

X-LINKED ADRENOLEUKODYSTROPHY IN A MOROCCAN PATIENT: GENETIC DIAGNOSIS LEADS TO PRESYMPTOMATIC TESTING AND FAMILY COUNSELING

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ABSTRACT

X-linked adrenoleukodystrophy (X-ALD) is a fatal neurodegenerative disorder caused by mutations in the adenosine triphosphate-binding cassette D1 (ABCD1) gene. In this study, we report the case of a Moroccan patient diagnosed with X-ALD due to a mutation in the *ABCD1* gene. This diagnosis enabled a genetic counseling with presymptomatic analysis of the disease in the patient's brother, facilitating proactive management for the family. This work expands the clinical and genetic spectrum of X-ALD and underscores the critical role of presymptomatic testing in the management of neurodegenerative diseases.

Key words: X-linked adrenoleukodystrophy, *ABCD1* gene, presymptomatic testing.

INTRODUCTION

X-linked adrenoleukodystrophy (X-ALD, OMIM: 300100) is a rare, progressive peroxisomal disorder characterized by a spectrum of clinical manifestations, including progressive myelopathy, peripheral neuropathy, endocrine dysfunction (such as adrenal insufficiency and, in some cases, testicular insufficiency), and, variably, progressive

leukodystrophy. The clinical presentation of X-ALD is highly variable, with no clear phenotype-genotype correlation [1,2].

X-ALD is an X-linked recessive disease caused by mutations in the adenosine triphosphate-binding cassette D1 gene (*ABCD1*, NM_000033.3), with over 1,200 different mutations reported to date (*ABCD1* Variants Database, 2023, www.x-ald.nl). The encoded *ABCD1* protein, a peroxisomal transmembrane protein, plays a critical role in the transport of very long-chain fatty acid CoA-esters (VLCFAs) from the cytosol into the peroxisome. A failure to transport these fatty acids prevents beta-oxidation and allows continued elongation of VLCFAs, leading to their abnormal accumulation in tissues. This accumulation predominantly affects the nervous system, adrenal cortex, and Leydig cells in the testes. However, the exact pathophysiological mechanisms underlying this accumulation remain poorly understood [3].

In this study, we report the clinical and genetic management of a Moroccan family, focusing on a patient with X-ALD in its cerebral form, Childhood Cerebral Adrenoleukodystrophy (cCALD). The patient carries a pathogenic *ABCD1* gene variant inherited from his mother. Additionally, a presymptomatic diagnosis was made for the patient's brother, aiming to optimize disease management and improve familial outcomes [2].

PATIENTS

Written informed consent was obtained from the patient, his brother, and their parents.

Clinical Presentation: The patient underwent a comprehensive clinical evaluation, which included symptom assessment, neurological examination (with cognitive function evaluation), neuroimaging, biochemical testing, and genetic analysis.

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Neuroimaging and Biochemical Testing:

Magnetic resonance imaging (MRI) was performed to identify pathological changes in the brain and spine. While plasma very long-chain fatty acid (VLCFA) measurements were unavailable, plasma adrenocorticotrophic hormone (ACTH) levels were assessed using a radioimmunoassay.

METHODS

Techniques

DNA Extraction and Sequencing Genomic DNA was extracted from peripheral blood samples using the QIAamp Blood DNA Mini Kit (QIAGEN, USA) following the manufacturer's protocol. DNA was enriched using the DNAprep with Enrichment Kit (Illumina) and sequenced with paired-end reads of 150 bp on a NovaSeq6000 sequencer (Illumina). Sequencing results were aligned to the reference human genome GRCh38 using the DRAGEN bioinformatics tool (Illumina). Duplicate reads were filtered, and variant calling was performed with DRAGEN.

Variant Analysis Data were aggregated and filtered using ANATOLE2 (in-house tool) for SNV/INDEL variations and AnnotSV for structural variants (e.g., exonic deletions or duplications). A trio-based approach was used, focusing on variants in exonic and nearby intronic regions (-10/+10 bp).

Data Quality The coverage rate achieved a depth of >20X for 96.75% of the targeted regions.

Panel Analysis Involving list of genes associated with neurodevelopmental disorders was curated using data from OMIM, PanelAPP, SysID, DDG2P, and PubMed. Exome data were first analyzed in silico based on this curated list of known or candidate genes related to neurodevelopmental disorders and developmental anomalies. The data are securely stored and may undergo secondary reanalysis in the future.

Sensitivity and Limitations

- Mosaic anomalies below 20% frequency are not detectable.
- Large-scale chromosomal rearrangements are not detected.
- Structural variations affecting only a single exon are not detectable.
- Chromosomal rearrangements, such as translocations or inversions, cannot be detected using this technique.

Interpretation

The genotypic data are interpreted according to the phenotypic information provided in the clinical information sheet. The interpretation and classification of varia-

tions may be reviewed and discussed in a multidisciplinary consultation meeting (MCM). The identified variations are classified according to the ANPGM recommendations, which are based on the ACMG guidelines. The nomenclature follows the HGVS (Human Genome Variation Society) recommendations [4,5].

Confirmation of Results Validation of pathogenic variants was performed using the Sanger method for the four family members. The identification of the pathogenic variation was further confirmed in the proband using an independent sample.

Databases

Class 3, class 4, and class 5 variations (as defined by the ACMG 2015 criteria) are submitted to the ClinVar database.

RESULTS

Clinical Information

The patient was an 11-year-old boy, the first of two siblings from a non-consanguineous family. Family pedigrees for all subjects are shown in Figure 1. He was followed by the pediatric neurology department for psychomotor deterioration and referred to the genetic department at the Clinical Research Center at Mohammed VI University Hospital due to a strong suspicion of adrenoleukodystrophy. The patient had no significant personal or family medical history before age of 10 [6].

The onset of symptoms dated back to the age of 10, following a road traffic accident. He began experiencing academic difficulties, including challenges with writing and comprehension, which progressively developed into spasticity and a feeling of heaviness in the right side of his body. On neurological examination, the patient exhibited significant pyramidal tract signs, including spastic paraparesis, increased muscle tone, heightened tendon reflexes, and positive Babinski's and Hoffman's signs. The clinical characteristics of the proband are summarized in Table 1.

A brain MRI revealed symmetrical signal abnormalities in the parieto-temporo-occipital white matter, extending to the splenium of the corpus callosum, the thalami, the internal capsules, and the corticospinal tracts of the midbrain (Figure 1). The patient's serum ACTH level was elevated, while the measurement of very long-chain fatty acids (C24:0; C22:0) could not be performed.

Mutations and Bioinformatics Analysis

Genetic variant was identified in the patient, consisting of a substitution of adenine (A) to guanine (G) at the splice site c.901-2A>G of the ABCD1 gene. The variant was detected in a hemizygous state. This varia-

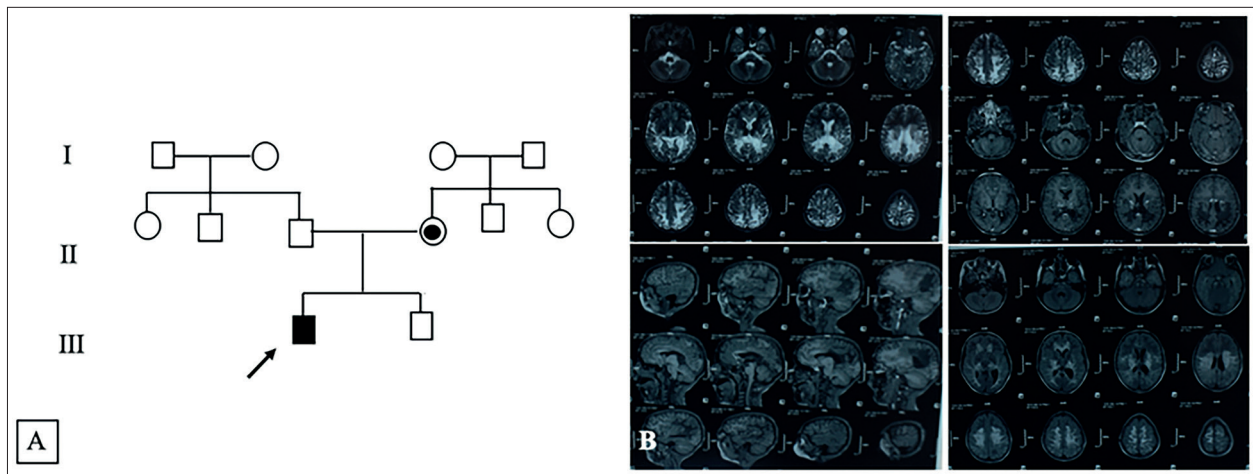


Figure 1. Family pedigree (A) with the Brain MRI Images of the patient showing signal abnormalities in the parieto-temporo-occipital white matter (B).

Table 1. Table summarizing the clinical signs of the patient.

	Patient symptoms
Gender	Male
Age at the diagnosis	12
Age at onset	10
Initial symptoms	Academic difficulties
Spinal symptoms	Spastic paraparesis
Peripheral neuropathy	Present
Cognitive impairment	Present
Sphincter dysfunction	Present
hypoadrenocorticism	Present
Muscle strength	Increased
Tendon reflexes	Exaggerated
Hoffman sign	Positive
Babinski sign	Positive
Sensory	Normal
Cerebral involvement in MRI	Present
Spinal involvement in MRI	Absent
Disease progression	Rapid

tion, NM_000033.4 (*ABCD1*): c.901-2A>G, is classified as pathogenic (Class 5) and associated with the patient's phenotype. The variant has already been reported in Clin-Var (Variation ID: 1323832) as pathogenic in the context of adrenoleukodystrophy (PMID: 16996397, 16199547 and 11748843). This sequence change affects an acceptor splice site in intron 1 of the *ABCD1* gene. It is expected to disrupt RNA splicing variants that disrupt the donor or acceptor splice site typically lead to a loss of protein function (PMID: 16199547), and loss-of-function variants in

ABCD1 are known to be pathogenic (PMID: 11748843). The adenine at position c.901-2 in the first intron of the *ABCD1* gene is highly conserved across all species and This variant is not present in population databases (gnomAD or 1000genome). For these reasons, this variant has been classified as Pathogenic [7].

For the majority of intronic variations, the consequences on mRNA splicing have only been inferred through in silico analysis, whereas experimental demonstration of their pathogenicity has been achieved through mRNA studies for only a few of them. The predicted protein consequences are a substitution or abolition of the canonical splice acceptor site and the synthesis of a partial protein or even the absence of protein synthesis [7-9].

The mother was found to carry the same hemizygous *ABCD1* variant. The variant was absent in both the father and the patient's brother (Figure 2)

DISCUSSION

X-ALD is a progressive neurodegenerative disorder caused by a congenital defect in the ATP-binding cassette transporter sub-family D member 1 gene (*ABCD1*), which encodes the adrenoleukodystrophy protein (ALDP). ALDP deficiency leads to abnormalities in very long-chain fatty acid (VLCFA) metabolism, resulting in VLCFA accumulation in critical parts of the body, such as the central nervous system (CNS, including brain white matter and spinal cord), testes, and adrenal cortex.

Despite the shared genetic cause, the clinical presentation of X-ALD can vary dramatically. ALDP deficiency may manifest as the severe cerebral pediatric form, Childhood Cerebral Adrenoleukodystrophy (cCALD), adult adrenomyeloneuropathy (AMN), or as Addison-only and

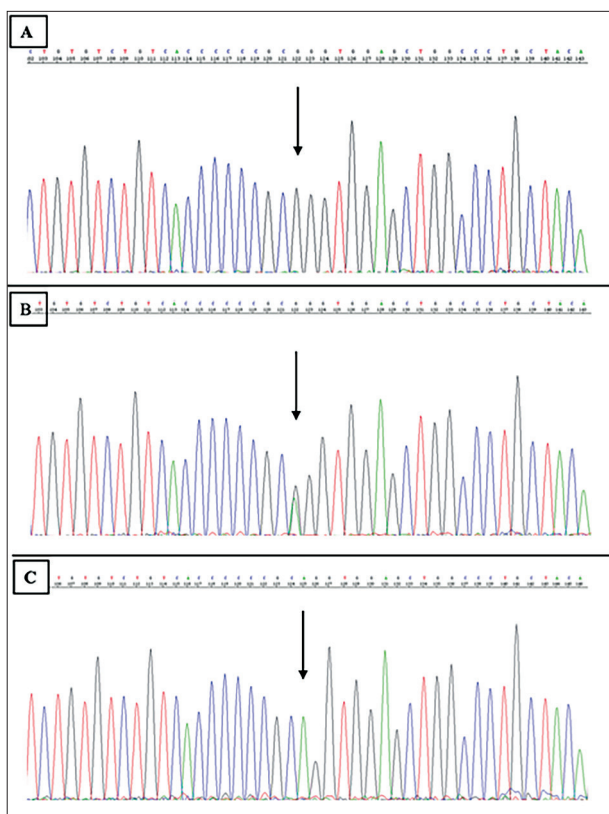


Figure 2. Electropherograms showing the pathogenic variant in the patient in a hemizygous state (A), in a heterozygous state in the mother (B), and the absence of the pathogenic variant in the healthy brother (C).

asymptomatic forms [1,2].

cCALD represents the most severe form of X-linked adrenoleukodystrophy (X-ALD), typically affecting boys between the ages of 4 and 8. It is characterized by progressive inflammatory demyelination in the cerebral white matter. Initial symptoms commonly include learning difficulties, behavioral changes, and attention deficits. As the disease advances, affected individuals may develop motor impairments, vision and hearing loss, seizures, and cognitive decline. In advanced stages, cCALD can lead to severe disability and significant loss of motor function [10,11].

X-ALD is an X-linked recessive disease caused by mutations in the *ABCD1* gene, located on the X chromosome (Xq28). The gene spans 19.9 kb and consists of 10 exons. It encodes a peroxisomal transmembrane protein of 745 amino acids, which follows the general structure of an ATP-binding cassette (ABC) transporter. As a result, males are more severely affected than females. However, some heterozygous females may exhibit symptoms due to skewed X-chromosome inactivation or other genetic factors [10,12,13]. Mutations in the *ABCD1* gene, which cause X-ALD, exhibit a wide spectrum, including missense,

frameshift, nonsense, and splice-site variants. The location and type of mutation often correlate with the clinical phenotype of X-ALD. However, identical variants can result in significantly diverse clinical presentations, suggesting the involvement of additional factors that influence disease expression. The majority of affected individuals carry mutations that result in the complete loss of ALDP protein function, while a smaller proportion may have milder mutations that allow some residual protein activity [14,15]. The management of X-ALD involves multiple approaches. Evidence clearly indicates that hematopoietic stem cell transplantation (HSCT) offers the best outcomes when performed on asymptomatic individuals with minimal but characteristic imaging findings of cCALD. Recently, an ex vivo gene therapy was approved, utilizing a lentiviral approach. This therapy involves transfecting a functional copy of the *ABCD1* gene into the patient's precursor hematopoietic cells. However, boys whose neurological disease has progressed too far at the time of diagnosis are not eligible for this targeted therapy [16]. In cases of adrenocortical insufficiency, corticosteroid replacement therapy is critical and can be lifesaving for males, though it is rarely required in females. Supportive care aimed at improving quality of life, maximizing function, and reducing complications is highly recommended. This care ideally involves a multi-disciplinary team of specialists in relevant fields.

The disorder is inherited in an X-linked manner. Consequently, if a female is a carrier of X-ALD, there is a 50% chance that her daughters will also be heterozygous carriers and a 50% chance that her sons will inherit X-ALD. Conversely, all daughters of an affected male will be carriers, while none of his sons will inherit the disorder. Wang and collaborators reported that, among 489 X-ALD families tested, 4.1% of the patients were affected by a de novo mutation in the index case, suggesting that the mutation occurred in the germ line. Additionally, less than 1% of cases demonstrated evidence of germinal mosaicism [17].

The patient described in this study exhibits the cerebral form of cCALD. Initial symptoms included learning and concentration difficulties, which progressed into a rapidly advancing neurological disorder accompanied by adrenal insufficiency. Corticosteroid replacement therapy and rehabilitation sessions were initiated. In the absence of biochemical analysis, the diagnosis was based on clinical presentation and radiological findings indicative of white matter involvement, characteristic of X-ALD. Molecular analysis subsequently confirmed the presence of a pathogenic variant in the *ABCD1* gene. Although a HSCT was considered, the patient was unable to undergo the procedure due to diagnostic challenges and the unavailability of specific tests. To provide comprehensive genetic counseling, molecular testing was conducted on the patient's

mother, revealing that she was a carrier of the same variant. So, she has a 50% risk of transmitting the variant to her children. With each pregnancy there is a 50% chance for each son to inherit the pathogenic variant and be affected and 50% chance for each daughter to inherit the pathogenic variant and be a carrier, or manifest some symptoms. As part of a comprehensive family care approach, a genetic analysis was offered to the mother's sister, who chose to postpone the test.

Considering the early onset of the disease and the availability of treatments to improve patient outcomes, presymptomatic testing was recommended for the patient's brother. Molecular analysis, however, did not detect the variant in the brother [18,19].

Our study underscores the importance of molecular analysis in confirming the diagnosis of X-ALD, while emphasizing its crucial role in genetic counseling provided to families. In addition to managing the affected child, families often express concern about the risks to siblings and future pregnancies. It is within this context that presymptomatic testing gains its full significance. Presymptomatic testing represents a predictive medicine approach, aimed at identifying a potential pathology before the onset of clinical symptoms in at-risk family members. However, the implications of a positive result on personal and family dynamics can be profound, particularly for minors. Current recommendations generally limit presymptomatic testing to situations where there is a demonstrated therapeutic benefit for the child. Although few studies have explored this aspect of X-ALD, Wang and collaborators conducted research on four families at risk of X-ALD and confirmed through molecular analysis that presymptomatic testing is an effective tool for determining the genotype of family members prior to symptom onset, enabling better management and support. In our case, performing the presymptomatic test on the patient's brother provided reassurance to the family. Through this work, in addition to describing the clinical presentation and expanding the existing data on *ABCD1* gene mutations, we highlight the significant impact such testing can have on affected families. This is particularly relevant given the currently limited data available on this aspect of X-ALD.

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