Clinical and Radiological Findings of a Cerebrotendinous Xanthomatosis Patient with a Novel p.A335V Mutation in the CYP27A1 Gene

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Abstract

We herein describe the case of a Japanese cerebrotendinous xanthomatosis (CTX) patient with a novel CYP27A1 gene mutation. The patient had been diagnosed with cataracts at 25 years of age and subsequently developed neurological symptoms in his forties, being referred to our hospital at 47 years of age. Upon admission, Achilles tendon xanthomas, cognitive impairment, dysphagia, dysarthria, dystonia, spasticity, muscle weakness and ataxia were observed. Brain MRI revealed abnormal signals in the dentate nuclei, periventricular white matter and pyramidal tract, and the serum cholestanol level was elevated. A CYP27A1 gene analysis identified compound heterozygosity for p.A335V, a novel mutation, and p.R405Q, a previously reported mutation. Making an early diagnosis of CTX is crucial, as the administration of chenodeoxycholic acid reverses metabolic derangement.

Key words: cerebrotendinous xanthomatosis, CYP27A1 gene, cholestanol, spasticity, ataxia, magnetic resonance imaging

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Introduction

Cerebrotendinous xanthomatosis (CTX, MIM# 213700) is an autosomal recessive lipid storage disease caused by a mutation in the CYP27A1 gene encoding the mitochondrial cytochrome P 450 enzyme, sterol 27-hydroxylase (CYP27A1, EC 1.14.13.15) (1, 2). CYP27A1 catalyzes the initial steps in the oxidation of the side chain of sterol intermediates in the pathway leading to the formation of bile acids in the liver. Therefore, its deficiency results in impaired bile acid synthesis and the increased production of cholesterol metabolites, such as cholestanol, which subsequently accumulate in the brain, tendons, lenses, bones and vessels. As a consequence of bile acid deficiency, affected patients usually experience chronic diarrhea during childhood (3). Progressive cholestanol deposition induces the formation of juvenile cataracts, tendinous xanthomas, osteoporosis, premature atherosclerosis and neurological symptoms, including psychomotor delays, cognitive impairment, ataxia, pyramidal and extrapyramidal symptoms, epilepsy, peripheral neuropathy and psychiatric disorders (3-8). To date, over 50 mutations in the CYP27A1 gene have been reported around the world (5, 9-14). We herein describe the clinical, neuroradiological and molecular biological findings of a Japanese CTX patient with a novel missense mutation in the CYP27A1 gene.

Case Report

The patient, a 47-year-old Japanese man, was the third child of non-consanguineous healthy parents and had a healthy sibling. He was born at term after a normal pregnancy and showed normal development. He had graduated

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from a regular community high school and began working as a factory laborer. However, he was diagnosed as having bilateral cataracts at 25 years of age. At 38 years of age, he broke his leg after a fall. At 40 years of age, he noticed an unsteady gait and dysarthria. At 43 years of age, he lost his job due to cognitive impairments and was admitted to a local hospital for treatment of deterioration of a gait disturbance. At 47 years of age, he developed dysphagia and was referred to our hospital.

Upon admission, bilateral enlargement of the Achilles tendons was observed (Fig. 1). An ophthalmologic examination showed bilateral cataracts, and neurologically, the patient exhibited severe dysphagia and dysarthria due to dystonia of the jaw and tongue. Severe dystonia was also noted in the right upper limb. Spasticity of the extremities with muscle wasting and weakness of the lower limbs, as well as a spastic and ataxic gait and positivity for Babinski and Chaddock signs, was also observed. The patient demonstrated neither myoclonus nor tremors. His intelligence, as assessed according to the Wechsler Adult Intelligence Scale, 3rd edition (WAIS-III), showed a Full Scale IQ of 61, Verbal IQ of 64, Performance IQ of 63, Verbal Comprehension Index of 69, Perceptual Organization Index of 70, Working Memory Index of 67 and Processing Speed Index of 54. The Mini-Mental State Examination (MMSE) score was 28. The discrepancy between the WAIS-III score and MMSE score appeared to have originated in a decreased processing speed. The findings of routine blood examinations, a cerebrospinal fluid assay, chest roentgenography and electrocardiography were all normal; however, the serum cholestanol level was remarkably elevated (35.6 μg/mL; normal, 1.91-3.51 μg/mL). In addition, the bone mineral density of the lumbar spine, as assessed on dual-energy X-ray absorptiometry, was remarkably decreased (0.754 g/cm², T-score -3.6), and an electroencephalogram (EEG) revealed a slow background activity composed of theta and delta waves; bursts of high-voltage activity were also frequently observed. Meanwhile, the motor evoked potential (MEP) showed a prolonged central motor conduction time, while the somatosensory evoked potential (SEP) evoked by right median nerve stimulation demonstrated a significant cortical response. Furthermore, brain MRI disclosed diffuse brain atrophy and bilateral hyperintense signals in the dentate nuclei, periventricular white matter and pyramidal tract, including the corona radiate, posterior limb of the internal capsule and cerebral peduncle, on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images (Fig. 2).

As the patient’s clinical findings were suggestive of CTX, a genetic analysis to detect this disorder was performed with
Figure 2. Brain MRI of the patient (FLAIR image, axial view). Diffuse brain atrophy and bilateral hyperintense signals were observed in the dentate nuclei, periventricular white matter and pyramidal tract, including the corona radiate, posterior limb of the internal capsule and cerebral peduncle.

informed consent. Consequently, DNA was extracted from peripheral leukocytes of the patient and his mother and brother, according to the standard protocol. We were unable to analyze the patient’s father, as he had died at 44 years of age. All nine exons of the CYP27A1 gene were amplified via polymerase chain reaction (PCR), as previously described (15). A direct sequence analysis of the PCR-amplified DNA of the patient identified two heterozygous missense mutations, c.1004C>T and c.1214G>A (Fig. 3A), which resulted in amino acid alterations of p.A335V and p.R405Q, respectively. The c.1214G>A (p.R405Q) mutation has been documented in three CTX families (4, 12, 13), whereas the c.1004C>T (p.A335V) mutation has not been previously described [SNPs reported in the database (dbSNP) of the National Center for Biotechnology information (NCBI) (16) and the University of California Santa Cruz (UCSC) Genome Browser (17)]. The possible impact of the novel p.A335V mutation on the structure and function of sterol 27-hydroxylase was assessed using a bioinformatics tool, Polymorphism Phenotyping-2 (PolyPhen-2) (18, 19), and predicted to likely be damaging (score: 1.00). The patient’s mother and brother were heterozygous for the c.1004C>T (p.A335V) mutation (Fig. 3B, C), indicating that the patient was compound heterozygous for the CYP27A1 gene mutation.

The patient was treated with 750 mg/day of chenodeoxycholic acid (CDCA) and 10 mg/day of atorvastatin calcium hydrate. One year later, a physical examination revealed no progression of his clinical manifestations, and the serum concentration of cholestanol decreased (4.1 μg/mL) to nearly the normal range.

Discussion

The present patient was initially suspected to have spinocerebellar degeneration or spastic paraplegia. However, the existence of Achilles tendon xanthomas, juvenile cataracts and osteoporosis, an elevated serum cholestanol level and characteristic brain MRI findings suggested a diagnosis of CTX. In previous radiological studies, MRI T2/FLAIR hyperintense signals in the periventricular white matter, dentate nuclei, globus pallidus and pyramidal tract have been identified to be characteristic findings of CTX, found in 71-100%, 76-79%, 63-86%, 67-86% of patients, respectively (5, 20, 21). In addition, lipid crystal clefts, perivascular macrophages, neuronal loss, demyelination, fibrosis and
reactive astrocytosis have been detected in these locations on microscopic examinations (20). The MRI findings of our patient were compatible with those of CTX.

In addition, a molecular genetic analysis of the CYP27A1 gene revealed that the patient was compound heterozygous for p.A335V and p.R405Q mutations, confirming the diagnosis of CTX. The c.1214G>A (p.R405Q) mutation has previously been reported in three CTX families (4, 12, 13). For example, Chen et al. reported a Japanese patient homozygous for the p.R405Q mutation who presented with typical CTX manifestations, including tendon xanthomas, cataracts, neurological dysfunction, an elevated plasma cholestanol level and an undetectable sterol 27-hydroxylase activity (12) as well as Japanese siblings compound heterozygous for p.R405Q and p.R474Q (4, 12). Korean siblings compound heterozygous for p.R405Q and p.H382QfsX26 have also been documented (13). The R405 residue is located near the adrenodoxin-binding site of the sterol 27-hydroxylase enzyme and is expected to affect the activity of the enzyme. In addition, a transient expression study of CYP27A1 cDNA recently revealed that p.R405Q proteins do not exhibit an enzymatic activity (12), compatible with the features of the typical CTX phenotype.

In contrast, the c.1004C>T (p.A335V) mutation is a novel missense mutation. The A335 residue is not related to the adrenodoxin-binding site (residues 384-398) or heme-binding site (residues 468-497) of the sterol 27-hydroxylase enzyme, where most missense mutations occur (14). However, in the present study, an in silico analysis revealed A335 to be a highly conserved amino acid residue (Table), while the amino acid substitution of A335 to V was predicted to alter the structure and function of the enzyme according to the bioinformatics tool, PolyPhen-2. Based on the clinical findings of our patient and the structural and functional properties of the p.A335V mutation, this novel mutation is considered to be pathogenic.

CTX may mimic neurodegenerative diseases, such as spinocerebellar degeneration, spastic paraplegia and Parkinson’s disease.

Figure 3. Direct nucleotide sequencing of the PCR-amplified CYP27A1 gene DNA of the patient (A) and his mother (B) and brother (C). The vertical arrow indicates nucleotide 1004 (exon 5), where a C→T transversion (c.1004C>T) resulted in an amino acid substitution, p.A335V (A-C). The arrowhead indicates nucleotide 1214 (exon 7), where a G→A transversion (c.1214G>A) resulted in an amino acid substitution, p.R405Q (A-C). The patient was compound heterozygous for the p.A335V and p.R405Q mutations.
References

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

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Table. Evolutionary Conservation of CYP27A1 Gene A335 Residue

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<th>A335 residue</th>
<th>Human</th>
<th>Chimpanzee</th>
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<th>Cat</th>
<th>Rabbit</th>
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disease; therefore, obtaining an early diagnosis of CTX is crucial, as treatment with CDCA reverses the metabolic derangement and may prevent or even improve the neurologic dysfunction associated with this disease (22-24). Importantly, treatment may be less effective once neurological symptoms are fully established, likely due to the irreversibility of the lesions (25, 26), and is more efficacious if started early in the disease course (27). CTX is likely an underdiagnosed disease, since its phenotype is often incomplete, especially in the early stage of the disease. Therefore, physicians should consider the potential for CTX in any individual presenting with normocholesterolemic xanthomatosis, juvenile cataracts and osteoporosis and neurological symptoms.