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CASE REPORT Open Access

# Identification of two Iranian siblings with cerebrotendinous xanthomatosis: a case report

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## Abstract

**Background** Cerebrotendinous xanthomatosis (CTX) is a rare autosomal recessive lipid storage disorder that leads to multisystem involvement. It is caused by mutations in the *CYP27A1* gene which encodes the mitochondrial enzyme sterol 27-hydroxylase.

**Case presentation** Herein we describe two affected CTX siblings with symptoms including seizures, severe diarrhea (steatorrhea), vomiting, and developmental motor delay, which was initially misdiagnosed as Short-Chain acyl-CoA dehydrogenase (SCAD) deficiency. However, to identify the possible genetic cause(s) of the disease, whole exome sequencing (WES) was performed. It was confirmed that these patients carried a nonsense variant (c.808C > T; p.Arg270Ter) of the *CYP27A1* gene. The variant in the *CYP27A1* gene was classified as pathogenic.

**Conclusion** We report rare cases of CTX with a novel mutation and summarize the clinical and molecular pathogenesis of this disease. Genetic analysis should be used as the conclusive method for CTX diagnosis because of the multisystem involvement and the lack of specific symptoms. The variant in these patients expands the molecular and phenotypic basis of a variant in CTX.

**Keywords** Cerebrotendinous xanthomatosis, *CYP27A1*, Cholestanol, Chenodeoxycholic acid novel mutation, Whole exome sequencing

## Background

Cerebrotendinous xanthomatosis (CTX: OMIM#213700) is a rare autosomal recessive bile acid biosynthesis disorder described in 1937. It is caused by sterol 27-hydroxylase deficiency (*CYP27A1*, EC 1.14.15.15), i.e., the mitochondrial cytochrome P 450 enzyme, due to mutations in the *CYP27A1* gene [1]. The *CYP27A1* gene spans 18.6 kb of DNA, which is located on chromosome 2q33-qter and contains nine exons and eight introns. A mature protein of 498 amino acids and 33-residue mitochondrial signal sequence constructs the sterol 27-hydroxylase enzyme, which maintains the binding site for heme and adrenodoxin. The prevalence of CTX is reported to be 3 to 5 per 100,000 but, it is probably underestimated [2, 3].

Regarding sterol 27-hydroxylase deficiency, the cholesterol and cholestanol metabolites accumulate in several tissues such as the central nervous system, muscle

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tendons, and eye lenses [4, 5]. Therefore, the clinical presentation of CTX is wide-ranging and highly heterogeneous, resulting in a difficult diagnosis, especially in the early stages. Broad systemic and neuropsychiatric clinical manifestations of CTX are reported including neonatal jaundice, cholestasis, juvenile bilateral cataracts, refractory diarrhea, osteoporosis, tendon xanthomas, seizures, developmental delay, intellectual disability, and progressive neuropsychiatric disturbances [6]. It is known that early diagnosis and treatment of chenodeoxycholic acid (CDCA), as a useful medicine, play a crucial role in the prevention of the development of neurological dysfunction [7].

In the present study, we report two CTX siblings with a delayed diagnosis who showed relative response after treatment with CDCA.

## Case presentation

This study recruited an Iranian family with two affected children a girl (proband) and her sister aged 7.5 and 6 years, respectively. The parents' marriage was consanguineous (first cousin). They enrolled in the database registry of Metabolic (no. 1400-7860), Shiraz University of Medical Sciences.

*Case 1* The 7.5-year-old girl was proband and she was delivered following an uneventful, normal, and term pregnancy with a birth weight of 2.85 kg and height of 49 cm.

At age one, the proband initially reported seizures, severe diarrhea (steatorrhea), vomiting, and developmental motor delay. She had vomiting accompanied by seizures two times. Biochemical results showed a high level of lactate (27 mg/dl), homocysteine (21.7 µmol/L), total cholesterol (280 mg/dL), and triglycerides (164 mg/dL) (Table 1). A fecal fat test confirmed the presence of steatorrhea. Organic acids and acylglycines profile showed a normal amount of methylmalonic acid, methylsuccinic acid, and mildly increased butyrylglycine (4.3 mmol/mol creatinine). The acylcarnitine profile in the plasma by LC–MS/MS indicated that the ratio C4/C0 (0.027, normal: < 0.018) was mildly increased. The alanine aminotransferase (ALT), aspartate aminotransferase (AST), urine creatinine, prothrombin time (PT), partial thromboplastin time (PTT), sodium, potassium, fasting blood sugar (FBS), and BUN were normal. Neurological examination demonstrated cognitive decline, increased deep tendon reflexes as well as mild muscle hypertonia. Many cutaneous xanthomas on the back were also detected.

Based on the above results, the metabolic disorder specifically the short-chain Acyl-CoA dehydrogenase deficiency (SCAD), was suggested. Despite all types of treatment, she had a progressive problem with walking and speaking, and overall cognition. Regarding the progressive and deteriorating disease, at the age of 5 years, molecular analysis was suggested for confirmation of diagnosis. Whole exome sequencing (WES) was performed to analyze all exons of protein-coding genes as well as some important other genomic regions. Analyses were performed using an Illumina Hiseq4000 (Illumina Inc., San Diego, CA, USA). It uses a 100 bp paired-end read with a mean depth of coverage of 55 × with 95.5%

**Table 1** Lab test results of the proband in the current case with CTX

| Analysis | Lab results | | | Reference range |
|---|---|---|---|---|
| | (1 years-old) | (5 years-old) | (7.5 years-old) | |
| Fasting blood sugar | 78 mg/dl | 91 mg/dl | 76 mg/dl | 70–99 mg/dl |
| Alanine aminotransferase | 10 IU/L | 12 IU/L | 12 IU/L | Up to 31 IU/L |
| Aspartate aminotransferase | 8 IU/L | 10 IU/L | 10 IU/L | Up to 31 IU/L |
| Triglyceride | 142 mg/dl | 199 mg/dl | 145 mg/dl | 40–160 mg/dl |
| Cholesterol | 195 mg/dl | 184 mg/dl | 135 mg/dl | 130–200 mg/dl |
| LDL | 105 mg/dl | 68 mg/dl | 32 mg/dl | 0–160 mg/dl |
| HDL | 49 mg/dl | 55 mg/dl | 49 mg/dl | 40–60 mg/dl |
| Uric acid | 2.1 mg/dl | 5.5 mg/dl | 3.1 mg/dl | 3.4–7 mg/dl |
| BUN | 11 mg/dL | 7 mg/dL | – | 5–17 mg/dL |
| Total protein | 7.3 g/dl | 4.2 g/dl | 7.7 g/dl/ | 4.1–7.9 g/dl |
| Creatinine | 0.43 mg/dL | 0.48 mg/dL | – | 0.31–0.6 mg/dL |
| Lactate | – | 27 mg/dL | – | Plasma:4.5–19.8 mg/dL |
| Homocysteine | – | 21.7 µmol/L | – | < 10 µmol/L |
| Potassium | 4.1 m.Eq/L | 4.8 m.Eq/L | – | 3.5–5.6 m.Eq/L |
| Sodium | 139 m.Eq/L | 139 m.Eq/L | – | 135–145 m.Eq/L |

and 91.5% coverage at 10× and 20×, respectively. The human genome 19 and the in-house database of 800 Iranian control samples were used as the reference. For in silico analysis, Polyphen2, scale-invariant feature transform (SIFT), and Mutation Taster were used. Genomic Evolutionary Rate Profiling (GERP) and Phastcons scores were used to evaluate the conservation of the variants. The variant interpretation was according to American College of Medical Genetics and Genomics (ACMG) guidelines [8]. Sanger sequencing was applied for validation of the identified variants using An ABI Prism 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). WES results showed that the patient was homozygous for the c.808C > T (p.Arg270Ter) variant in exon 4 of the *CYP27A1* gene [9]. In silico analysis revealed that the variant, c.808C > T (p.Arg270Ter) was pathogenic, which would have possibly resulted in premature termination of the protein (Fig. 1). In addition, Sanger sequencing analyzed the samples from the parents. It should be mentioned that both parents were asymptomatic.

As a result, high-dose CDCA treatment (5 mg/kg/day 3 times per day) was initiated for the patient. After 4 months, her spinal cord xanthomas began to diminish, also improvement in her intellectual ability and movement was observed. However, because of non-compliance, CDCA treatment was repeatedly discontinued; CDCA treatment was stopped, diarrhea recurred, and cognitive abilities and walking worsened.

In the last follow-up, at the age of 7.5 years old, she still had difficulty walking and needed help to get up and climb the stairs. She also had speech problems and a learning disorder in school. However, other problems such as muscle hypertonia, and cognitive problems were diminished.

*Case 2* A 6-year-old female was the youngest sister of the proband. She was born at 35 weeks of gestational age after a normal and uneventful pregnancy. Her symptoms and signs were very similar to her siblings including severe diarrhea (steatorrhea), vomiting, and motor delay. At the age of two, she had a high HDL level (71 mg/dL, normal range: 40–60) and aspartate aminotransferase (AST) (41 IU/L, normal range: 1–31). Like case 1, she was misdiagnosed as short-chain Acyl-CoA dehydrogenase deficiency (SCAD) and treated accordingly. After performing molecular analysis and WES, at the age of 3.5, she was diagnosed with CTX. She was carrying the same homozygous variant c.808C > T (p.Arg270Ter) in exon 4 of *CYP27A1* gene as case 1. Treatment with a high dose of CDCA (5 mg/kg/day 3 times per day) started. After treatment, she showed a dramatic response and quick improvement and all symptoms disappeared in less than one month.

In the last follow-up, at the age of six, she had no problem in walking, speaking, cognition, and learning at school.

## Discussion and conclusions

CTX is an inherited lipid storage disorder with a wide range of manifestations, which can show variable types, manifestations, and severity, even within the same

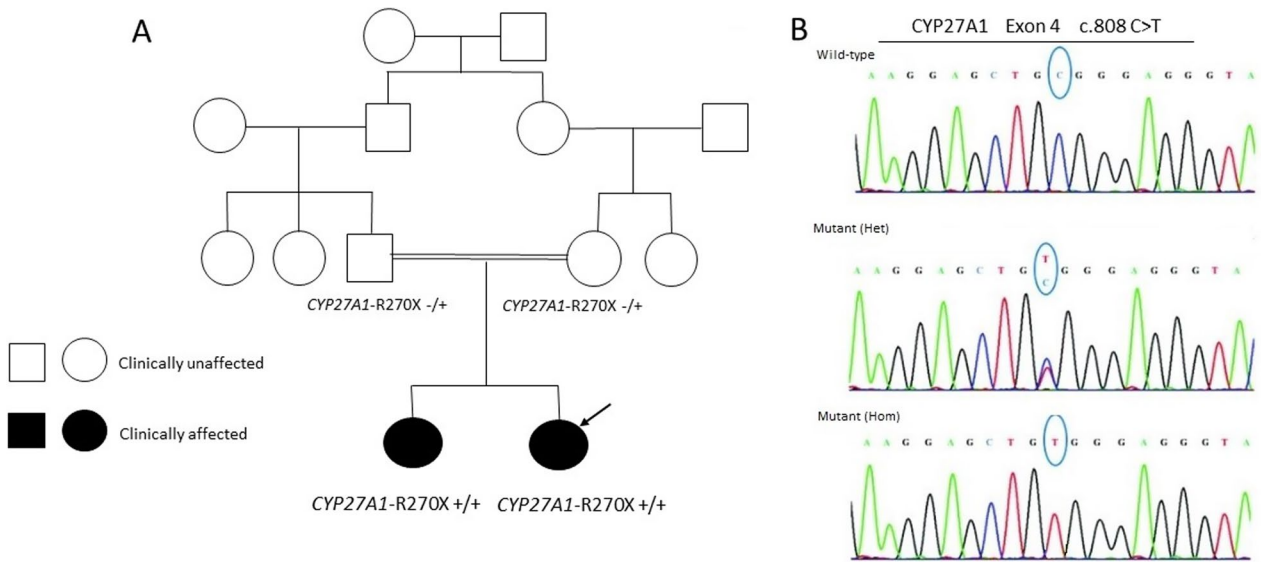


**Fig. 1** Genetic analysis identified a *CYP27A1* variant. **A** Pedigree of the family in the *CYP27A1* variant carrier. Black arrow shows proband, **B** DNA chromatogram shows a homozygous C-to-T transition at nucleotide 808 of *CYP27A1*, predicting a substitution of an arginine for stop codon at residue 270 (p.Arg270Ter). + indicates variant positive, − indicates variant negative. (CYP27A1: NG_007959.1)

family. The majority of common clinical presentations are chronic diarrhea, progressive neurologic dysfunction, cataract, and tendon xanthoma. However, the reported results of more than 300 patients so far have demonstrated that the diagnosis of CTX is still challenging because of varied clinical manifestations [10]. Herein, we are reporting a homozygous variant in the *CYP27A1* gene, leading to the loss of function of sterol 27-hydroxylase, in patients that are born in a consanguineous family of Iranian descent.

As to clinical manifestations, it has been reported that cataract is the first symptom, which is frequently appears in the first decade while tendon xanthomas manifest in the second or third decade of life. However, in our patient, xanthomas were the initial manifestation on the spinal cord as well as seizures at the age of one. It should be mentioned that infantile spasms have been reported as an underappreciated symptom in infants with CTX [11].

As reported, they showed cognitive impairment from early infancy. Other highlighted signs included behavioral changes, dementia, hallucinations, aggression, agitation, and depression reported between puberty and the third decade of life. This has not appeared in our patients so far. In addition to the mentioned clinical symptoms, increased butyrylglycine and vomiting have been observed in our cases, which lead to misdiagnosis as a SCAD first. Vomiting is a rare clinical manifestation for CTX patients, which has been not reported previously. Furthermore, there is a lot of controversy about SCAD deficiency and, whether it should be considered a clinical entity or not is controversial. Many reports consider it a non-disease [12, 13]. Therefore, for the differential diagnosis, early genetic analysis in complex cases should be performed.

Early and correct diagnosis of CTX by next-generation sequencing (NGS) is crucial and effective. NGS is an accurate, efficient, and cost-effective method for the diagnosis of inherited metabolic disorders. It is documented that NGS can be useful for simultaneous sequencing of the group of candidate genes, especially for clinically and genetically heterogeneous diseases caused by a group of genes involving a common metabolic pathway. An early genetic diagnosis by NGS besides clinical suspicion, laboratory, biochemical confirmation of CTX, and imaging findings results in correct diagnosis and treatment, cost, and time effectiveness [14]. Until now, more than 250 variants are reported in CTX patients, and 85 variants are pathogenic or likely pathogenic [15]. Variants including missense, nonsense, frameshift, splice site, duplications, deletion, indel, and insertion have been reported so far. Variants were detected in all 9 exons and introns 2,4,6,7, and 8 of the *CYP27A1* gene; however, the splice site variant had the highest prevalence, and exon 4 had the highest number of mutations, based on ClinVar database [16]. It is worth mentioning that the most prevalent variant among spinal CTX patients is reported to be the Arg395Cys allele [15].

It is important that when genetic analysis of *CYP27A1* confirmed the CTX, its treatment by CDCA should be started as soon as possible. Treatment with CDCA has been documented to improve or even prevent clinical signs of CTX in the early stage of the disease. It can improve symptoms by direct inhibition of CYP7A1 hydroxylation of cholesterol and has a negative feedback on cholesterol biosynthesis, to decrease the rate of bile acid synthesis (and production of cholestanol) resulting in decreased accumulation of cholestenol in the tissues. It has appeared that early treatment by CDCA can prevent the development of neurologic symptoms in CTX patients [17].

In conclusion, our study reported two Iranian patients who suffered from CTX. A pathogenic variant in the *CYP27A1* gene was identified, which is represented by a new clinical manifestation in our CTX patients. Regarding the progress and utility of NGS application, early detection before the onset and identifying more patients is predicted. It can also help to reduce the complications of this disease and increase our knowledge about rare inherited metabolic diseases.

**Abbreviations**

| | |
|---|---|
| CA | Cholic acid |
| CDCA | Chenodeoxycholic acid |
| CTX | Cerebrotendinous xanthomatosis |
| MR | Mitral regurgitation |
| NGS | Next-generation sequencing |
| SCAD | Short-Chain acyl-CoA dehydrogenase |
| WES | Whole exome sequencing |

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**Author contributions**
ZB contributed to conception of the manuscript, acquisition of data, drafting and final approval the manuscript. HM contributed to acquisition of data, revising the manuscript. SI contributed to revising the manuscript. MHI contributed to acquisition of data, revising the manuscript. BG contributed to acquisition of data, revision and final approval of the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**
The data that support the findings of this study are available from Molecular Genetics Laboratory, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are, however, available from the authors upon reasonable request and with permission of Molecular Genetics Laboratory. The datasets generated and/or analyzed during the current study are available in the Genbank repository (GRCh37/hg19, https://www.ncbi.nlm.nih.gov/genome/guide/human/) for *CYP27A1*: NG_007959.1.

## Declarations

**Ethics approval and consent to participate**
This study was conducted in accordance with the Declaration of Helsinki. It was approved by the Ethics Committee of Shiraz University of Medical Sciences. The researcher informed the parents of the children about the objectives of the study, examination and investigations that have been done. Also, the confidentiality of their information, their rights not to participate in the study were respected and written informed consent was obtained.

**Consent for publication**
Written informed consent was obtained from their parent or legal guardian for publication of this case report and accompanying images.

**Competing interests**
The authors declare that they have no competing interest.

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## Publisher's Note