INTRODUCTION

X-linked adrenoleukodystrophy (ALD) is one of the most common peroxisomal disorders caused by mutations within \textit{ABCD1} (OMIM 300371) gene (Berger & Gartner, 2006). It is characterized by abnormal accumulation of the very long-chain fatty acids (VLCFA) in white matter, adrenal glands, fibroblasts, and plasma (Berger & Gartner, 2006). Clinically, ALD presents with various phenotypes, ranging from asymptomatic type to rapidly progressive childhood cerebral form. However, no remarkable abnormality in cerebral white matter usually makes it difficult to distinguish adult ALD from hereditary spastic paraplegia (HSP).

Methods: We analyzed the features of seven Chinese ALD patients who had a primary phenotype of spastic paraplegia. Sequencing was performed in the probands and their familial members. Detailed clinical, VLCFAs test, hormone test, magnetic resonance imaging, and electromyogram are presented.

Results: We reported seven ALD patients from a Chinese cohort of 142 HSP patients. Genetic investigations revealed five known \textit{ABCD1} mutations (c.346G>C, c.521A>G, c.829G>T, c.1415_1416delAG, and c.1849C>T) and two novel mutations (c.454C>G, c.1452_1482del). Further auxiliary testing revealed that they had higher VLCFA and/or adrenal insufficiency.

Conclusions: Our findings expand the mutation spectrum of \textit{ABCD1} and indicate that ALD represent a significant portion (4.9%, 7/142) of the spastic paraplegia entities. ALD should be considered in male patients with spastic paraplegia, even if there was no positive family history.

KEYWORDS

\textit{ABCD1}, hereditary spastic paraplegia, peroxisomal disease, very long-chain fatty acids, X-linked adrenoleukodystrophy
various phenotypes, including childhood cerebral adrenoleukodystrophy, adolescent cerebral adrenoleukodystrophy, adult cerebral adrenoleukodystrophy, adrenomyeloneuropathy (AMN), olivo-ponto-cerebellar ALD, Addison’s only, and asymptomatic ALD (Niu, Ni, & Wu, 2013). In clinical practice, no remarkable abnormality in cerebral white matter usually makes it difficult to distinguish adult AMN from hereditary spastic paraplegia (HSP). In this study, we reported seven Chinese ALD patients with ABCD1 mutations and a phenotype mimicking HSP.

2 MATERIALS AND METHODS

2.1 Ethical compliance

This study was approved by the Ethics Committees of Second Affiliated Hospital, Zhejiang University School of Medicine. Written informed consent was obtained from each participant.

2.2 Clinical and genetic analysis

A total of 142 Chinese participants with clinically diagnosed HSP were consecutively recruited from the Second Affiliated Hospital of Zhejiang University School of Medicine from April 2015 to June 2019. The clinical evaluations were performed by at least two senior neurologists. Genomic DNA was extracted from the peripheral blood, using QIAamp genomic DNA kits (Qiagen). Targeted next-generation sequencing (NGS), Sanger sequencing, and cosegregation analysis were carried out in every proband and available familial members, as previously described. Five hundred individuals without neurological disorders were collected as controls. Variant analysis was based on the American College of Medical Genetics and Genomics (ACMG) guidelines. Patients detected to carry ABCD1 mutations were further underwent VLCFAs test, hormone test, magnetic resonance imaging (MRI), and electromyogram. Variants were described at cDNA and protein level using reference sequences NM_000033.4 and NP_000024.2, respectively.

3 RESULTS

3.1 Identification of mutations by NGS and Sanger sequencing

Among the 142 patients with diagnosis of HSP, we found seven index patients carrying ABCD1 mutations, including five known pathogenic mutations (c.346G>C, c.521A>G, c.829G>T, c.1415_1416delAG, and c.1849C>T) and two novel mutations (c.454C>G, c.1452_1482del). The pedigrees of these seven probands were shown in Figure 1. The mutation c.454C>G (p.R152G) was absent in the

ExAC, 1000G, genomAD, and our 500 in-house controls. It was predicted to be deleterious by SIFT, Polyphen-2, Mutation Taster, and CADD. According to ACMG, it was classified as “likely pathogenic.” The truncation mutation c.1452_1482del (p.P487Wfs*61) was also absent in the above database and should be classified as “pathogenic.” Among the five identified missense mutations, four mutations (c.346G>C, c.454C>G, c.521A>G, and c.829G>T) were localized in exon 1 of ABCD1. The two truncating mutations (c.1415_1416del, c.1452_1482del) were localized in exon 5. Interestingly, in the case with c.346G>C mutation, we did not detect the mutation in either of his parents, implying a possible de novo mutagenesis in this family. Unfortunately, functional studies of these novel mutations were not performed due to condition limitations.

3.2 Clinical features of patients carrying ABCD1 mutations

The detailed clinical features for seven patients were summarized in Table 1. One patient had a positive history, while the others were sporadic cases. All seven cases were male and had an initial diagnosis of HSP. The mean age at onset was 32 years (ranging from 24 to 45 years). None of them showed cognitive deficit or obvious evidence of white matter lesions in brain MRI. The level of VLCFA was increased in five patients, and not available in two patients (Table S1). Cortisol levels were normal in all patients. However, there was a definite increase in ACTH level in two cases carrying c.521A>G and c.1452_1482del.

Case 1, a 31-year-old male, had 5-year history of rigidity and weakness in lower limbs, gait difficulty, and urination dysfunction. Neurological examinations revealed enhanced muscle tone, brisk tendon reflexes, positive Babinski signs and clonus. Hypesthesia of topesthesia and vibration sense was also observed. Brain MRI and whole spine MRI revealed no obvious lesion. Electromyography showed impaired peripheral nerves injury of upper and lower limbs.

Case 2 was a 49-year-old man who developed progressive gait difficulties over the past 8 years. Neurological examinations revealed increased muscle tension, hyperreflexia, positive ankle clonus, and Babinski sign. The sensory system examination and coordination were intact. Brain MRI revealed no abnormality. Electromyography showed decreased the sensory conduction velocity of bilateral median nerve and left ulnar nerve. The levels of VLCFA were high: C26:0 level was 3.41 nmol/ml, C24:C22 was 1.79, C26:C22 was 0.067.

Case 3 was a 34-year-old male who complained numbness of lower limbs at the age 23 years. Five years after the onset, he required assistance to walk and had a problem with urinary incontinence. No remarkable change was observed in brain and lumbar MRI. The results of the VLCFAs were
increased. The pituitary gland also produced high levels of adrenocorticotropic hormone, luteinizing hormone and prolactin, which are listed in Table S1. His affected brother developed gait difficulty at age 18 years and died in bed at the age 28 years.

Case 4 was a 28-year-old male who experienced weakness of lower limbs and sexual dysfunction for 2 years. Neurological examinations revealed slight nystagmus, brisk tendon reflex, positive ankle clonus. MRI of the brain and whole spine revealed unremarkable findings. Electromyography revealed peripheral neuropathy in both lower limbs. His levels of VLCFAs were elevated: C26:0 level was 4.92 nmol/ml, C24:C22 was 1.59, C26:C22 was 0.092.

Case 5 was a 52-year-old male who experienced weakness of lower limbs starting from 7 years ago. Neurological examination revealed scissors gait, increased muscle tension, hyperreflexia, and ankle clonus in lower limbs. Babinski sign and Hoffmann’s sign were positive bilaterally. He did not show paresthesia or cognitive impairment. MRI showed no obvious abnormality. Electromyography showed reduced conduction velocity of sensory and motor nerve in lower limbs. There were no data about adrenocorticotropic hormone, serum cortisol, and plasma VLCFA.
## Table 1: The clinical features of patients with ABCD1 mutations

| Patient | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 |
|---------|--------|--------|--------|--------|--------|--------|--------|
| **Variants** | c.346G>C (p.G116R) | c.454C>G (p.R152G) | c.521A>G (p.Y174C) | c.829G>T (p.G277W) | c.1415_1416delAG (p.Q472Rfs*83) | c.1452_1482del (p.P487Wfs*61) | c.1849C>T (p.R617C) |
| **AAO (years)** | 26 | 41 | 23 | 26 | 45 | 24 | 36 |
| **DD (years)** | 5 | 8 | 11 | 2 | 7 | 7 | 4 |
| **Family history** | No | No | Yes | No | No | No | No |
| **Phenotype** | AMN | AMN | AMN | AMN | AMN | AMN | AMN |
| **Initial symptoms** | Spasm | Weakness; unstable walk | Weakness; unstable walk | Weakness | Weakness | Weakness | Spasm |
| **UL reflex** | +++ | +++ | ++++ | +++ | ++++ | ++++ | ++ |
| **LL reflex** | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| **Ankle clonus** | + | + | NA | + | + | + | + |
| **Babinski sign** | + | + | − | + | + | + | + |
| **Cognitive impairment** | No | No | No | No | No | No | No |
| **MRI** | N/N/N/N | N/N/N/N | N/NA/NA/NA | N/N/N/N | N/N/N/N | N/N/N/N | N/Thinnet/ N/N |
| **EMG** | Yes | Yes | NA | Yes | Yes | Yes | Yes |

Abbreviations: AAO, age at onset; AMN, adrenomyeloneuropathy; DD, disease duration; EMG, electromyography; LL, lower limbs; MRI, brain MRI/cervical spine MRI/thoracic spine MRI/lumbar spine MRI; MRI, magnetic resonance imaging; N, no obvious abnormality; NA, not available; UL, upper limbs.
Case 6 was a 31-year-old male who started the disease with a progressive gait difficulty at the age 24 years. On the neurological examinations, he showed spastic gait, weakness, and increased muscle tension in lower limbs. Hoffman sign, Babinski signs, and ankle clonuses were bilaterally positive. Vibration sensation and position sensation were weakened. His cortisol was normal, but his adrenocorticotrophic hormone, serum cortisol, and plasma VLCFA were also not obtained.

Case 7 was a 40-year-old male who suffered from rigidity for more than 4 years. He complained about weakness, abnormal gait, and ankle pain at the age 39 years. His muscle tension was increased, with hyperreflexia in knee and ankle. Babinski sign was positive bilaterally. MRI indicated that the thoracic medulla was atrophic extensively. Electromyography for more than 4 years. He complained about weakness, abnormal gait, and ankle pain at the age 39 years. His muscle tension was increased, with hyperreflexia in knee and ankle. Babinski sign was positive bilaterally. MRI indicated that the thoracic medulla was atrophic extensively. Electromyography of upper and lower limbs showed no peripheral nerve damage. His levels of adrenocorticotrophic hormone, serum cortisol, and plasma VLCFA were also not obtained.

4 | DISCUSSION

ALD is a metabolic disease with high clinical heterogeneity. It is caused by defect in peroxisomal ATP-binding cassette-transporter adrenoleukodystrophy protein, which is coded by \( ABCD1 \) and plays an important role in facilitating VLCFA to peroxisomes for \( \beta \)-oxidation (Berger & Gartner, 2006). A retrospective and international study suggests that adrenal insufficiency can occur in nearly 80% ALD patients (Huffnagel et al., 2019). Most men and women with ALD have a slowly progressive spinal cord disease, AMN, which typically begin in the 30’s for men and postmenopausal for women. 35% of ALD males may develop a rapidly progressive inflammatory cerebral demyelination peaking in the ages 3–10 years of age. About 20% of adult males with AMN also develop cerebral disease that rapidly progresses to disability and death (Kemp et al., 2001). It is challenging to diagnose AMN when there is no evidence of adrenal insufficiency or accumulation of VLCFA. Recently, Chen et al. (2019) reported 7 ALD patients who had a phenotype of HSP and \( ABCD1 \) mutations. Only four cases had adrenocortical insufficiency, whereas the other three presented normal adrenocortical function. Therefore, for sporadic spastic paraplegia, AMN should also be considered in addition to HSP. As a biochemical marker of ALD, VLCFA is an indispensable test, and ACTH and testosterone also need to be tested in patients with baldness or sexual dysfunction. Of course, genetic investigations are also an effective diagnostic method. Given the large number of genes and the relatively high diagnostic yield, whole-genome sequencing (WGS) may be particularly relevant to diagnosing spastic paraplegia (Kim et al., 2019). However, due to the high price of WGS, targeted gene panels and whole-exome sequencing (WES) is usual the preferred choice, followed by multiplex ligation-dependent probe amplification (MLPA). Of note, a significant proportion of HSP cases could not obtain a genetic diagnosis after WES and MLPA (Shribman, Reid, Crosby, Houlden, & Warner, 2019). WGS is then required to identify new causative gene or intronic variants.

To date, 812 nonrecurrent mutations in \( ABCD1 \) gene have been reported including missense (46%), frameshift (23%), nonsense (13%), and amino acid insertions/deletions (6%) (https://adrenoleukodystrophy.info/). Mutations are distributed throughout the entire \( ABCD1 \) gene unevenly and most missense mutations cluster in some exons (1b, 1c, 6, 7, 8, and 9) (Kemp et al., 2001). The amino acids encoded by exon 1b and 1c are located in transmembrane domain (TMD) and the amino acids encoded by exons 6, 7, 8, and 9 are located in nucleotide binding domain (NBD) (Kemp et al., 2001). TMD is associated to substrate binding, whereas NBD is involved in dimerizing and ATP binding, both two are necessary for the functional ABC transporters (Geillon et al., 2014).

In Chinese ALD patients, more than half of the mutations localized in exon 1 and 6 (Niu et al., 2013). And mutations in exon 6 are higher than that listed in the worldwide database, suggesting exon 6 is another possible hotspot exon in the Asian populations (Niu et al., 2013). The truncating mutation c.1415_1416delAG in family 5 was identified as the most frequent mutation in the word (Kemp et al., 2001), which resulted in a nonfunctional truncated protein by a premature stop codon at amino acid position 554 (Kemp et al., 1994).

Previous studies have shown that there was no clear genotype-phenotype correlation in ALD. Patients with the hotspot mutation (c.1415_1416delAG) in the world had presented a variety of phenotypes (Kemp, Huffnagel, Linthorst, Wanders, & Engelen, 2016). Apparent intrafamilial phenotypic variation had also been described within individual kindreds, even in identical twins (Wiesinger, Eichler, & Berger, 2015). Many nonrecurrent mutations, high de novo mutation rate, as well as environmental factors or modifying genes, make the genotype–phenotype correlation more difficult to research (Wiesinger et al., 2015). Unfortunately, no curative disease-modifying therapy has been found to prevent or slow the progression of spinal cord disease in AMN. There are only symptomatic treatments to relieve pain, spasm, and urinary incontinence. Hematopoietic cell transplantation (HCT) is effective at halting the progression of brain disease in males with ALD only if done at the first signs of progressive brain lesions and before neurological disability (Mahmood, Raymond, Dubey, Peters, & Moser, 2007). Allogeneic hematopoietic stem cell transplantation, with or without autologous \( ABCD1 \) gene therapy, can halt the cerebral demyelination if done early before neurological symptoms or advanced brain disease occurs (Miller et al., 2011). Early diagnosis through
family screening of at-risk males, as of 2015 in the USA new-
cal and material support, drafting and critical revision of the
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ting the manuscript. Dr. Dong: data acquisition, interpretation
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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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ORCID

Hong-Fu Li https://orcid.org/0000-0002-2203-0046
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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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