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QUIZ CASES

A novel missense mutation in the ABCD1 gene of a Chinese boy diagnosed with X-linked adrenoleukodystrophy: case report

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Introduction

X-linked adrenoleukodystrophy (X-ALD) is the most common peroxisomal disease, caused by mutations in ABCD1, a gene located on the X chromosome that codes for adrenoleukodystrophy protein (ALDP) [1], which belongs to the adenosine triphosphate (ATP) binding cassette (ABC) transporter protein superfamily [2] and is connected with very long chain fatty acids (VLCFAs) beta oxidation in peroxisomes. As a consequence, a slight dysfunction of ALDP results in the accumulation of very long chain fatty acids (VLCFAs) in tissues throughout the body. The most severely affected tissues are the myelin in the central nervous system, the adrenal cortex, and the Leydig cells in the testes [3]. X-ALD can present in a wide range of phenotypic expression. The total frequency of mutant carriers for hemizygotes plus heterozygotes is estimated to be 1:16,800 [4]. The different presentations are complicated by the pattern of X-linked recessive inheritance. The most frequent clinical phenotypes of X-ALD are adrenomyeloneuropathy (AMN) and childhood cerebral ALD (CCALD), accounting for 70–80% [5]. Although the detection of an ABCD1 mutation identifies an individual who is affected with a form of X-ALD, there is no genotype-phenotype correlation [6]. The clinical presentation of X-ALD can vary greatly, making diagnosis difficult. Therefore, the mutation analysis of ABCD1 is the most accurate approach to confirm the disease at present [7]. In the present study, we report a Chinese patient with X-ALD derived from a novel missense point mutation that caused by cDNA nucleotide change 1017 G > C in exon 2 (c.1017G > C).

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Materials and methods

The information of the patient was obtained from Xiangya Hospital (Changsha, China). The diagnosis of X-ALD was conformed by the clinical manifestation, VLCFAs analysis, craniocerebral MRI images, and gene detection of ABCD1 gene. Genomic DNA of the patient and his families were extracted from whole blood samples using a standard phenol-chloroform method [8].

Ten coding exons as well as the flanking regions of exon-intron boundaries of ABCD1 gene were detected by conventional Sanger sequencing analysis. The primers for PCR to amplify the ten fragments from ABCD1 gene were similar to that of previous publications [9]. First of all, DNA samples were amplified in a total volume of 50 μl containing: 10× PCR buffer, 100 ng of genomic DNA, 0.4 mM dNTP, 0.2 μM of each primer, 1.25 U Taq DNA polymerase, 1.2 mM $MgCl_2$. After an initial denaturation for 5 min at 95°C, PCR were performed as following procedure for 35 cycles: denaturation for 30 s at 94 °C, annealing for 30 s at 62 °C, extension for 45 s at 72 °C and final extension for 10 min at 72 °C. BigDye Terminator Cycle Sequencing Kit and the ABI3100 Genetic sequence analyzer (Applied Biosystems, Foster City, CA) were applied to purifying and sequencing the products using the forward and reverse primers. The PCR primers were designed on account of the National Center for Biotechnology Information (NCBI) website: NM_000033;exon2.

Case presentation

The patient is a 14-year-old boy suffering a progressive diminution of his bilateral vision simultaneously for 1 year without discomfort in head, dyskinesia, dysesthesia, or cutaneous pigmentation. With the development of the impaired vision, the patient appeared to be lack of alertness and poor academic performance. The neurological examination showed hyperfunction in bilateral patellar and ankle reflex. Bare eye sight

Table 1 VLCFAs analysis in serum

VLCFAs	Patient	Normal control
C22	54.9 nmol/ml	≤ 96.3 nmol/ml
C24	81.3 nmol/ml	≤ 91.4 nmol/ml
C26	3.20 nmol/ml ↑	≤ 1.30 nmol/ml
C24/C22	1.48 ↑	≤ 1.39
C26/C22	0.058 ↑	≤ 0.023

in both sides are 0.1. The patient is normal in eye movements in all directions, no nystagmus on both eyes. Direct and indirect light pupillary reflex are present and sensitive. No Babinski's sign or ankle clonus.

His laboratory data revealed a declining cortisol level (4.43 μg/dL, normal range, 6.2–19.4 μg/dL) and a normal plasma adrenocorticotropic hormone (ACTH) level (6.45 pmol/L, normal range, 1.6–13.9 pmol/L) in the morning, and in the afternoon, a normal cortisol level (4.90 μg/dL, normal range, 2.3–11.9 μg/dL) and an elevated ACTH level (32.14 pmol/L, normal range, 1.6–13.9 pmol/L) and normal cortisol level (12.9 pg/mL; normal range, 7.2–63.3 pg/mL). VLCFAs analysis (Table 1) by liquid chromatography-tandem mass spectrometry (LC-MS/MS) showed high levels of C26 as well as C24/C22 and C26/C22 ratio in serum. Complete blood counts, urine and stool routines, erythrocyte sedimentation rate (ESR), immunological function, coagulation function, multivitamin levels, plasma electrolytes, and the liver, kidney, and thyroid function tests were normal.

Visual evoked potential (VEP) amplitudes of P100 decreased in both visual pathways. Electrocardiogram (ECG) revealed intrasinus wandering pacemaker. Renal CT noncontrast-enhanced scan showed right renal cyst, and the left is normal. An accessory spleen was found in abdominal color Doppler ultrasound. What is more, MRI images detected in T1W1, T2W1, and flair and showed extensive, symmetric demyelination in the alba region of bilateral posterior periventricular areas, the bilateral thalamus, splenium corporis callosum, and the pedunculus cerebri (Fig. 1).

Surprisingly, genetic analysis of the patient exhibited a novel point mutation in the ABCD1 gene that has never been discovered. The missense mutation G > C at the 1017th encoding area (C.1017G > C) led to 339th amino acide change (from tryptophan to cysteine). The results from SIFT, PolyPhen_2, and REVEL, the softwares for protein function prediction, proved to be harmful, harmful, harmful respectively. There is no family history for X-ALD and he was born from unrelated parents. According to the pedigree analysis, the mutation was absent in his father, but his mother was a genetically confirmed carrier of this mutation (Fig. 2).

Discussion

X-ALD is very rare and has been identified among all ethnic groups and on all continents. Few cases about X-ALD have

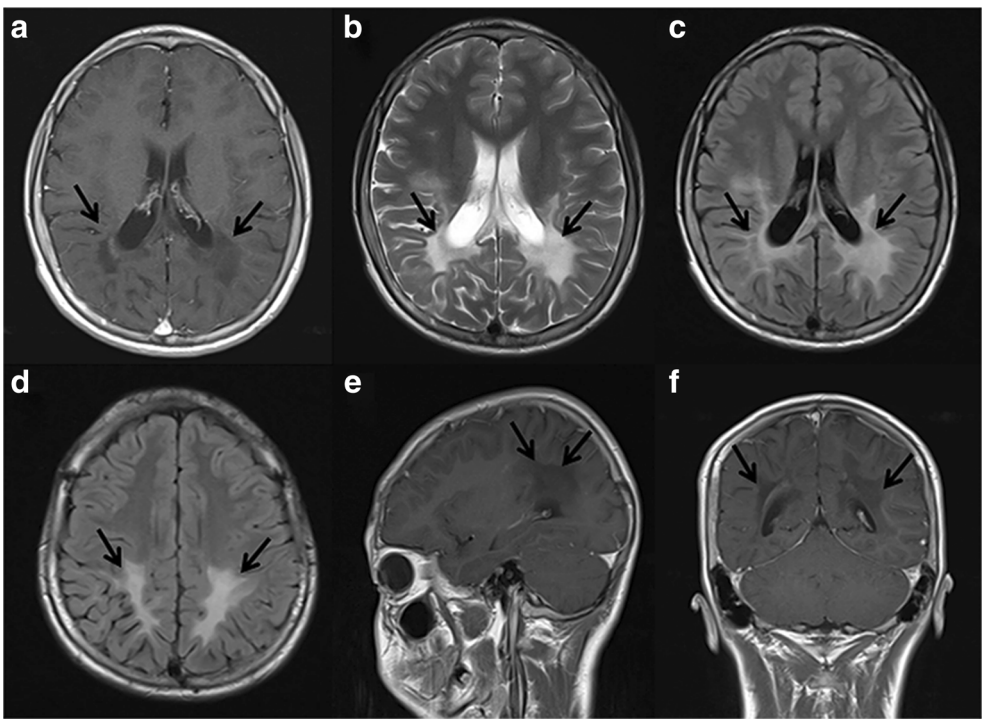

Fig. 1 MRI images of the brain tissue of the patient. The arrows show a low signal in axial T1 (**a**) and high signal intensity in T2 (**b**) and flair (**c**, **d**) in cross sectional MRI images, and a low signal in T1 in sagittal (**e**) and coronal (**f**) images within the white matter of bilateral posterior periventricular areas and parietal-occipital areas

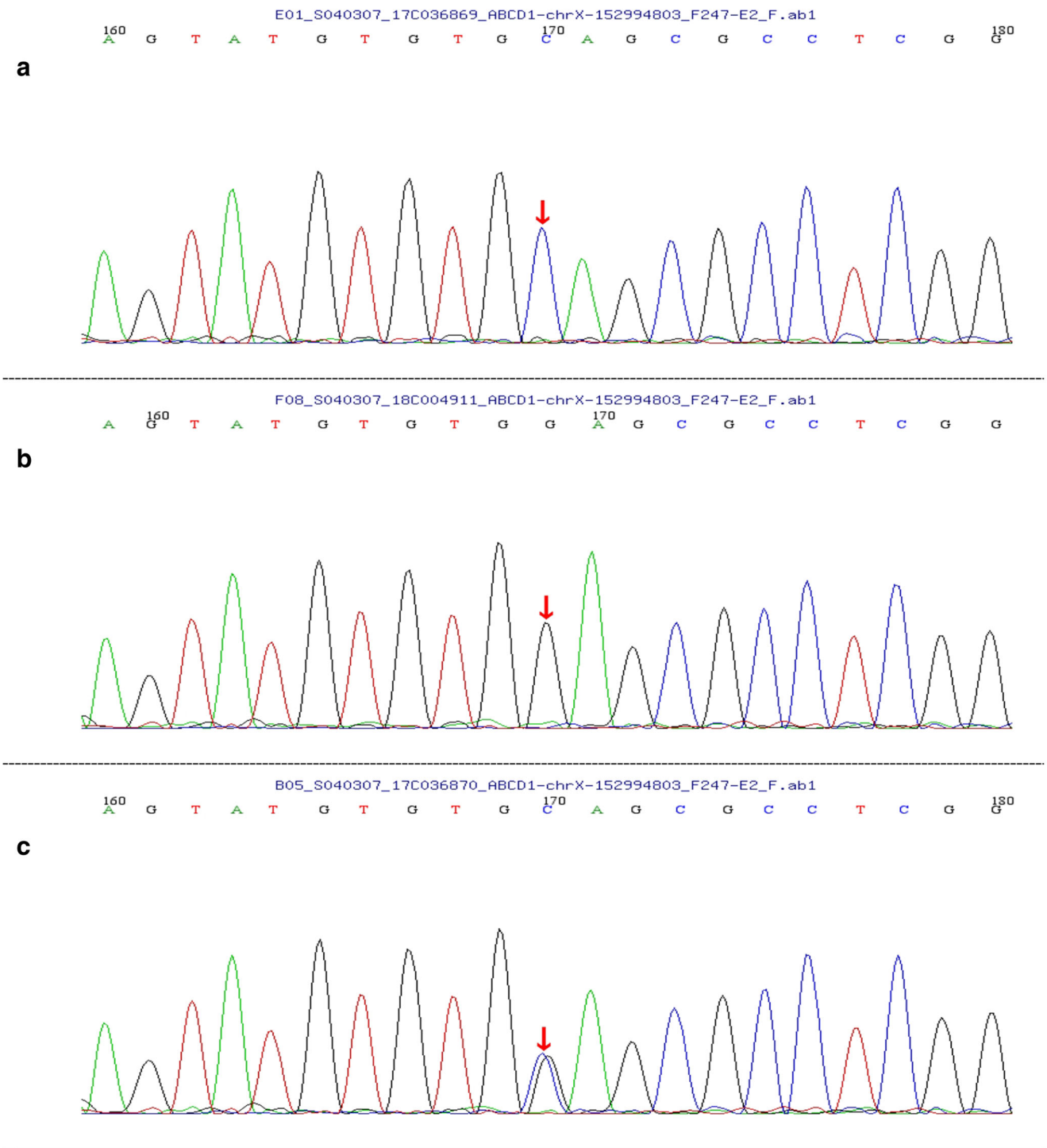

Fig. 2 Result of pedigree analysis. The X-ALD patient turned out to be X-linked heterogametic, and the mutational copy (c.1017G > C) stemmed from his heterozygous mother. **a** ABCD1 gene of the patient. **b** ABCD1 gene of the patient's father. **c** ABCD1 gene of the patient's mother. Red arrows indicate position of the mutation

been reported in China. In our study, we report a boy who was clinically diagnosed with CCALD. CCALD is the second most frequent phenotype (31–35%), which presents in childhood with visual disturbances, auditory impairment, mental decline, spastic tetraparesis, cerebellar ataxia, and seizures. It may originally lead to decline of school performance in boys and adolescents. These early symptoms are often ignored or misdiagnosed, causing delay in diagnosis of CCALD [5]. The boy also had no obvious symptoms previously to attract the attention of his family. However,

this time he had developed a severe visual disturbance when visiting the hospital. At this stage, progression is extremely rapid and fatal. Due to severe inflammatory demyelinating process, the neurologic function of the patients with CCALD drops rapidly. Demyelinating lesions are usually found in the white matter of the cerebrum. In 80% of patients, the initial demyelinating lesions are confined to the splenium of the corpus callosum, and further involvement of adjacent occipital white matter [10]. Most patients have poor prognosis, usually death occurs several years after onset of symptoms [11].

To date, more than 1000 mutations have been reported. Among all of them, 78 are located in exon 2, and 55 of these 78 mutations are pathogenic (https://adrenoleukodystrophy.info/mutations-and-variants-in-abcd1). Our study identified a novel aberrant missense mutation (c.1017G > C) in exon 2 of the ABCD1 gene causing the CCALD, which is one of the unpublished data in the X-ALD database (https://adrenoleukodystrophy.info/mutations-and-variants-in-abcd1). The missense mutation causes a substitution of the 339th amino acid from tryptophan to cysteine (p.W339C). Not all missense mutations lead to appreciable protein changes, especially when an amino acid is replaced by an amino acid of similar chemical properties or the amino acid substitution occurs in a region of the protein which does not significantly affect the protein secondary structure or function. Therefore, we use the protein function prediction software SIFT, PolyPhen_2, and REVEL to predict this mutation respectively, and all results of them are harmful. So this mutation may render the resulting adrenoleukodystrophy protein (ALDP) nonfunctional, which is also consistent with the patient's progressive clinical manifestations. The mutation was inherited from his asymptomatic mother. Therefore, for the female carriers, the timely use of prenatal diagnosis to prevent unnecessary new cases of this severe genetic disease is available. Furthermore, identifying more novel mutations in the ABCD1 gene could expand the gene spectrum of people with X-ALD.

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Abbreviations ABC, adenosine triphosphate binding cassette; ACTH, adrenocorticotropic hormone; ALD, Adrenoleukodystrophy; ALDP, adrenoleukodystrophy protein; AMN, adrenomyeloneuropathy; ATP, adenosine triphosphate; CCALD, childhood cerebral adrenoleukodystrophy; ECG, electrocardiogram; ESR, erythrocyte sedimentation rate; NCBI, National Center for Biotechnology Information; VEP, visual evoked potential; VLCFAs, very long chain fatty acids; X-ALD, X-linked adrenoleukodystrophy

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