INTERNAL MEDICINE

□ CASE REPORT □

Identification of a Novel Missense Mutation in the Sterol 27-Hydroxylase Gene in Two Japanese Patients with Cerebrotendinous Xanthomatosis

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Abstract

Cerebrotendinous xanthomatosis (CTX) is a rare autosomal recessive sterol storage disease caused by a mutated sterol 27-hydroxylase (CYP27A1) gene. We analyzed the CYP27A1 gene in two Japanese CTX patients. The CYP27A1 gene was amplified by PCR and screened by PCR-SSCP. The nucleotide sequence was analyzed to confirm mutations. Case 1 was a compound heterozygote for Arg104Gln in exon 2 and Arg441Gln in exon 8. To our knowledge, this is the first report in which the Arg104Gln mutation is identified in CTX patients. Probably case 2 would be a compound heterozygote for Arg441Trp in exon 8 and a mutation that was not identified.

Key words: cerebrotendinous xanthomatosis, cholestanol, gene mutation, 27-hydroxylase, xanthoma

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Introduction

Cerebrotendinous xanthomatosis (CTX) is a rare autosomal recessive sterol storage disease caused by a mutated sterol 27-hydroxylase (CYP27A1) gene (1). Japanese CTX patients account for nearly one-third of all the CTX cases reported in the world (2). This disease is characterized by the accumulation of cholesterol and cholestanol in various tissues. Accumulation in the central nervous system leads to neurological dysfunction including dementia, spinal cord paresis, and cerebellar ataxia (3). Accumulation in other tissues causes tendon xanthomas, premature atherosclerosis, and juvenile cataracts (1, 2, 4).

Here, we describe two unrelated Japanese patients affected by CTX and provide the results of our mutational analysis of the CYP27A1 gene.

Case Report

Case presentations

Case 1: The patient was a 65-year-old man with a history of gait disturbance that appeared when he was about 50 years old, and who was referred to our hospital in May 2000 because of severe Achilles tendon thickness that had become detectable when he was 30. Serum cholestanol level was increased (7.7 μg/mL) and the ratio of cholestanol to cholesterol, which is an accurate index for diagnosing CTX (5), was also increased (0.4%) (Table 1). He showed tendon xantomas in bilateral knees and Achilles tendon, and right ring finger (Fig. 1). Furthermore, he showed mild to

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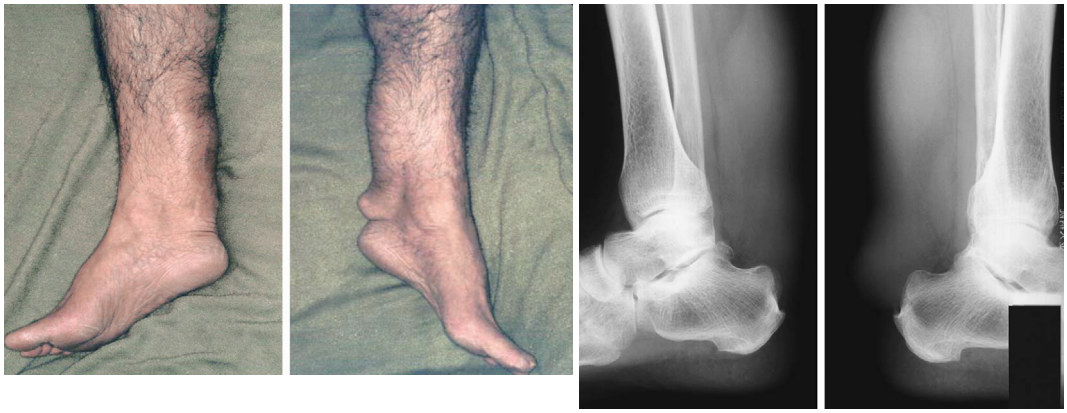

Figure 1. **Pictures of the legs and X-ray of the Achilles tendon of case 1. Massive Achilles tendon xanthomas and thickness were observed.**

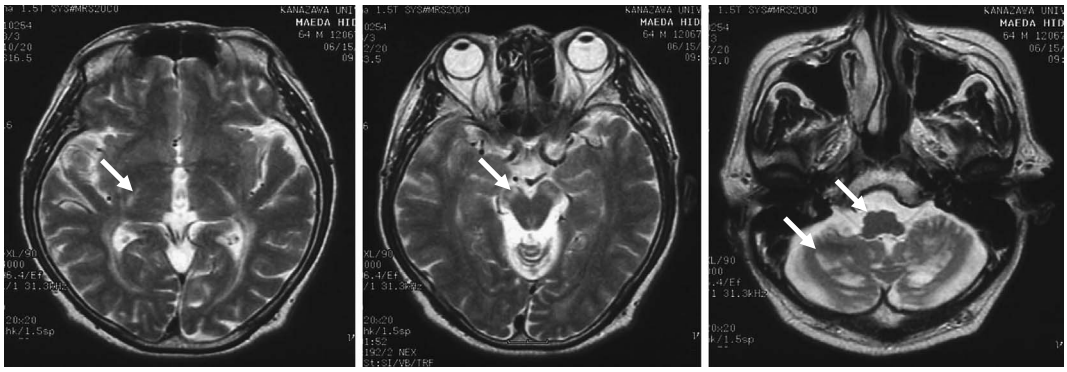

Figure 2. **T2-weighted magnetic resonance image of the brain in case 1. Cerebral and cerebellar atrophy was evident. The arrows indicate hyperintensive signals on both sides of the internal capsule, cerebral peduncle, pons, and cerebellar hemisphere.**

Table 1. **Clinical Characteristics of Two Patients with CTX**

	Case 1	Case 2
Age (year)	65	67
Gender	Male	Male
TC (mg/dL)	191	167
HDL-C (mg/dL)	60	26
TG (mg/dL)	62	124
Apo AI (mg/dL)	155	84
Apo AII (mg/dL)	32.1	20.5
Apo B (mg/dL)	99	114
Apo CII (mg/dL)	4.3	2.6
Apo CIII (mg/dL)	8.8	3.3
Apo E (mg/dL)	5.2	3.9
LDL-R activity (%)	120	-
CETP (μg/mL)	2.3	2.4
Cholestanol (μg/mL)	7.7	11.0
Sitosterol (μg/mL)	1.4	5.4
Cholestanol / cholesterol (%)	0.4	0.7

CTX, cerebrotendinous xanthomatosis; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; apo, apolipoprotein; LDL-R, low-density lipoprotein receptor; CETP, cholesteryl ester transfer protein; -, not examined.
All values were obtained during the pre-treatment period.

moderate dementia [Revised Hasegawa Dementia Scale (HDS-R) 14 points], pyramidal-tract signs (positive Babinski and Chaddock reflexes), and cerebellar signs (poor result in the finger-to-nose test and knee-heel test, and ataxic gait). T2-weighted magnetic resonance image (MRI) showed hyperintensive signals on both sides of the internal capsule, cerebral peduncle, pons and cerebellar hemisphere, and these were associated with cerebral and cerebellar atrophy (Fig. 2). There was no known consanguinity in previous generations.

Case 2: The patient was a 67-year-old man who had started to develop xanthomas in bilateral elbows, the back of both hands, knees and legs when he was about 37 years old. He was referred to our hospital in August 2000 because of the increase in the number of xanthomas and newly noticed symptoms of dementia. Serum cholestanol level (11.0 μg/mL) and the ratio of cholestanol to cholesterol (0.7%) were increased (Table 1). He showed tendon xantomas in bilateral elbows, the back of both hands, knees, instep of both feet, soles, and Achilles tendon (Fig. 3). Furthermore, he showed severe dementia (HDS-R 4 points), pyramidal-tract signs (positive Babinski and Chaddock reflexes), and cerebellar signs (normal result in the finger-to-nose test, but poor result in the knee-heel test and ataxic gait). There was no family history of CTX and his parents' marriage was not consanguineous.

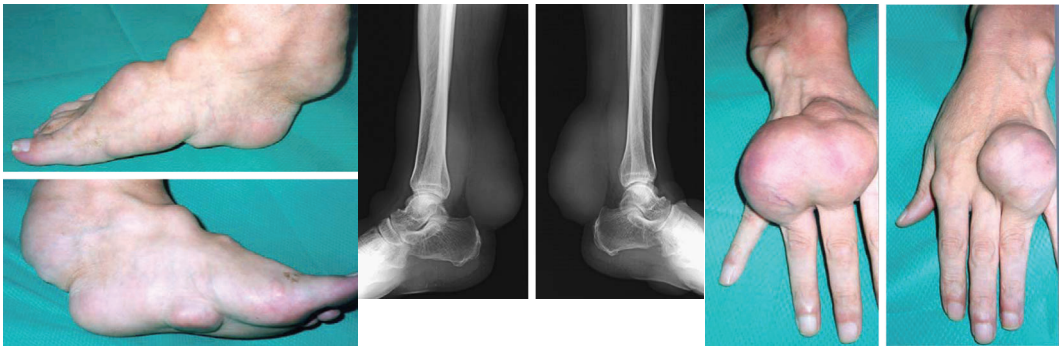

Figure 3. **Pictures of the legs and hands, and X-ray of the Achilles tendon of case 2. Massive xanthomas of the Achilles tendon and hands, and Achilles tendon thickness were observed.**

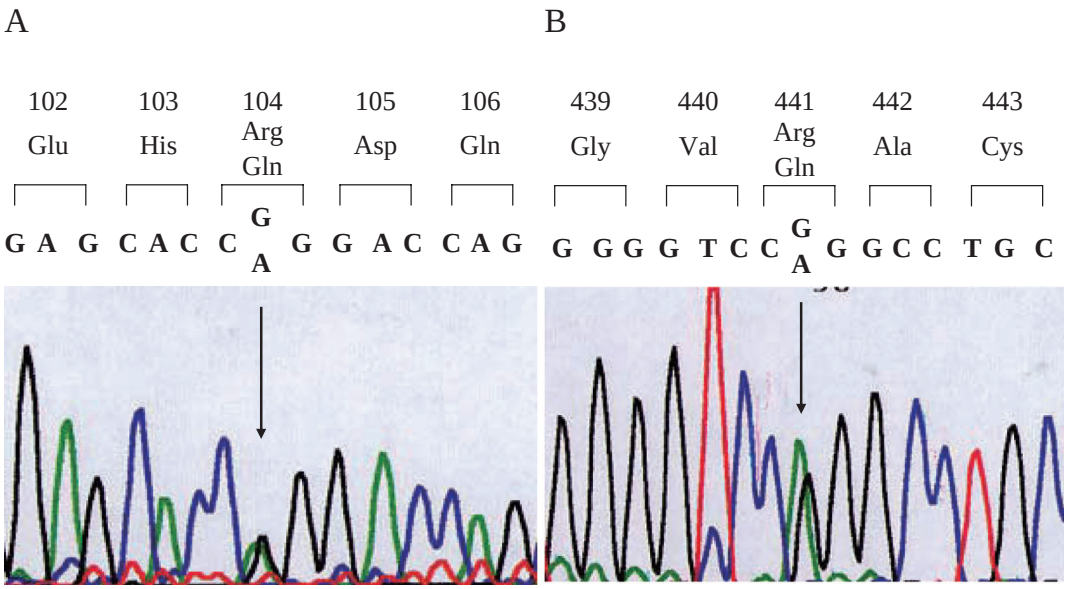

Figure 4. **Direct sequence analysis of exon 2 (panel A) and exon 8 (panel B) of the sterol 27-hydroxylase gene. Each curve indicates adenine (A), cytosine (C), guanine (G), and thymine (T). The graph shows the results for case 1. The arrow indicates the A peak of the mutant and G peak of the wild type.**

Genetic analysis

Written informed consent was obtained from the patients and/or family members. The Ethics Committee of our hospital approved the study protocol. Blood samples were collected from the patients and from 100 unaffected and unrelated Japanese volunteers. Genomic DNA was isolated from peripheral blood leukocytes according to standard procedures and was used as a template for polymerase chain reaction (PCR). The sequence information was obtained from the report of Cali et al (6) and the CYP27A1 gene arrangement registered with the Gene Bank database. All 9 exons, the promoter, and poly-A of the CYP27A1 gene were amplified by PCR and variant conformers were detected by PCR-single strand conformational polymorphism (PCR-SSCP) (7). The nucleotide sequence was analyzed using the dideoxynucleotide chain termination method by Thermo sequence II (Amersham Pharmatica Biotech, Cleveland, OH) in ABI 310 automated DNA sequencer (Perkin-Elmer, Foster City, CA) to confirm the mutations.

Three point mutations were identified in the CYP27A1 gene. One was a G-to-A mutation in exon 2, resulting in amino acid substitution of Arg (CGG) to Gln (CAG) at codon 104 (R104Q) (Fig. 4A), the other was a G-to-A mutation in exon 8, resulting in amino acid substitution of Arg (CGG) to Gln (CAG) at codon 441 (R441Q) (Fig. 4B), and another was a C-to-T mutation in exon 8, resulting in amino acid substitution of Arg (CGG) to Trp (TGG) at codon 441 (R441W) (Fig. 5). To our knowledge, R104Q is the first mutation identified in CTX patients. These three different mutations in the CYP27A1 gene were further confirmed by the PCR-restriction fragment length polymorphism (PCR-RFLP) method. The mutation of R104Q predicted the loss of *Hpa* II restriction site. Case 1 showed both 199 bp and 161 bp fragments after the digestion of the PCR products in exon 2 with *Hpa* II, which indicated the heterozygote for R 104Q (data not shown). The mutation of R441Q predicted the creation of a novel recognition site for *Aat* I. Case 1

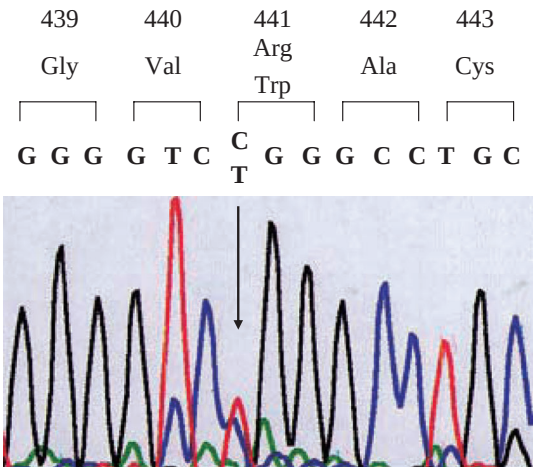

Figure 5. Direct sequence analysis of exon 8 of the sterol 27-hydroxylase gene. The graph shows the results for case 2. The arrow indicates the T peak of the mutant and C peak of the wild type.

showed both 292 bp and 178 bp fragments after the digestion of the PCR products in exon 8 with *Aat* I, which indicated the heterozygote for R441Q (data not shown). Therefore, case 1 was a compound heterozygote for R104Q and R441Q. The mutation of R441W predicted the loss of *Hpa* II restriction site. Case 2 showed both 292 bp and 182 bp fragments after the digestion of the PCR products in exon 8 with *Hpa* II, which indicated the heterozygote for R441W (data not shown). Probably case 2 would be a compound heterozygote for R441W and a mutation that was not identified. Unfortunately, we did not have the opportunity to examine the parents of both cases. R104Q was not detected in the 200 alleles from the 100 control volunteers. Therefore, we considered this mutation of the CYP27A1 gene was the cause of CTX.

Discussion

At present about 50 mutations have been identified in about 200 patients, which are spread across all exons, with the exception of exon 9 (8, 9). We identified one novel and two previously reported missense mutations in the CYP27A1 gene in this study. R441Q and R441W were reported by Kim et al in 1994 (10). R441Q and R441W seem to be predominant in Japanese CTX patients (11). The CYP 27A1 is a member of the large mitochondrial cytochrome P 450 family and two functional domains, that are the adrenodoxin-binding site (residues 351-365) and the heme-binding site (residues 435-464), have been identified (12, 13). Lee et al reported that most of the missense mutations occurred in these two domains (8). R441Q, R441 W and R104Q were located in the heme-binding site (8). Nakashima et al previously reported cases of CTX caused by a mutation at codon 104 (R104W) (14). This position has a 100% conserved positive charge in all known vertebral cytochrome P450s. Although R104Q mutation was not detected in our control panel of 200 alleles, further studies, including actual measurement of enzyme activity and site-directed mutagenesis of this region of the CYP27A1 gene, should be conducted to prove that R104Q is, in fact, responsible for the defective or decreased enzyme activity. We found only a heterozygote for R441W in case 2. Probably this patient would be a compound heterozygote for R441W and a mutation that was not identified. Examination of copy number by array CGH is necessary to detect whether microdeletion exists or not in another allele of R441W. Furthermore, multiplex ligation-dependent probe amplification (MLPA) may be useful because deletion, insertion or duplication of one exon cannot be defined. Sugama et al reported a heterozygous mutation (R441Q) in the CYP27A1 gene in a patient with clinical CTX (15). They speculate that another enzyme at the same level as CYP27A1 or cofactors, such as ferredoxin reductase or ferredoxin, may be involved in their case. It is possible that our case 2 also may actually have a heterozygous mutation in the CYP27A1 gene.

Development of symptoms in patients with CTX is extremely variable and it has not yet been possible to correlate the varying symptoms with mutations in specific parts of the CYP27A1 gene (9). Verrips et al also reported that the genotype-phenotype analysis did not reveal any correlation (16). In CTX the same mutation may result in different phenotypes, or mutations at different sites of the CYP27A1 gene may result in the same or in different phenotypes. It has been suggested that environmental factors are responsible for these clinical differences (17). The most important clinical finding of CTX patients is bilateral Achilles tendon xanthomas as 95% of CTX patients show this feature at the time of diagnosis (2). On the other hand, tendon xanthomas, in particular Achilles tendon xanthomas, are common in familial hypercholesterolemia (FH), which is caused by a mutated low-density lipoprotein (LDL) receptor gene. FH patients whose clinical features mimic those of CTX, have been reported (18). In addition, serum cholestanol is increased in FH (19). Thus, it is important to analyze the CYP27A1 gene and the LDL receptor gene to distinguish CTX from FH. We performed genetic analysis of LDL receptor gene in both cases. However, LDL receptor gene showed no abnormal findings.

The neurological disorders observed in CTX patients have been reported to be due to xanthomas in the brain (1). Kinoshita et al found that the multiple xanthomas observed in CTX were induced by increased oxidized LDL and low activity of the cholesteryl ester transfer protein (20). von Bahr et al showed that the CYP27A1 itself was important for the efflux of both cholesterol and cholestanol from xanthomas (21). Recently, it was reported that 27-hydroxycholesterol was a ligand for the nuclear receptor liver X receptor (LXR) (22, 23) and 27-hydroxycholesterol generated in macrophages was identified as an important endogenous ligand for up-regulating cholesterol efflux via the ATP-binding cassette (ABC) transporter pathway (22). ABC transporters have been shown to be responsive to the nuclear

receptor LXR (24). The markedly accelerated development of xanthomas and the premature atherosclerosis observed in patients with CTX can now be explained at least in part by the loss of 27-hydroxycholesterol/LXR-mediated cholesterol efflux from macrophages (23).

In summary, we have performed a mutational analysis of all 9 exons, the promoter, and poly-A of the CYP27A1 gene in two Japanese patients with CTX. One novel (R104Q) and two previously reported missense mutations (R441Q and R441W) were identified in the CYP27A1 gene.

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