [12, 27], slightly longer in E200K and shorter in T188K. This again highlights that the genotypes of the mutations in *PRNP* greatly affect not only the clinical phenotypes but also the duration of gPrDs.

Compared with many Western countries, the brain postmortem rate in China is extremely low, which is believed to be associated with traditional Chinese customs [12]. Except for a few cases of D178N FFI, G114V, and 7 extra OR insertion [21, 28–30], the neuropathological features of most Chinese gPrDs remain unclear. This lack of neuropathological data may also influence the diagnosis of mutations with low penetration, such as 1 OR mutation, V180I, and M232R. V180I and M232R are frequently detected in Japanese [11], and are usually considered to be polymorphisms. We did not find the mutations V180I and M232R in our referred cases in the past 15 years of surveillance. Three kinds of mutation with 1 OR have been found in our surveillance system. One with 1 extra OR insertion was still alive after 4 years of follow-up, after which contact was lost. Another with 1 OR deletion displayed the clinical phenotype of Parkinson disease and was eventually diagnosed as having this disease. The third contained 1 OR deletion and a G114V mutation, and displayed sCJD-like features. This again indicates that the mutations with 1 OR are polymorphisms rather than causal mutations.

The E196A variant has been considered a neutral polymorphism in the East Asian population. The allele frequency of this variant is ~0.1% among Chinese individuals [31]. The absence of a family history in 16 of the Chinese patients with the E196A variant in this study also supports the inference that they are sCJD cases but with this variant. However, E196A was the fourth most frequent *PRNP* variant, accounting for 7.3% of Chinese gPrD cases. In addition, cases with the E196A variant accounted for approximately 0.8% of all Chinese PrD cases diagnosed in the CNS-CJD at the same time (218 gCJD and 1793 sCJD, unpublished data), which is much higher than the 0.1% allele frequency among Chinese based on the ExAC data [31]. Whether E196A is just a benign variant or increases the risk of prion disease like R148H, T188R, and V203I needs more studies with a large sample size. For convenience of presentation, we used E196A gCJD in this report to compare the features with other types of gPrD.

It is well known that the distributions of the *PRNP* variants have ethnocorrelations. The data here and our previous studies [12, 13] have repeatedly proposed that T188K, D178N, E200K, E196A, and P102L are the most frequent mutations in Chinese, mostly Han Chinese who account for 92% of Chinese at approximately 1.3 billion. This disease profile is different from Europeans, in whom E200K, V210I, and D178N predominate [4, 6, 8] and Americans, in whom E200K, P102L, and D178N predominate [32]. Meanwhile, as approximately 93% of Chinese are homozygotic for codon 129 MM [12], some subtypes of gPrD with 129MV or VV polymorphism, such as D178N–129V gCJD, are fairly hard to detect. The *PRNP* variant profile in China is also distinct from that in Japan. The frequent mutations V180I and M232R in Japan [5, 11, 33] are either rare (only one V180I case) or have never been identified in China. Another common GSS-associated mutant in Japanese, P105L, is also rare in Chinese. Although the T188K mutant was first reported in Germany in 2000 [34], only a few cases have been described worldwide since then, apart from in China. The E196A variant was first reported in China in 2014 [35, 36], and since then, almost no such cases have been reported in other countries. The high prevalence of T188K and E196A mutations in Chinese PrDs is a unique feature of the PrD profile in China.

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Availability of Data and Materials Data are available on reasonable request. All anonymized data from this study will be shared on request from any qualified investigator. Data reuse is permitted only for academic purposes.

Competing interests The authors declare that they have no competing interests.

Patient Consent Use of patients' information stored by the China National Surveillance for CJD (CNS-CJD) was approved by the Research Ethics Committee of the National Institute for Viral Disease Control and Prevention, China CDC. Written informed consent for each case was given mostly by a member of the patient's family according to the requirements of CJD surveillance.

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