Identifying Gene Mutations in Autosomal Recessive Cerebellar Ataxias and Extending the Mutational Spectrum in China

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Research

Keywords: Autosomal recessive cerebellar ataxias, gene mutations, clinic phenotype, sequencing.

Posted Date: July 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-577033/v1

Abstract

Background: Autosomal recessive cerebellar ataxias (ARCA) are heterogeneous, complex, disabling neurodegenerative diseases characterized by autosomal recessive inheritance and cerebellar ataxia. Numerous mutations are described in several populations. However, in China, few data are available concerning ARCA. In this study, we aimed to identify ARCA-associated ataxia by targeted next-generation sequencing or whole-exome sequencing in a Chinese cohort, trying to determine clinical and genetic characteristics of Chinese patients with ARCA.

Results: We identified 15 different mutations in 7 unrelated patients, of which 12 were novel, including seven missense mutations, three frameshift mutations, two splicing mutations, two nonsense mutations, and one inframe deletion mutation. The most frequent gene was *ATM* (3 patients), followed by *SACS* (2 patients), *SYNE1* (2 patients), and *SETX* (1 patient). Specifically, 1 patient harbored mutations in both *ATM* and *SYNE1*. The phenotype was mainly cerebellar ataxia in all these cases. However, peripheral neuropathy, dystonia, oculomotor abnormalities, pyramidal tract dysfunction, cognitive impairment, and epilepsy were also revealed. Patients who harbored different gene mutations showed mutational heterogeneity.

Conclusions: Our results indicate that ARCA-associated gene mutations are uncommon with additional clinical features in the Chinese population, and advanced sequencing is required to aid the diagnosis of undetermined cerebellar ataxia in Chinese patients.

Introduction

Autosomal recessive cerebellar ataxias (ARCA) are heterogeneous, complex, disabling inherited neurodegenerative diseases characterized by autosomal recessive inheritance, cerebellar ataxia, and early onset of disease [1]. ARCA should be considered in any patient younger than 30 years old with a gradually worsening disorder of gait and balance or with the development of excessive clumsiness, but some patients also showed the late-onset forms [2].

Clinically, the large heterogeneity of clinical presentations and the great overlap between different pathologies bring complexity to the clinical diagnosis of ARCA, with various neurologic signs including cerebellar ataxia, peripheral neuropathy, chorea, dystonia, oculomotor abnormalities, pyramidal tract dysfunction, mental retardation, cognitive impairment, and epilepsy. The clinical manifestations are as varied as the possible underlying mutations.

Genetically, more than 30 causative genes/ loci have been identified in ARCA [3]. Some of these genes are responsible for well-known ataxia forms, while others underlie very rare forms. Furthermore, a significant proportion of patients remain with unidentified ARCA.

The diagnostic workup is therefore complex and can be challenging. The prevalence of different forms of ARCA varies by geographic region and ethnicity. These factors need to be considered in the diagnostic strategy. For example, Friedreich's ataxia (FA) is the most prevalent genetic ataxia in Caucasians, with an estimated prevalence of 3–4 in 100000, but this subtype is extremely rare in China [4]. The recently developed targeted next-generation sequencing (NGS) and whole exome sequencing technologies have helped to unravel the biological bases of genetic ataxias and uncover new genetic causes.

Here, using whole-exome sequencing or targeted panel sequencing, we screened the causative mutations of ARCA among a cohort of 109 unrelated index patients with unexplained autosomal recessive or sporadic ataxia in China, aiming to identify ARCA patients in China and describe their clinical characteristics.

Method

Subjects

A cohort of 109 unrelated index patients was enrolled from the Department of Neurology, West China Hospital, Sichuan University, from January 2014 to July 2018. The clinical presentations were examined and evaluated by at least two senior neurologists. The laboratory tests for thyroid function, levels of vitamin B12 and E, serology for syphilis and HIV, autoimmune antibodies, tumor markers, paraneoplastic antibodies were carried out, and electromyography (EMG) brain magnetic resonance imaging (MRI) scanning. Patients were assigned to an ARCA diagnosis if they presented cerebellar features (progressive ataxia, nystagmus, intentional tremor, dysarthria, and cerebellar atrophy on brain MRI), and specific phenotypes of ataxic genetic disorder (polyneuropathy or myopathy, telangiectasia, cognitive decline, oculomotor apraxia, or abnormal findings upon fundus examination). The familial histories were carefully screened. More specific genetic tests were performed to exclude patients with repeat expansion disorders, including SCA1, SCA2, SCA3, SCA6,

SCA7, SCA8, SCA10, SCA12, SCA17, SCA31, SCA36, DRPLA. Patients enrolled from January 2014 to December 2016 were screened by targeted panel sequencing, and patients enrolled from January 2017 to July 2018 were screened by whole-exome sequencing, because increasing evidence showed the priority of the whole-exome sequencing in undiagnosed ataxias over the targeted panel sequencing [5]. In addition, 500 healthy subjects of matched geographical ancestry were recruited as controls. Written informed consent was obtained from all subjects engaged in this study and Ethic Committee approved this study of West China Hospital, Sichuan University.

Genetic Testing

DNA Preparation: Genomic DNA was extracted from 3 mL peripheral blood using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Additional samples were taken from affected or unaffected relatives to examine for segregation analysis. Targeted Panel Sequencing: Targeted capture, library preparation, and sequence amplification of 39 ARCA causative genes were then performed as previously described [9]. Whole-Exome Sequencing: Genomic DNA was fragmented into 250–300 bp length fragments by sonication. The DNA fragments were processed by end-repairing, a-tailing and adaptor ligation, a 4-cycle pre capture PCR amplification, and enriched by xGen Exome Research Panel. Paired-end sequencing (150 bp) was performed on the Illumina HiSeq X-ten platform. The raw data were filtered for non-synonymous variants, indels, and putative splice site variants in coding regions and splice site regions with sufficient read depth (> = 10×) and genotype quality (> = 99). Variant analysis: The number of variants was narrowed down according to minor allele frequency (MAF) with the cutoff set at < 1% based on the following population databases: 1000 Genomes Project, Exome Sequencing Project 6500, and ExAC, dbSNP, and gnomAD. Variants near splicing donor/recipient sites and frameshift indels were given particular attention since they could cause pathogenic changes, such as exon-skipping or frameshifts. Benign variants were identified by Mutation Taster, SIFT, and PolyPhen-2 software. To estimate the potential for pathogenicity among novel variants, we evaluated variants based on the ACMG guidelines [6]. We defined the following 4 pathogenicity groups: (1) probable or definitive causative variants; (2) possibly causative variants; (3) variants of clinically unknown significance (VUS); and (4) no diagnosis. Sanger sequencing: Sanger sequencing was performed in affected individuals and their parents or unaffected siblings to validate the filtered variants, aiming to confirm segregation or mutation phase for compound heterozygous or de novo mutations. Five hundred healthy controls were sequenced for the detected mutations.

Results

Fifteen mutations were detected from 7 out of 109 unrelated index ARCA patients, consisting of seven missense mutations, three frameshift mutations, two splicing mutations, two nonsense mutations, one inframe deletion mutation. Of these, 3 variants were previously described and 12 variants were novel (Table 1). Variations in a total of 4 different genes were identified in our cohort. The most frequent were *ATM* (3 patients), followed by *SACS* (2 patients) and *SYNE1* (2 patients), and mutations were also found in *SETX* (1 patient). Specifically, 1 patient harbored mutations in both *ATM* and *SYNE1*. Of these, we identified 1 definite pathogenic variant, 12 probable causative variants, and 2 possible causative variants. All these possible to definite variants are listed in Table 1. In addition, these variants were absent in 500 matched controls. In total, of the 109 patients who met the inclusion criteria, 7 (6.42%) patients were confirmed to have a genetic diagnosis.

Table 1
Molecular analysis of ARCA-associated gene mutations

Patient	Gene	Location	Variant	Predicted Amino Acid Change	Mutation Type	Pathogenicity class according to ACMG	Reference
Case 1	*SYNE1*	Exon 63	c. 10105A > G	p. M3369V	Missense	Possible	Novel
		Exon 41	c. 8592C > A	p. N1964K	Missense	Possible	Novel
Case 2	*ATM*	Exon 9	c.1163A > C	p.K388T	Missense	Probable	Novel
		Exon 10	c.1402_c.1403delAA	p.K468Efs*18	Frameshift	Probable	Broeks A, et al. Jeddane L, et al.
Case 3	*ATM*	-	c.4236 + 1_c.4236 + 2insT	-	Splicing	Probable	Novel
		Exon 2	c.5644C > T	p.R1882X,1175	Nonsense	Probable	Buzin CH, et al. Coutinho G, et al. Mitui M, et al.
Case 4	*SYNE1*	-	c.24642 + 3A > G	-	Splicing	Probable	Novel
		Exon 81	c.15469G > A	p.E5157K	Missense	Probable	Novel
	ATM	Exon 9	c.1163A > C	p.K388T	Missense	Probable	Novel
		Exon 38	c.5697C > A	p.C1899X,1158	Nonsense	Probable	Novel
Case 5	*SACS*	Exon 8	c. 13085T > G	p.I4362R	Missense	Probable	Novel
		Exon 8	c.5236dupA	p.T1746fs	Frameshift	Probable	Novel
Case 6	*SACS*	Exon 10	c.4756_4760delAATCA	p.N1586fs	Frameshift	Definite	Novel
Case 7	*SETX*	Exon 23	c.7034_c.7036delTAA	p.I2345_T2346delinsT	Inframe deletion	Probable	Novel
		Exon 6	c.502C > T	p.R168W	Missense	Probable	Szpisjak L, et al.

SYNE1 **gene**

Case 1 he was aware of mildly slurred speech when he was 19 years old. In the following six years, he slowly developed gait abnormalities, balance difficulties and clumsiness of the hands. Through neurological examination, he had dysarthria with cerebellar ataxic speech, dysmetria in upper and lower limbs, lower limb pyramidal signs with spasticity, hyperreflexia and positive Babinski sign on the left side, and reduced vibration sense in distal lower extremities. Nerve conduction studies showed reduced median and sural sensory nerve action potential (SNAP). His MoCA score was 23/30 with poor performance on visuospatial skills and memory. MRI showed atrophy in the cerebellum and prominent thin corpus callosum. His parents are not consanguine. Both his parents and his elder sister did not show any abnormal neurological symptoms or signs. He was found to carry two compound heterozygote mutations of c.5892C > A and c.10105A > G in the *SYNE1* gene, and these compound heterozygotes were not described previously. The missense mutation c.5892C > A located in exon 41 is predicted to lead to an amino acid change at position 1964 from Asn to Lys. The missense mutation c.10105A > G located in exon 63 is predicted to cause a Met to Val non-synonymous substitution at position 3369. Further validation confirmed that his father harbored the mutation c.5892C > A. His mother carried the other mutation c.10105A > G. His affected elder sister only carried the mutation of c.5892C > A.

ATM **gene**

Case 2, she was a 22-year-old woman from a nonconsanguineous family with healthy parents. She reported occasional experience of falling due to imbalance since childhood. At age 20, she showed difficulties in handwriting, with shaking and clumsiness. She also developed an unstable gait and slurred speech. Ocular telangiectasias were observed. Neurological examination showed the presence of cerebellar ataxia, hyperactive patellar reflexes and mild hypertonia at lower limbs. No oculomotor abnormalities and sensory deficits were present. Increased AFP levels (29.20 ng/ml) were found. MRI of the brain revealed diffuse atrophy of the cerebellum, and the EMG test indicated demyelination with axonal injury of sensory fibers. Two heterozygous sequence variations were identified in *ATM* gene: variant c.1163A > C and variant c.1402_c.1403delAA. The missense c.1163A > C variant located in exon 9 is predicted to lead a Lys to Thr at position 388. The c.1402_c.1403delAA variant located in exon 10 leads to a frameshift mutation, which was reported in previous studies [7–8]. Both variants were confirmed via Sanger sequencing, the father for variant c.1163A > C and the mother for variant c.1402_c.1403delAA.

Case 3, he was an 8-year-old boy from a nonconsanguineous family. This patient first presented for medical evaluation at 8 months of age with parental concerns of developmental delay, including gross motor, speech and language domains. He walked at 2 years of age. He can ambulate independently but not steady. During the whole childhood, he presented with prominent gait instability. He also had symptoms of impaired cognitive function, delayed language acquisition, and he always suffered from recurrent infections. Telangiectasias in the conjunctiva was observed. Neurological examinations revealed clinical signs of cerebellar ataxia. The MRI showed diffuse cerebellar atrophy with sparing of cerebellar peduncles and the brainstem. The increased AFP levels (69.70 ng/ml) were found. Two heterozygous sequence variations were identified in *ATM* gene: variant c.4236 + 1_c.4236 + 2insT and variant c.5644C > T. The splicing mutation c.4236 + 1_c.4236 + 2insT located in splicing site IVS28 is predicted to lead to abnormal shear of mRNA, and this mutation was not reported previously. The nonsense variant located in exon 37 leads to Arg to termination codon, and this mutation was reported in previous studies [9]. Both variants were confirmed via Sanger sequencing in his brother and parents. His parents were healthy, and the father had variant c.5644C > T and the mother had variant c.4236 + 1_c.4236 + 2insT. His little brother was affected, and carried both variants.

ATM **gene and** SYNE1 **gene**

Case 4, she presented with unstable walking when she was 6 years old, but the symptom was mild, and did not affect walking obviously. At age 16, she showed involuntary neck deviation to the left side and neck rigidity. The symptoms got worse when she was nervous and became relieved when she touched the left side of the neck. One year later, her instability in walking became worse after a fall. Neurological examinations revealed clinical signs of cervical dystonia and cerebellar ataxia. The MRI showed diffuse cerebellar atrophy. Increased AFP levels (92.93ng/ml) were also found. A splice site variant c.24642 + 3A > G and a missense variant c.15469G > A of *SYNE1* gene were detected in this patient as compound heterozygotes, which were not reported previously. The c.24642 + 3A > G variation located in intron 136 is close to the splice site, leading the third base A mutated into G. The c.15469G > A variant located in exon 81 is predicted to lead to a Glu to Lys at position 5157. Segregation analysis showed that her father harbored the mutation c.24642 + 3A > G, and her mother carried the other mutation c.15469G > A, respectively. Her parents were not affected by the disease, and the consanguinity was denied. Interestingly, this patient also shared the compound heterozygote mutations of c.1163A > C and c.5697C > A in the *ATM* gene. These compound heterozygote mutations were not reported previously. The missense c.1163A > C variant located in exon 9 is predicted to lead a Lys to Thr at position 388, and the c.5697C > A variant located in exon 38 is expected to lead to a truncating mutation of Cys at position 1899. Segregation analysis showed that her father harbored the mutation c.5697C > A, and her mother carried the other mutation c.1163A > C.

SACS **gene**

Case 5, she was a 21-year-old woman from a nonconsanguineous family with healthy parents and an affected younger brother. She showed unsteady walk at age 3. Especially she had difficulty determining the location of the feet when climbing stairs. She first reported the paroxysmal upper limb rigidity at age 7; the symptoms usually lasted about 30 seconds, with loss of consciousness. The EEG showed epileptic discharges. At age 13, she complained of obvious stiffness of lower limbs, having difficulties in climbing stairs. At age 18, her symptoms became more severee with a spastic gait. The walking difficulties were aggravating, and she fell down frequently. She was unable to hold objects steadily with both hands, and choked occasionally. Physical examination showed vision impairment in both eyes, with horizontal nystagmus and slow scanning. She had action tremor in both arms, hyperreflexia in upper and lower limbs, and increased muscle tone and positive Babinski's sign in both sides. The muscle strength was decreased to grade 4. The Finger-nose test and heel knee-shin test were clumsy, with dysmetria on both sides. She also presented with a spastic gait and claw foot. The motor nerve conduction velocities were decreased in the right tibial nerve and right medium nerve. The compound muscle action potentials

amplitude (CMAP) was decreased in the right tibial nerve and right common peroneal nerve. The sensory nerve conduction showed no elicitation of a waveform in the right medium nerve, right ulnar nerve, and sural nerve. No elicitation of F waves was found in the right common peroneal nerve. MRI was characterized by the atrophy of the cerebellum and brain stem, with a widening of the sulci and enlargement of fourth ventricle enlargement; and extensive atrophy of cervical and thoracic spinal cord. The brother was 10-years-old, and he also had walking instability, but the symptoms were minor, which did not affect his normal life. Neurological examinations revealed horizontal nystagmus, mild spastic gait, but normal ocular movements. EMG and MRI results were similar to his sister. Two heterozygous sequence variations were identified in the *SACS* gene: variant c. 13085T > G and variant c. 5236dupA. The missense c. 13085T > G variant is predicted to lead an Ile to Arg at position 4362. Variant c. 5236dupA was expected to lead to frameshift mutation of Thr at position 5236, causing the protein to lose its normal function. Both mutations were not reported in previous studies, and were confirmed via Sanger sequencing; the father carried variant c. 5236dupA and the mother carried variant c. 13085T > G and the brother carried both variants.

Case 6, he was a 23-year-old boy from a consanguineous family. His father was his mother's cousin. This patient had gait instability once he could walk, and he was easy to fall. During school, he could ambulate independently, but he could not attend the physical education class. He had an unsteady gait, and was unable to tandem walk. Romberg's test was positive, and the finger-nose test and heel knee-shin test were clumsy. Muscle strength was normal, but muscle tone was increased. He presented with spasticity and hyperreflexia, more pronounced in the lower extremities with bilaterally positive Babinski's sign. The MRI showed marked cerebellar atrophy, predominantly in the vermis and hemispheres, with an enlarged fourth ventricle. EMG test revealed decreased conduction velocity and evoked potential of both motor and sensory nerve. Genetic investigations revealed a homozygous c.4756_4760delAATCA mutation in the *SACS* gene. This variation was expected to lead to frameshift mutation of Asn at position 1586, causing the protein to lose its normal function.

SETX **gene**

Case 7, he was a 30-year-old male who had 5 years of progressive gait difficulty. His developmental milestones were non-significant until the age of 25, when he exhibited unstable gait and slurred speech. Two years after the onset, he recognized that his gait disturbance was worsened and he could not use chopsticks stably. He had no dysphagia or bucking after drinking. Neurological examinations revealed horizontal nystagmus. The muscle strength and tension were normal. The Finger-nose test and heel-knee-shin test were abnormal. Romberg's sign was positive. The deep tendon reflexes were decreased in his lower limbs, and Babinski's sign was negative. The AFP levels were found to be increased (83.90 ng/ml). EMG test revealed reduced conduction velocity and CMAP of the motor nerve. The evoked potential of sensory nerves was decreased. MRI showed diffuse cerebellar atrophy. Two compound heterozygous mutations were identified, c.7034_c.7036delTAA and c.502C > T. Variant c.7034_c.7036delTAA located in exon 23, was an inframe deletion mutation, leading to loss of TAA bases from position 2345 to 2346, and this mutant has not been documented in previous studies. The variant c.502C > T was a missense mutation, and was predicted to lead an Arg located in exon 6 to Trp at position 168. A previous study also reported that a female Caucasian patient with ataxia with oculomotor apraxia type 2 (AOA2) phenotype harbored this c. 502C > T mutation in *SETX* gene [10]. A further validation revealed that the patient's father carried c.7034_c.7036delTAA mutation, while his mother carried c.502C > T mutation.

Discussion

SYNE1 **gene mutations**

SYNE1 gene located on chromosome 6p25 is one of the biggest genes in the human genome with 147 exons encoding a 27652 bp mRNA that translates into an 8797 amino acid protein [11]. Although SYNE1 protein is expressed in various tissues, it is particularly abundant in the cerebellum. Mutations in the *SYNE1* gene were first identified to be responsible for a recessively inherited pure cerebellar ataxia in individuals originating from Beauce, France and Quebec [12]. However, patients from Japan, Turkey and Brazil with homozygous truncating *SYNE1* mutations were reported to present with a motor neuron phenotype in addition to cerebellar ataxia [13–16]. Recently a large European multi-center study found that 81% non-French-Canadians exhibited additional non-cerebellar features, including motor neuron features, slow saccades, and mental retardation [17]. Moreover, a subsequent study also reported that all 8 patients with *SYNE1* variants presented with cerebellar ataxia plus phenotype [18]. In our study, patient 1 presented with progressive cerebellar ataxia, lower limb spasticity, brisk tendon reflex, axonal polyneuropathies and cognitive decline with thin corpus callosum. Our finding of *SYNE1* mutations in Chinese populations extended the phenotypic spectrum and ethnic diversity underlying *SYNE1*. In this patient, we identified two novel compound heterozygote mutations (Asn1964Lys and Met3369Val, 2 missense mutations) in the *SYNE1*

gene. To minimize the coincidence of this large gene, we first performed a segregation study, and then screened these variants in 500 healthy controls. Therefore, these variants were considered 'possible pathogenic' based on the ACMG guidelines [6], and they were absent in non-Chinese population databases.

ATM gene mutations

ATM gene has been localized on chromosome 11q22.3, and this gene spans 150 kb of genomic DNA and the 13 kb mRNA, codes a Ser/Thr kinase involved in DNA repair [19–20]. Ataxia-Telangiectasia (AT) is a rare autosomal recessive disorder caused by mutations in the *ATM* gene. AT is a multisystem neurodegenerative disease, and affected children usually have a severe neurological disability, including cerebellar ataxia, extrapyramidal features, oculomotor dyspraxia and polyneuropathy. Patient 2 had normal eye movements, with limb ataxia and peripheral neuropathy. In addition, she also had presented with additional extrapyramidal features, including dystonic tremor, especially when she was writing. Inpatient 2, we identified two novel compound heterozygote mutations. The missense c.1163A > C variant could lead a Lys to Thr change at position 388. Furthermore, this variant was considered 'probable pathogenic' based on the ACMG guidelines [6], and the MAF of this variant was 0 according to the population databases. The other variant, c. 1402_c. 1403delAA was reported in a study including 22 unrelated families of 27 patients with AT [21]. Classic (or typical) AT presents with a severe phenotype. However, disease severity in patient 2 was milder compared to the classic form. A range of neurological systems was affected in this patient, including cerebellum, peripheral nerves, and extrapyramidal system; but not severe. This patient may have variant AT, which has been described as a cause of neurological dysfunction [22]. A study included 9 AT patients with retained ATM kinase activity, and found that they had a milder neurological phenotype [23]. The missense mutation may produce a mutant ATM protein with activity, and the frameshift mutation may allow the expression of some normal ATM protein; and we speculated that the presence of some ATM kinase activity might be associated with a milder neurological phenotype. However, we did not examine the expression of ATM protein and the ATM kinase activity in the present study. Inpatient 3, the splicing mutation c.4236 + 1_c.4236 + 2insT located in splicing site IVS28, and it could lead to abnormal shear of mRNA. In addition, the c.5644C > T nonsense mutation was detected, and this mutation could lead to the truncation of ATM protein at codon 1882 and was reported in previous studies [9, 24–25]. In a cohort from Morocco, the c.5644C > T mutation was the most frequently detected, and all of the patients were homozygous for this mutation, and all these patients showed classical phenotype [8]. Patient 3 presented a classical phenotype, with onset in infancy, cerebellar ataxia, oculocutaneous telangiectasia, and recurrent infections. In addition, this patient showed symptoms of cognitive impairment and language deficit. The genotype-phenotype correlation between mutation c.4236 + 1_c.4236 + 2insT and other atypical neurological features was not clear, but there is an idea that this specific mutation could cause a high risk of cognitive dysfunction.

SYNE1 and ATM combined mutations

Patient 4 display a milder phenotype, and showed predominant extrapyramidal signs. Cerebellar ataxia developed later in the course of the disease, and oculocutaneous telangiectasias were absent. She exhibited marked neck dystonia, which was treated with baclofen, but the symptoms were not improved. She also had scoliosis. Exome sequencing identified compound heterozygotes (c.24642 + 3A > G and c.15469G > A) in *SYNE1* and compound heterozygotes (c.1163A > C and c.5697C > A) in *ATM*. These four variants were considered 'probable pathogenic' based on the ACMG guidelines [6]. Interestingly, the missense c.1163A > C variant in *ATM* was also shared by patient 2, present with variant AT. Patient 2 showed a phenotype of variant AT, a mixture of ataxia and additional extrapyramidal features. It is possible that the second genetic determinant caused by *SYNE1* may have combined its effect with the *ATM* mutations to give the atypical phenotype in patient 4. *SYNE1* involved in ARCA was the first identified gene responsible for a recessively inherited, pure cerebellar ataxia. Extra-cerebellar neurological and non-neurological dysfunctions were also reported in *SYNE*1-associated ARCA, including motor neuron disorder, mental retardation and cognitive impairment, depression, dystonia, kyphosis, scoliosis, splanchnectopia et al. [26]. Therefore, it is proposed that *SNYE1* ataxia should be recognized as multisystemic ataxia [18]. In our study, patient 4 also present with prominent dystonia and scoliosis. However, we did not perform yeast experiments to demonstrate that if the mutated proteins are functionally impaired. The variant phenotype of patient 4 may be due to the combined effect of different mutated genes (*SYNE1* and *ATM*) associated with ataxia or related disorders, but functional assays are required for the interpretation of the data.

SACS gene mutations

SACS gene identified in the gigantic exon 10, encodes the protein sacsin [27]. Mutations in *the SACS* gene were linked to autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS), which is characterized by the very early onset triad of cerebellar ataxia, pyramidal tract signs in lower limbs, and axonal-demyelinating sensorimotor peripheral neuropathy [27], combined with increased visibility of retinal nerve fibers. Symptoms usually appear before the age of 5 years, but occasionally in adulthood [28]. Patient 5 had the onset at the age of 3, with symptoms of the unsteady walk and cerebellar ataxia, and then she developed epileptic seizures. At the age

of 18, she presented with nystagmus, increased muscle tone and hyperreflexia in lower limbs, and scissors gait. Her young brother showed unsteady walk since childhood, but the disease progressed slowly, and his symptoms were mild. Patient 5 showed symptoms of epilepsy at the age of 7, but her brother still had no epilepsy seizures during the follow-up, suggesting that different patients in the family with the same genetic mutations in the *SACS* gene could have certain differences in clinical phenotypes. These two patients in this family had no symptoms of muscle atrophy and retinal abnormalities. They both showed ataxia, spasticity, and peripheral neuropathy. The IQ test of patients 5 indicated mild deficit without a mental disorder. These findings were different from the previous studies concerning cognitive dysfunction, mental disorder, and epilepsy. The phenotype is more variable than originally recognized, and this variability can make ARSACS difficult to diagnose, especially to distinguish from hereditary spastic paraplegia and spinal cord cerebellar ataxia. We identified 2 SACS variants, both of them were novel. Variant c. 5236dupA is probably pathogenic and predicted to cause protein loss of function, leading, if translated, to a premature stop codon. Variant c. 13085T > G is predicted to be have an impact on sacsin by several computational algorithms. Considering the rarity in population databanks, this variant is reported as probable pathogenic. Patient 6 with ARSACS showed the main clinical features of this disease, including ataxia, spasticity, and peripheral neuropathy with onset at the age of 2. He was from a consanguineous family; a homozygous c.4756_4760delAATCA mutation was detected. This variant was absent in population databases, and predicted to be definite pathogenic. Characterizing ARSACS phenotypes and reporting novel mutations are important to improve the diagnosis of this ever-expanding spectrum disorder and figure out its pathogenesis.

SETX gene mutations

SETX gene has at least three isoforms, and encodes protein syntaxin. The longest isoform encodes a protein of 2,706 amino acids [29]. Senataxin has been suggested to play important roles in the regulation of transcription, DNA damage responses, mRNA splicing, meiotic recombination, and gene silencing [29–32]. AOA2 is a less common form of ARCA that is clinically characterized by progressive cerebellar ataxia, peripheral neuropathy, oculomotor apraxia, and elevated serum AFP. The age at onset varies, normally juvenile-onset. Recessively inherited mutations in *SETX* are associated with AOA2. Patient 7 presents with an unsteady walk, dysarthria, and nystagmus. EMG test revealed peripheral nerve damage. Moreover, elevated serum AFP and prominent cerebellar atrophy were also observed. Taken together, these manifestations were in line with the features of AOA2. However, patient 7 had disease onset at the age of 25, unlike the juvenile-onset in AOA2. The study reported that patients with AOA2 were affected anywhere between 10–20 years of age [29]. Furthermore, he had horizontal nystagmus, but he had no defeat of controlled, voluntary, and purposeful eye movement. Normally, oculomotor apraxia is considered to be a specific feature of AOA2, but studies showed that approximately only half of AOA2 cases were reported to have oculomotor apraxia [33–35]. Inpatient 7, the late-onset and no exhibition of abnormal eye movements maybe his unique phenotype, and further research on the relationship between the phenotype and genotype is required. In this study, the identified p.I2345_T2346delinsT and p.R168W mutations were located in exon 23 and exon 6, respectively. These two mutations brought about the loss of senataxin's essential role in RNA processing and DNA repair, and were predicted to be disease-causing. They were regarded as probably pathogenic mutations, according to the ACMG guidelines [6].

Conclusions

ARCA is a large group of neurodegenerative disorders characterized by autosomal recessive inheritance and cerebellar ataxia. Clinically, ARCA is associated with diverse involvement of central and peripheral nervous systems and many systemic features. ARCA is rare, and the overall prevalence is estimated to be about 5 to 6 per 100,000 [36]. Despite the rarity, the phenotypes of ARCA were heterogeneous, including age at onset, manifestations, and disease progression. More than 30 causative genes have been identified in ARCA to date[1, 3]. In the present study, we only identified 4 causative genes in our Chinese population. Furthermore, the clinical features of these patients were varying largely. Therefore, it is challenging to decide which gene should be screened solely depending on the patients' clinical features. For this reason, the high throughput whole-exome sequencing and targeted NGS have shown a distinct advantages in molecularly diagnosing inherited diseases [37]. Our results provide the first insights into the identification of ARCA-associated gene mutations underlying undetermined ataxias in Chinese patients. We extended the ethnic, phenotypic, and mutational spectrum of ARCA-associated cerebellar ataxia. However, further functional investigations were needed to elucidate the pathogenicity of the mutations we identified.

Declarations

Acknowledgments

We thank all the patients who participated in this study and their families.

Funding

This work is supported by the National key Research and development program of China (Grant No.

2017YFC09007703) .

Consent for publication

This study was approved by the Ethical Committee of West China Hospital of Sichuan University. All patients gave their written informed consent to participate in the investigation.

Availability of data and materials

The data and materials used in the current study are stored in the corresponding author, but all the data is not available due to privacy protection.

Ethical approval

This study was approved by the Ethics Committee of West China Hospital, Sichuan University.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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