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ORIGINAL ARTICLE

Follow-up study of 22 Chinese children with Alexander disease and analysis of parental origin of *de novo* *GFAP* mutations

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To delineate the phenotype and genotype in Chinese children with type I Alexander disease (AxD) and the parental origin of *de novo* glial fibrillary acidic protein (*GFAP*) mutations. Twenty-two children with clinically diagnosed type I AxD were followed up for 1.66–6.62 years. Allele-specific PCR was used for the analysis of parental origin of the allele harboring the *de novo* mutation. Phenotype of these patients were consistent with type I AxD described in other population, with developmental delay (motor delay in 81.82%, cognitive delay in 63.64%), macrocephaly (100%), seizures (95.45%), paroxysmal deterioration (27.27%) and typical brain magnetic resonance imaging (100%). Progression was slower than reported. At 8.55 years of age (5.29–13.25), all patients who underwent the second follow-up were alive. Eleven heterozygous missense mutations of *GFAP* were identified in 21 patients, with three novel mutations. Reported hot spot mutations, p.R79, p.R239 and p.R88, were also identified in Chinese patients. Mutations were *de novo* in all but one case. The mother of a proband was demonstrated to be a presymptomatic patient with type II AxD with a p.R79H mutation. Ninety percent of *de novo* mutations were on the paternal allele demonstrated by allele-specific PCR. This is the largest follow-up study on Chinese children with AxD. The phenotypes of these patients are consistent with reports in other populations. *GFAP* mutations were identified in 95.46% of Chinese children with clinically diagnosed type I AxD. Our data suggested a male germ-line transmission.
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Keywords: Alexander disease; Chinese; follow-up study; *GFAP*; parental origin

INTRODUCTION

Alexander disease (AxD) is a rare progressive leukoencephalopathy inherited in an autosomal dominant manner. Age-dependent clinical subtypes have been defined for almost 30 years, including infantile (onset before 2 years of age), juvenile (2–14 years) and adult AxD (late juvenile to adult).[1] Overlaps between age-dependent subtypes, especially between juvenile and adult AxD, have been described.[2] Revised clinical subtypes defined as type I and II were proposed in 2011 based on a careful review of 215 AxD cases.[3] Type I is characterized by early onset (usually <4 years of age), macrocephaly, seizures, developmental delay, paroxysmal deterioration and typical magnetic resonance imaging (MRI) findings. Type II is characterized by later onset, autonomic dysfunction, ocular movement abnormalities, bulbar symptoms and atypical MRI findings. The pathological hallmark of AxD is the accumulation of Rosenthal fibers in astrocytes, composed of glial fibrillary acidic protein (GFAP), heat shock protein 27 and αB-crystallin.[4,5] Diagnosis was on the basis of the neuropathological features before the identification of *GFAP* mutations in 2001.[6] Nearly all ($>90\%$) cases with AxD harbor heterozygous *GFAP* mutations,[7] and most of these mutations occur *de novo*.[8] In this study, we performed a 1.66–6.62 year follow-up of 22 Chinese children with type I AxD and analyzed the parental origin of the allele bearing *de novo* mutations.

PATIENTS AND METHODS

Clinical diagnosis, assessment and follow-up

Clinical diagnosis of AxD was made on the basis of typical MRI features proposed by van der Knaap.[9] We enrolled patients since 2005. In December 2010 and February 2012, follow-up was performed.

Motor function assessment was based on the Gross Motor Function Classification System (GMFCS), Chinese version.[10] Levels I-V was classified according to the motor function and age of the children. For patients who had acquired the skill of walking, level I was classified as walking independently indoors and outdoors, level II as walking independently but with limitations on uneven surfaces and inclines, level III as walking with assistance, level IV as ambulance using a power wheelchair and level V as loss of independent mobility.

Gesell or Wechsler Intelligence Scale for Children was used for assessment of the cognitive function. The scale for cognitive function was classified as C0 (normal, IQ (intelligence quotient)/DQ (development quotient)≥ 70), C1 (mild impairment, IQ/DQ 55–69), C2 (moderate impairment, IQ/DQ 40–54) and C3 (severe impairment, IQ/DQ<40).

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Standard protocol approval and patient consent

The study was approved by the clinical research ethics committee of Peking University First Hospital, and informed consent was obtained from all parents.

GFAP gene sequencing

Genomic DNA was extracted from the white blood cells. The sequence of *GFAP* was obtained from the UCSC Genome Bioinformatics database (NM_002055). Nine exons and flanking introns were sequenced. Novel mutations found in patients were tested in 100 controls.

Analyzing the parental origin of *de novo GFAP* mutations

To determine the parental origin of the chromosome bearing the *de novo* mutation, allele-specific PCR was performed in 10 cases (Table 1). For each mutant nucleotide, informative single-nucleotide polymorphisms within 2 kb region flanking the mutation were selected. Subsequently, with one of the primer pair ending with the alternative nucleotide of the informative single-nucleotide polymorphism (for example, rs139617907, A or T), and a common primer, allele-specific PCR was performed to amplify the single-allele containing either the wild-type or mutant site. The parental origin of the mutation was determined based on the presence of the informative single-nucleotide polymorphism within the specifically amplified mutant allele. Each amplification was repeated for three times.

RESULTS

Twenty-two patients were clinically diagnosed with type I AxD, including 13 boys and 9 girls. All individuals were unrelated and children of non-consanguineous parents.

Phenotype of patients

Disease onset and signs at first visit. The developmental milestones of the motor function were slow in 81.82% ($n=22$) in the first year of life, with cognitive developmental delay in 63.64%. At the first visit, the average age was 4.83 years (0.54–12.08). The chief complaints were developmental delay in 5(22.73%), recurrent seizures in 14 (63.64%) or acute onset of walking disability after a falling in 3(13.64%). Macrocephaly was noticed in most patients, with head circumference of 44.00–58.00 cm, 6.33% (1.32–13.95%) above average. Physical examination showed hypertonia in 7 (31.82%, $n=21$), hypotonia in 2 (9.52%), hyperreflexia in 12 (57.14%) and Babinski sign in 10 (47.62%). At the first visit, GMFCS I was scored in 13 (59.09%, $n=22$), II in 5 (22.73%), IV in 1 (4.54%), and V in 3 (13.64%). Eight cases (36.36%, $n=22$) showed no cognitive impairment. Mild cognitive dysfunction (C1) was found in 8 (36.36%), C2 in 2 (9.10%), and C3 in 4 (18.18%). Brain MRI was performed at an age of 0.67–6.00 years (Figure 1). Blood biochemistry and metabolic test were unremarkable in all cases. Symptoms and sign at first visit were shown in Table 2.

Disease progression. 0.13–5.41 years after the first visit, 19 patients underwent the first follow-up, with an average age of 7.34 years (1.38–14.71). 1.66–6.62 years after the first visit, 14 underwent the second follow-up, with the average age 8.55 years (5.29–13.25). Some cases were not followed because of failure to make contact.

Progression of motor and cognitive impairment. At the first follow-up, motor function was classified as GMFCS I in 10 (52.63%, $n=19$), II in 6 (31.58%), IV in 1 (5.26%) and V in 2 (10.53%). Compared with the first visit, 14 (73.68%) remained stable, 3 (15.79%) showed regression and 2 (10.53%) showed improvement. Most patients (84.21%) preserved the ability to walk independently. At the second follow-up 1.25 years later, 6 (42.86%, $n=14$) were classified as GMFCS I, 3 (21.43%) as II, 2 (14.29%) as III and 3 (21.43%) as IV. Compared with the first follow-up, 10 (71.43%) remained stable, while 4 (28.57%) showed regression and could not walk by themselves.

Table1 Primers and results of allele-specific PCR

Case	*Mutation*	Single-nucleotide polymorphism	*Allele-specific PCR primers*		*Products size(bp)*	*Paternal/Maternal allele bearing the mutation*
			Forward primer(5′→3′)	*Reverse primer(5′→3′)*		
3	R88C	rs111979924	5′-CTCTGGGCACAGTGACCTC-3′	5′-AGGCTCAGAATAGGTGAGCTC***G***-3′ 5′-AGGCTCAGAATAGGTGAGCTC***A***-3′	977	M
4	R239C	rs78435425	5′-CTTGTGACCCCCATCAAGTT-3′	5′-ATTGCCTCATACTGCGTGC***G***-3′ 5′-ATTGCCTCATACTGCGTGC***A***-3′	1240	*P*
5	R239C	rs139617907	5′-AGAGACACTCAGAGAGAGGAGAG***A***-3′ 5′-AGAGACACTCAGAGAGAGGAGAG***T***-3′	5′-TATTCTCCCAGCTTCCTCCA-3′	470	*P*
8	R79L	rs111979924	5′-CTCTGGGCACAGTGACCTC-3′	5′-AGGCTCAGAATAGGTGAGCTC***G***-3′ 5′-AGGCTCAGAATAGGTGAGCTC***A***-3′	977	*P*
10	R88S	rs2070935	5′-TAGAGAGAGGGTCCTCTTG***A***-3′ 5′-TAGAGAGAGGGTCCTCTTG***C***-3′	5′-CACTCCTTCTTGGGGATTCA-3′	808	*P*
15	N77K	rs28485918	5′-CCTATCAGGACCTCCACTGC-3′	5′-TGTAGCTGGCAAAGCGGTC***A***-3′ 5′-TGTAGCTGGCAAAGCGGTC***T***-3′	1110	*P*
18	R239H	rs111979924	5′-TGAATCCCCAAGAAGGAGTG-3′	5′-CATTGCCTCATACTGCGTG***C***-3′ 5′-CATTGCCTCATACTGCGTG***T***-3′	1668	*P*
19	R79C	rs111979924	5′-GATGATGGAGCTCAATGAC***C***-3′ 5′-GATGATGGAGCTCAATGAC***T***-3′	5′-CCTCTGATCCCAGGTAACC***A***-3′	811	*P*
20	R239H	rs111979924	5′-TGAATCCCCAAGAAGGAGTG-3′	5′-CATTGCCTCATACTGCGTG***C***-3′ 5′-CATTGCCTCATACTGCGTG***T***-3′	1668	*P*
21	R239H	rs111979924	5′-TGAATCCCCAAGAAGGAGTG-3′	5′-CATTGCCTCATACTGCGTG***C***-3′ 5′-CATTGCCTCATACTGCGTG***T***-3′	1668	*P*

Abbreviations: M, maternal; P, paternal; The 3′ ending of the specific primers are shown in bold and italic.

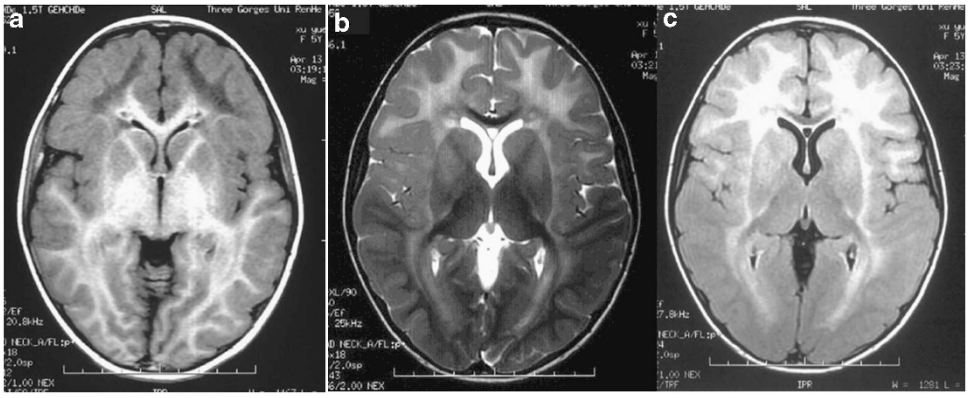

Figure 1 Typical brain magnetic resonance imaging in one of our patients with type I AxD. (**a**) (T1 image), (**b**) (T2 image) and (**c**) (Falir image) illustrate the characteristic abnormalities in white matter with predominance in the frontal lobe, and swelling of the basal ganglia.

Table 2 Clinical features of 22 AxD patients

			Symptoms and signs on first visit						*First follow-up*		*Second follow-up*		
					Head circumferences								
Patient	*Sex*	*Developmental delay*	*Age (year)*	*Complaint*	*cm*	*L (%)*	*Symptoms*	*Babinski sign*	*Age (year)*	*Symptoms*	*Age (year)*	*Symptoms*	*Episodic aggravations*
1	F	−	6.42	S	53.5	5.88	GI.C0.S	−	11.83	GI.C0.S	13.04	GI.C0.S	−
2	M	−	2.67	U	NA	NA	GI.C0	NA	7.83	GI.C0	9.04	GI.C0	+
3	M	−	7.00	S	53.5	5.73	GI.C0.S	+	NA	NA	NA	NA	−
4	M	+	1.83	DD	50	3.80	GI.C2	+	5.50	GII.C0.S	6.63	GIV.C1.S	+
5	M	+	1.67	S	51	7.14	GI.C3.S	−	5.17	GII.C2.S	6.29	GII.C2.S	−
6	M	+	0.75	S	44	2.33	GV.C3.S	−	4.17	GIV.C3	5.29	GIV.C2	−
7	M	+	3.25	DD	53	7.29	GI.C0.S	+	6.58	GI.C0.D	7.75	GIII.C1.D	+
8	M	+	5.83	S	NA	NA	GI.C0.S	−	8.67	GI.C1	9.83	GI.C1	−
9	F	+	5.17	S	55	12.02	GII.C1.S	−	7.75	GII.C1.S	8.92	GII.C1.S	−
10	M	+	2.25	U	50	3.31	GII.C1.S	+	4.50	GI.C1	5.67	GI.C1	+
11	M	+	5.75	S	54	6.09	GI.C1.S	−	7.33	GI.C1.S	8.54	GI.C1.S	+
12	M	+	12.08	S	54	6.09	GI.C1.S	−	14.71	GII.C1.	NA	NA	
13	F	+	10.88	S	58	13.95	GI.C1.S	−	12.08	GI.C1	13.25	GI.C1	−
14	M	+	10.17	S	54	5.26	GII.C2.S	+	11.17	GII.C2.S	12.33	GII.C2.S	−
15	F	−	5.33	U	53	7.94	GI.C0.S	+	5.92	GI.C0	7.08	GIII.C0	+
16	M	+	4.42	DD	51	1.80	GI.C0.S	−	4.92	GI.C0.D	6.08	GIV.C2.D.S	−
17	F	+	6.42	S	54	9.98	GI.C0.S	+	8.33	GI.C0	NA	NA	−
18	F	+	2.67	DD	49.5	3.13	GV.C3.S	+	3.17	GV.C3.D	NA	NA	−
19	F	+	8.33	DD	55	10.67	GII.C1.S	+	8.54	GII.C1	NA	NA	−
20	F	+	1.25	S	46	1.32	GV.C3.S	−	1.38	GV.C3	NA	NA	−
21	M	+	0.54	S	49	8.65	GIV.C1.S	+	NA	NA	NA	NA	−
22	F	+	1.67	S	48.5	4.30	GII.C1.S	−	NA	NA	NA	NA	−

Abbreviations: AxD, Alexander disease; C0-3, cognitive scale; DD, developmental delay; D, dysphagia; F, female; GI–V, GMFSC scale; L, larger than age-matched average; M, male; NA, not available; +, positive; −, negative; S, seizures; U, unable to walk after falling.

At the first follow-up, 7 (36.84%, $n=19$) showed no cognitive impairment, 7 (36.84%) were classified as C1, 2 (10.53%) as C2 and 3 (15.79%) as C3. Compared with the first visit, most patients (15, 78.95%) remained stable, 2 (10.53%) showed regression and 2 (10.53%) showed improvement. At the second follow-up visit, there were only 3 (21.43%, $n=14$) without cognitive impairment, 7 were classified as C1 (50.00%), and 4 as C2 (28.57%). Compared with the first follow-up, 10 (71.43%) remained stable, 3 (21.43%) showed decline and 1 (7.14%) showed improvement.

For the 14 cases underwent second follow-up, change in constituent ratio of motor and cognitive scaling showed a gradual increase of patients with functional impairments (Figure 2).

Paroxysmal aggravations and seizures. Paroxysmal aggravations presented in 27.27% ($n=22$). These were characterized by acute onset of walking disability and weakness of limbs after infection or trauma, followed by slow recovery. Seizures were present in 95.45% ($n=22$) until the last follow-up. They were characterized by partial motor or generalized tonic-clonic seizures. The first seizure occurred at an average of 1.56 years of age (0.33–5.00). Seizures were precipitated by fever in 42.86%. Seizure frequency varied among individuals, from once per month to once per year. Antiepileptic drugs, such as valproate, topiramate or levetiracetam, were prescribed for 16 patients. At the second follow-up, 42.86% still had seizures.

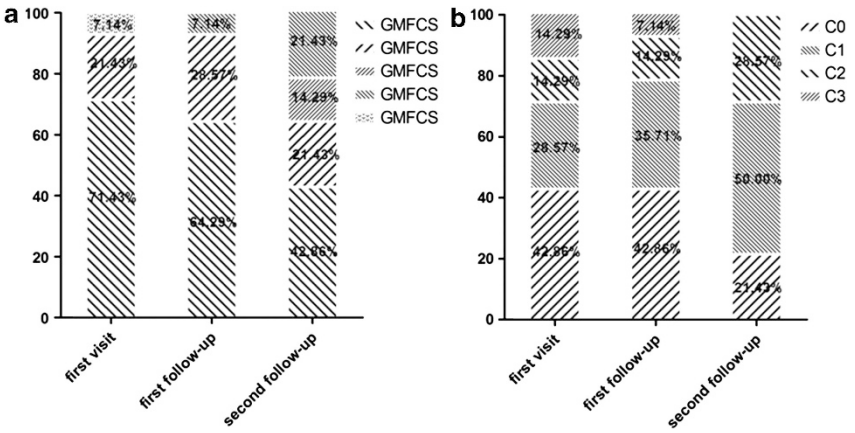

Figure 2 Constituent ratio of motor and cognitive scaling at first visit and follow-up visits. For the 14 cases underwent second follow-up, change in constituent ratio of motor (**a**) and cognitive scaling (**b**) showed a gradual increase of patients with functional impairments.

Genotype of patients

Heterozygous missense mutations of *GFAP* were detected in 21 (95.45%, $n=22$) (Table 3). Eleven mutations were identified, of which three were novel. Mutations affected p.R79 in 6 (28.57%, $n=21$), p.R239 in 5 (23.81%) and p.R88 in 5 (23.81%). Mutations were identified in exon 1 in 13 (61.91%), exon 4 in 5 (23.81%), exon 6 in 2 (9.52%) and exon 7 in 1 (4.76%). All mutations were *de novo* except in patient 17. The girl inherited the mutation p.R79H from her mother. The mother was 29-years-old with average IQ, and without symptoms of ocular movement abnormalities, bulbar symptoms, seizures or cognitive decline at the time of follow-up. Brain MRI showed abnormalities in deep white matter and atrophy of the medulla oblongata and upper cervical spinal cord (Figure 3).

Parental origin of the allele harbor the *de novo* mutation

Allele-specific PCR was performed in 10 cases with available informative single-nucleotide polymorphisms in the 2 kb region flanking the mutations. In 90% of cases ($n=10$), mutations occurred on the paternal allele (Table 1).

Table 3 *GFAP* mutations in 22 AxD patients

Cases	*Exon*	*Nucleotide change*	*Amino-acid change*	*Location in protein domain*	*Parental derivation*	*Novel/ reported*
1	e1	c.262C>T	p.R88C	Coil-1A	—	Reported
2	e1	c.262C>T	p.R88C	Coil-1A	—	Reported
3	e1	c.262C>T	p.R88C	Coil-1A	—	Reported
4	e4	c.715C>T	p.R239C	Coil-2A	—	Reported
5	e4	c.715C>T	p.R239C	Coil-2A	—	Reported
6	e7	c.1154C>T	p.S385F	C-terminal	—	Novel
7	e6	c.1119G>C	p.E373D	C-terminal	—	Novel
8	e1	c.236G>T	p.R79L	Coil-1A	—	Reported
9	e1	c.226C>T	p.L76F	Coil-1A	—	Reported
10	e1	c.262C>A	p.R88S	Coil-1A	—	Reported
11	e1	c.262C>T	p.R88C	Coil-1A	—	Reported
12	—	—	—	—	—	—
13	e1	c.236G>A	p.R79H	Coil-1A	—	Reported
14	e1	c.235C>T	p.R79C	Coil-1A	—	Reported
15	e1	c.231T>A	p.N77K	Coil-1A	—	Novel
16	e1	c.235C>T	p.R79C	Coil-1A	—	Reported
17	e1	c.236G>A	p.R79H	Coil-1A	Maternal	Reported
18	e4	c.716G>A	p.R239H	Coil-2A	—	Reported
19	e1	c.235C>T	p.R79C	Coil-1A	—	Reported
20	e4	c.716G>A	p.R239H	Coil-2A	—	Reported
21	e4	c.716G>A	p.R239H	Coil-2A	—	Reported
22	e6	c.1120G>C	p.E374Q	C-terminal	—	Reported

Abbreviations: AxD, Alexander disease; GFAP, glial fibrillary acidic protein; —, negative.

DISCUSSION

Phenotype of AxD

Modified classification systems of clinical subtype of AxD have recently been proposed, slightly different from the conventional age-dependent system. Yoshida *et al.*[11] proposed a guideline to classify AxD into cerebral (type 1), bulbospinal (type 2) and intermediate (type 3) on the basis of neurological and MRI findings. Patients between types 1 and 2, and long-term survivors with infantile AxD were considered as type 3. A survey in Japan revealed that type 1 AxD accounted for 34.3% of cases, type 2 accounted for 45.7% and type 3 accounted for 20%. Based on a latent class analysis on 215 AxD patients to statistically define AxD subtypes, Prust *et al.* recently proposed to categorize patients as either type I or type II.[3,8] The modified classification focuses less on the age of onset and more on constellations of symptoms and MRI features. The estimated prevalence rates of types I and II are 60% and 40%, respectively.

As diagnosis was made on the basis of typical MRI features, all children in our study were classified as type I according to Prust's system. Type I is usually characterized by early onset, macrocephaly, seizures, developmental delay, paroxysmal deterioration and typical MRI findings. The phenotypes of our 22 Chinese patients were consistent with the typical features. Motor development was delayed in 81.82%, and cognitive delay in 63.64%. Macrocephaly was noticed in most patients. Seizures have been reported in 92% of infantile patients, 39.1% with juvenile AxD and 16.1% with adult AxD.[1,12] In our study, 95.45% experienced recurrent seizures, with 42.86% precipitated by fever. The first seizure occurred at as early as 4 months of age, with an average of 1.56 years. The earlier onset and higher prevalence of seizures in type I AxD is probably due to the early involvement of subcortical white matter. Paroxysmal deterioration was more common in type I than in type II.[3] Episodic aggravations presented in 27.27% of our patients. Paroxysmal deterioration after febrile illness or head trauma has been reported in other hereditary leukoencephalopathies, especially in vanishing white matter disease, which is supposed to be related to endoplasmic reticulum (ER) stress in glial cells.[13,14] Disease progression is

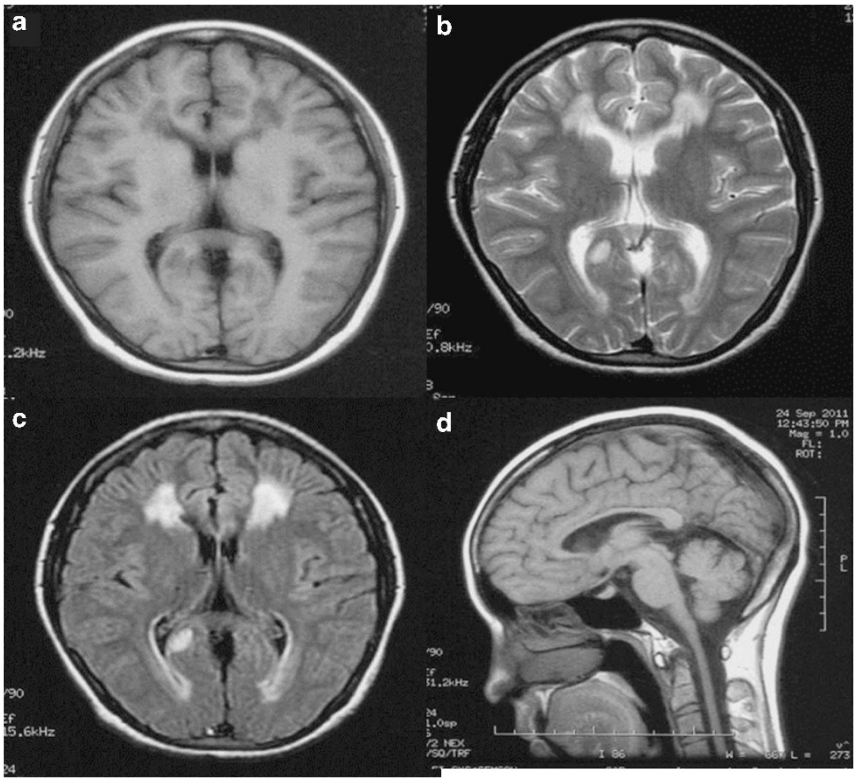

Figure 3 The brain magnetic resonance imaging of the presymptomatic mother with type II AxD. (**a**) (T1 image), (**b**) (T2 image) and (**c**) (Flair image) showed abnormalities in periventricular white matter, and atrophy of the medulla oblongata and upper cervical spinal cord (**d**).

much more rapid in type I than in type II. The survival analysis in Prust's study revealed a mean survival of 14.0 ± 1.8 years in type I. At 8.55 years of age (5.29–13.25), all patients who underwent the second follow-up in our study were alive. The motor or cognitive function was slowly gradually impaired in most patients, with GMFCS I still preserved in 42.86%, and C0 in 21.43%. Further follow-up of this cohort is needed to fully understand the progressive process in Chinese patients.

Type II AxD is characterized by late onset, autonomic dysfunction, ocular movement abnormalities, bulbar symptoms, palatal myoclonus and atypical MRI findings. Signal abnormalities and/or atrophy of the medulla oblongata and cervical spinal cord are common features on MRI.[15,16] Some atypical imaging features were reported, like focal lesions, abnormal signals in cerebellum and calcifying lesions in the sub-cortex and cortex.[17–19] The survival analysis in Prust's study revealed a mean survival of 25.0 ± 2.1 years. It was reported that half of the late-onset cases were familial, whereas familial cases are very rare in infantile type.[20,21] In our study, the mother of patient 17 was demonstrated to have a heterozygous *GFAP* mutation p.R79H. She was 29-years-old without any symptoms at the time of the follow-up. Brain MRI showed common features of Type II AxD. Given the 100% penetrance of *GFAP* mutations, she should be a presymptomatic patient. The age of disease onset in type II AxD is estimated to be 21.64 ± 2.35 years,[3] with a maximum age of 63 years.[1] Follow-up of the mother is needed. To our knowledge, this is the second case of late-onset AxD with a p.R79H mutation worldwide.

Genotype of AxD

GFAP encodes glial fibrillary acidic protein. It is an astrocytic intermediate filament that forms the cytoskeleton together with microfilaments and microtubules, with participates in cytoskeleton restructuring, cell adhesion and myelination.[22] It is composed of a central α-helical rod domain flanked by a non-α-helical N-terminal head and C-terminal tail domains. The rod domain comprises four helical domains (1A, 1B, 2A and 2B), separated by short linkers (L1, L12 and L2).[23] Possible pathogenic mechanisms underlying AxD include GFAP aggregation, oxidative stress, autophagic response in glia and decreased transfer of glutamine from astrocytes to neurons.[24–26]

GFAP sequencing can identify mutations in >90% of AxD patients. To date, almost 100 different mutations have been reported.[11] More than half involved in one of the hot spots including p.R239 (20.3%), p.R79 (16.6%), p.R88 (7.9%) and p. R416 (5.6%).[3] In our study, mutations affected p.R79 in 28.57%, p.R239 in 23.81% and p.R88 in 23.81%, with a higher prevalence of p.R88 mutations. All mutations identified in our study are missense, consistent with other reports. Small deletions and short in-frame insertions have been found in a few patients.[8] It was reported that *GFAP* mutations were identified in exon 1 in 45.2% of cases, exon 3 in 3.3%, exon 4 in 27.2%, exon 5 in 1.8%, exon 6 in 16.0%, exon 7 in <1% and exon 8 in 7.5%. No mutations were found in exons 2 and 9.[3] In our study, mutations were identified in exon 1(61.90%), exon 4(23.81%), exon 6 (9.52%), and exon7 (4.76%). The distribution of mutations across the GFAP domains was reported as follows: N-terminal head (<1% of cases), coil-1A (43.7%), coil 1B (4.2%), L12 (<1%), coil-2A (23.7%), L2 (<1%), coil 2B (13.0%), and C-terminal tail (13.5%). No mutations have been identified in the L1 region.[3] The mutations identified in our cases were distributed in coil-1A (61.90%), coil-2A (23.80%), and C-terminal tail (14.29%). We could not identify a *GFAP* mutation by sequencing in one patient.

Mutations in untranslated regions or deep introns, or copy number variations (especially duplication of *GFAP*), probably underlie the disease in nearly 10% of mutation-negative patients.

Genotype–phenotype correlation

It was reported that type I shows higher incidence of p.R79 and p.R239 mutations than type II.[3] A few studies analyzed the phenotypic difference between mutations in p.R79 and p.R239. Patients with p.R239 mutations appear to be more severe.[1,27,28] Like other inherited disorders, the genotype of *GFAP* is absolutely not the only determinant factor for phenotype. Patient 17 and her mother, who shared the same genotype, showed totally different phenotype. Therefore, other genetic or environmental factors influence the phenotype.

Tendency for *de novo GFAP* mutation on the paternal allele

In 90% of analyzed cases in our study, the *de novo* mutations were harbored on the paternal allele. This result is consistent with a previous research, which showed that *de novo GFAP* mutations were on the paternal allele in 85.7% of cases ($n = 28$).[29] It is possible that *GFAP* mutations have a high frequency of male germ-line transmission. *De novo* mutations tend to occur on the paternal allele in other genetic neurological diseases, like Rett syndrome and Dravet syndrome.[30,31] There are several potential explanations for this male-biased mutation. Most point mutations at a CpG nucleotide pair are caused by methylation.[32] Methylation occurs more during spermatogenesis.[33] This may explain why 43.6% of *GFAP* mutations occurred at CpG methylation sites.[11] More cell divisions in the life history of sperm also increase the possibility of errors.[34]

CONCLUSIONS

This is the largest follow-up study on Chinese children with type I AxD. The phenotypes of these patients are consistent with reports in other populations. The mother of a proband was demonstrated to be a presymptomatic patient with type II AxD, who is the second case of late-onset AxD with a p.R79H mutation worldwide. Reported hot spot mutations of *GFAP*, p.R79, p.R239 and p.R88, were also identified in Chinese patients. Our data showed that 90% of *de novo GFAP* mutations were on the paternal allele, suggesting male germ-line transmission. More patients and a longer follow-up period are needed for a better understanding of the clinical and genetic characteristics of AxD.

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