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ORIGINAL COMMUNICATION

Atypical familial amyotrophic lateral sclerosis with initial symptoms of pain or tremor in a Chinese family harboring VAPB-P56S mutation

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Abstract Amyotrophic lateral sclerosis (ALS) is the most prevalent fatal motor neuron disease and ~10 % of cases are hereditary. Mutations associated with ALS have been identified in more than 20 genes, but ALS type 8 (ALS8), which is caused by mutations in vesicle-associated membrane protein-associated protein B (VAPB), is rare. To date, the dominant missense mutation P56S, which is in the major sperm protein domain of VAPB, has been described in nine families of Portuguese-Brazilian origin and one family of German origin. Here, we report a Chinese family spanning three generations with ALS8 caused by the same VAPB-P56S mutation detected in these cohorts, but which in its initial manifestation displays different features. We also detected a R545Q variant of optineurin (OPTN) in this family and which was previously considered a pathogenic mutation. However, our analysis showed that OPTN-R545Q is benign and that VAPB-P56S accounts for the phenotype. Haplotype tests revealed that VAPB-P56S in the Chinese family has arisen independently from the Brazilian cohorts. To our knowledge, this is the first study to report ALS caused by a VAPB mutation in a Chinese population.

Keywords Amyotrophic lateral sclerosis · ALS type 8 · Vesicle-associated membrane protein-associated protein B · Optineurin

Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive fatal neurological disease and the most common form of motor neuron disease. It is characterized by progressive neuronal loss and degeneration of motor neurons. The decrease of motor neurons leads to loss of control of voluntary muscle movement and eventually results in death owing to respiratory failure in the later stages of the disease. Although the first ALS case was described more than a century ago [1], its underlying mechanism is still unclear. Approximately 10 % of ALS cases are familial, whereas the rest of the cases are sporadic. Since the first SOD1 variant in ALS was established as the first causative gene for ALS, mutations causing familial ALS have been identified in more than 20 genes [2]. ALS type 8 (ALS8) displays a pattern of dominantly inherited motor neuron disease. It was first identified in a large Brazilian family in 2004 and traced to a heterozygous missense mutation p.P56S of vesicle-associated membrane protein-associated protein B (VAPB) and displays a wide range of phenotypes including late-onset spinal muscular atrophy, atypical ALS, or typical ALS [3]. As of 2008, an additional eight families with ALS8 caused by the same mutation were identified and again were all of Portuguese/Brazilian origin with autonomic abnormalities [3–5]. Patients with ALS8 that descended from outside the Brazilian population were not reported until 2 years later when a German patient with ALS8 caused by the same mutation in VAPB was identified. The mutation did not originate from the same founder in the Brazilian population

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[6]. Subsequent studies found ALS8 caused by mutations of VAPB in patients from Japan, the United Kingdom, and the Netherlands [7–9]. However, it is unknown whether the mutations in these three families were inherited from the same ancestors of the Brazilian or German cases. Here, we report VAPB-P56S causing ALS8 in a Chinese family and with different initial manifestations from all previously reported cohorts, and which is not inherited from the same ancestor as the patients in the Brazilian population. This is the first report of ALS caused by a VAPB mutation in a Chinese population.

Methods

Pedigree

The index patient was a 57-year-old female Chinese with progressive pain, weakness, and cramps (II-5 in Fig. 1). Her father exhibited similar symptoms. An extended family investigation was performed. We carried out a detailed clinical assessment of 19 individuals spanning three generations. Age of onset was defined as the age at which the patient first noticed any symptoms of the disease, including weakness, atrophy, fasciculation, cramps, pain, and tremor. The muscle power degree was determined according to the Medical Research Council grading scale. There was no family record of Portuguese, Brazilian, or German ancestors in at least four previous generations. All family members originated from southern mainland China. The study was conducted after receiving written informed consent from the patients. This study was approved by the Institutional Ethics Committee of Xuan Wu Hospital, Capital Medical University, Beijing, China.

Laboratory tests

Laboratory examinations of the index case and patient II-7 included: electrocardiography, echocardiography, electromyography (EMG), and routine laboratory tests. Biochemical analysis included: serum creatine kinase, electrolytes, fasting glucose, blood urea, nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, thyroxine, thyroid-stimulating hormone, sedimentation rate, and C-reactive protein. EMG was done in II-9.

Molecular tests

Whole-exome sequencing: DNA was extracted from anticoagulated blood from affected and unaffected individuals using a standard phenol–chloroform method. Indexed genomic DNA (gDNA) libraries were prepared from patient gDNA using the TruSeq DNA Preparation kit (Illumina, USA) and exome captured using the TruSeqExome Enrichment kit (Illumina, USA) according to the manufacturer's protocol. Sequencing was performed with 100-bp paired-end reads on a HiSEq 2000 (Illumina, USA). Reads were aligned to the human reference genome with NovoAlign (Novocraft Technologies, USA). Variants were named with SAMtools15 and annotated with SeattleSeq. Pathogenicity predictions for variants were obtained from SeattleSeq and SIFT16. Sequencing coverage depth was calculated using BEDTools17 and genomic coordinates provided by Illumina. Control exomes were from patients undergoing exome analysis for diseases other than ALS. All reported genomic locations are from GRCh37/hg19. Segregation of mutations was assessed with standard polymerase chain reaction-based sequencing using Primer3Plus.

Haplotype analysis

The available information concerning microsatellite markers D20S100, D20S1148, D20S430, D20S164, D20S171, and D20S173 was extracted from the National Center for Biotechnology Information database. The primers for polymerase chain reaction amplification and SNaPshot extension reactions were designed with the National Center for Biotechnology Information sequence database using Primer 5 software.

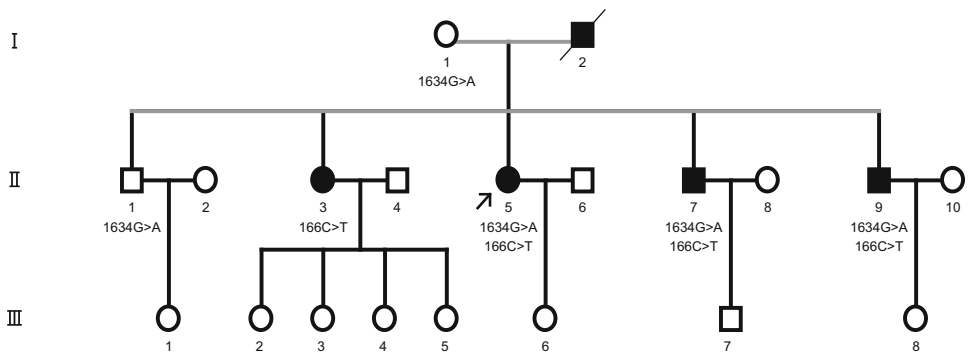

Fig. 1 Pedigree of the family. I-1,II-1, and III-1are normal, who have the heterozygous c.1634G>A/p.R545Q in *OPTN* gene; II-3,5,7,9 have symptoms of ALS, II-3 has a heterozygous c.166C>T/p.P56S in *VAPB* gene, II-5,7,9 have the heterozygous c.1634G>A/p.R545Q in *OPTN* gene and the heterozygous c.166C>T/p.P56S in *VAPB* gene

Results

Clinical features and course

Our clinical findings for the individual patients are summarized in Table 1. The age at onset ranged from 42 to 51 years. I-2 died aged 54 years. The only symptom in II-9 was pain in limbs and lower back and his EMG test was normal. II-3, 5, and 7 were positively diagnosed according to El Escorial criteria. I-2, II-3, and II-7 had tremor, which was the first symptom experienced by these three patients. All patients noticed tremor during their 30 s. The second early symptom in affected patients was pain in limbs and lower back, which was the first symptom experienced by II-5 and similar to that described by II-9. They described the pain as sore, electrical, or miserable. II-3, 5, and 7 then experienced pain in the pelvic girdle and had proximal lower limb weakness. They noted difficulty in climbing stairs as well as rising from a squatting position. Weakness in the shoulder girdle and proximate upper extremity were observed in all affected patients. Distal lower limb weakness was also observed. II-3 and II-7 were unable to walk on their toes. II-5 could not walk on her toes or heels. II-3 and II-5 had distal upper limb weakness. All had cramps and pyramidal tract signs. Cramps were painful and mainly localized in the lower limbs, but were also present in the abdomen and upper limbs and mostly appeared with active movements. Furthermore, all three patients had severe constipation and required stimulation of the rectum, indicating autonomic nerve involvement in the family. The disease course ranged from 6 to 12 years in all five patients. The disability slowly progressed over the years and the prognosis was relatively benign. I-2 died of ventilator paralysis 12 years after onset. None of the individuals in the third generation had symptoms, and the eldest was 37 years old.

Laboratory examinations

The serum creatine kinase level was 296 IU/L in the index patient II-5 and 242 IU/L in patient II-7; this was mildly elevated from the normal range which is between 18 and 198 IU/L. Creatine kinase levels in other family members were not detected. Needle electromyography revealed chronic denervations of muscles of upper limbs, lower limbs, and paravertebral muscles, with normal repetitive nerve stimulations in patient II-5 and patient II-7, but which was normal in II-9. The upper and lower extremity sensory and motor nerve conduction studies were normal.

Mutation analysis

Whole-exome sequencing in the index patient revealed a heterozygous c.166C>T/p.P56S missense mutation in *VAPB* gene (NM_001195677), which encodes vesicle-associated membrane protein-associated protein B, and a

Table 1 Clinical findings in five affected individuals

Case	I-2	II-3	II-5	II-7	II-9
Sex	M	F	F	M	M
Age at examination (year)/died	54 died	59	57	53	50
Age at onset (year)	42	50	51	47	44
Progression	12	9	6	6	6
First symptoms	Tremor	Tremor	Pain	Tremor	Pain
Pain	–	Yes	Yes	Yes	Yes
Tremor	Yes	Yes	No	Yes	No
Fasciculations	–	Yes	Yes	Yes	Yes
Cramps	–	Yes	Yes	Yes	No
Proximal arm	–	4	3	4	5
Distal arm	–	4	4	5	5
Proximal leg	–	3	2	3	5
Distal leg	–	4	4	4	5
Bulbar signs	No	No	No	No	No
Tendon reflex	–	Hyperactive	Hyperactive	Hyperactive	Hyperactive
Pyramidal signs	–	Yes	Yes	Yes	No
Muscle atrophy	Yes	Prox leg	Prox leg	Yes	No
Constipation	–	Yes	Yes	Yes	No
Als-FRS-R scale	–	31	26	31	40

– not known

heterozygous c.1634G>A/p.R545Q in *OPTN* gene (NM_001008211.1), which encodes optineurin. Both variants were confirmed by Sanger sequencing. VAPB-P56S was not present in 300 controls of Chinese origin and had only been previously described in ALS patients of Brazilian and German descent [3, 4, 6, 8]. The variant p.R545Q in the OPTN gene was reported to be pathogenic in an adult-onset familial patient with primary open-angle glaucoma [10], but our study showed that OPTN-R545Q is carried in 9.3 % of normal controls and is predicted to be benign by both Mutation Taster (http://www.mutationtaster.org/) and Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/). Further sequencing of other family members revealed VAPB-P56S was carried in three symptomatic individuals (II-3, II-7, and II-9) in the second generation. OPTN-R545Q was carried in asymptomatic individuals I-1 and II-1. As observed in the index patient, the two other symptomatic VAPB-P56S carriers, II-7 and II-9, also carry the OPTN-R545Q mutation.

Haplotype analysis

VAPB-P56S has been found in different ethnicities. We performed haplotype analysis on the index patient using microsatellite markers D20S100, D20S164, D20S171, D20S173, D20S430, and D20S1148. We found that the patient had a different number of repeats for each marker from the Brazilian families (Table 2).

Discussion

We identify two hetero-allelic variants in a Chinese ALS8 family, one in the vesicle-associated membrane protein (VAMP)-associated protein (VAPB-P56S) and the other in optineurin (OPTN-R545Q). VAPB-P56S has been previously found in non-Chinese ALS patients [4, 5]. Our patients represent a unique phenotype of ALS8 because their initial symptoms are different from reported cases. In this family, VAPB-P56S was carried in both four symptomatic individuals in the second generation and five asymptomatic individuals in the third generation. OPTN-R545Q has been considered a causative mutation for adult-onset primary open-angle glaucoma [10] and for ALS type 12 [11]. However, in this study, individuals harboring OPTN-R545Q variant alone showed no symptoms of glaucoma or ALS. Furthermore, 9.3 % of the healthy control group are OPTN-R545Q carriers, which is in agreement with an earlier study on screen testing of the same mutation in Asian controls [12]. Genetic testing in a large cohort of Brazilian patients shows a lack of association between OPTN-R545Q and primary open-angle glaucoma [13]. Therefore, we conclude that OPTN-R545Q is a benign variant and VAPB-P56S determines the phenotype of ALS8 in the Chinese family. Haplotype analysis shows different numbers of repeats for multiple satellite markers flanking the VAPB gene in the index patient of the Brazilian population, indicating that the deleterious VAPB-P56S mutation in the Chinese family has arisen independently from Brazilian patients. To our knowledge, this is the first study to report a Chinese cohort with ALS caused by a VAPB mutation.

Whereas most patients initially manifest symptoms of weakness in limbs or bulbar muscles, the patients in this Chinese ALS family have an uncommon initial symptom of either pain or tremor. Two-thirds of ALS patients eventually have pain [14], but, to our knowledge, this study is the first to report ALS patients manifesting with the initial symptom of pain. In fact, the only symptom in II-9 was pain in the limbs and lumbar sacral part. Moreover, in this family, the index patient had the most severe ALS phenotype and experienced the most pain. Our finding is consistent with other types of ALS patients whose pain interfered with daily activities [15]. However, we found no correlation between the duration of the disease and the severity of pain, which is in agreement with previous reports in other types of ALS patients [15, 16]. Our patients described the pain as sore, electrical or miserable.

Table 2 Haplotype comparison of the Chinese index patient and the patients from Brazilian families with a VAPB-P56S mutation

Marker	Chinese index patient	Brazilian families[a]							
		1	2	3	4	5	6	7	8
D20S100	13 14	3 5	2 5	1 5	4 5	2 3	2 5	3 5	1 3
D20S1148	13 17	4 5	3 5	6 5	3 5	4 5	4 5	4 5	3 5
D20S430	4 7	7 6	8 6	2 6	6 6	1 6	7 6	7 6	7 6
D20S164	14 15	3 3	4 3	4 3	3 3	4 3	4 3	4 3	4 3
D20S171	23 24	7 4	3 4	4 4	1 4	2 4	5 4	4 4	3 4
D20S173	19 20	1 2	2 2	6 2	2 2	2 2	2 2	2 2	2 2

Numbers are shown as repeat numbers

[a] From Nishimura et al. [23]

Electrical or miserable pain stemming from small-fiber neuropathy occurs in 79 % of other types of ALS patients [17]. Our results suggest that the VAPB-P56S mutation can affect the sensory function of distal epidermal nerve fibers at an early stage of the disease. Another common complaint in this family was severe constipation. This could be a consequence of the dysfunction of the enteric nervous plexus owing to the involvement of the efferent pathways responsible for autonomic control of gut movements [5]. Ample evidence exists for subclinical dysfunction of cardiovascular, gastrointestinal, sexual, salivary, and lacrimal regulation, even in early ALS cases [5, 18]. Complaints reported by ALS patients such as constipation, diffuse abdominal pain, and a feeling of fullness or nausea may be attributed to autonomic involvement [19]. The VAPB-P56S mutation has been identified in a family displaying proximal SMA with dysautonomia [5]. Our data indicate that VAPB-P56S impairs the autonomic nervous system in ALS patients.

VAPB-P56S-causing motor neuron diseases display heterogeneity of clinical phenotypes. A study of 24 patients carrying VAPB-P56S in seven kindred of the Brazilian population showed intra- and interfamilial heterogeneity of phenotypes with late-onset spinal muscular atrophy (LSMA), typical ALS, or atypical ALS [3]. The main clinical findings of LSMA are late onset (age 35–55 years) with proximal weakness, fasciculations, cramps, muscular atrophy and absent reflexes, without bulbar or pyramidal tract sign involvement [3]. The clinical features of LSMA were confirmed in more Brazilian patients [20]. Typical ALS is characterized by both lower motor neuron and pyramidal signs, with a survival time of less than 5 years. Atypical ALS patients manifest an average age at onset of 38 years (range 25–44 years) and bulbar signs (dysphagia) are seen in two-thirds of subjects. In addition to ALS manifestations, some patients had essential tremor and a slow course of progression, which is not seen in typical ALS. Our patients manifest unique symptoms at onset and fall into the category of atypical ALS.

The genotype and phenotype correlation of ALS8 has not been established. VAPB, as a member vesicle-associated membrane protein family of ER tail-anchored transmembrane proteins, is highly conserved and ubiquitously expressed. The wide spectrum of VAPB interacting proteins attributes a great variety of physiological functions. Four VAPB mutations, P56S and T46I in the major sperm protein domain [3, 7], S160del in the C-terminal half of the protein [4], and V234I in the transmembrane domain [21] have been identified. Many hypotheses have been proposed for the pathophysiology of VAPB-P56S based on the interaction of VAPB with its many different protein partners [22], however, the mechanism for each phenotype remains elusive. It is helpful to understand the wide spectrum of phenotypes in motor neuron disease caused by VAPB-P56S, but it also suggests that other factors besides dysfunction of mutant VAPB need to be considered. Further characterization of more Chinese ALS patients will answer whether the initial symptom of pain, observed in the current ALS8 family, is a predominant characteristic in ALS patients in the Chinese. Nevertheless, *VAPB* gene screening should be considered as a rare differential diagnosis in familial ALS in China.

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Compliance with ethical standards

Conflicts of interest All the authors listed have approved the content of the manuscript. There is no conflict of interest declared by any of the authors.

Ethical standards The study was conducted after receiving written informed consent from patients. In addition, this study was approved by the Institutional Ethics Committee of Xuan Wu Hospital, Capital Medical University, Beijing, China.

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