SHORT REPORT

Mutation of the sterol 27-hydroxylase gene (*CYP27*) results in truncation of mRNA expressed in leucocytes in a Japanese family with cerebrotendinous xanthomatosis

K Shiga, R Fukuyama, S Kimura, K Nakajima, S Fushiki

Abstract

***Objectives*—A Japanese family with cerebrotendinous xanthomatosis (CTX) was investigated for a sequence alteration in the sterol 27-hydroxylase gene (*CYP27*). The expression of *CYP27* has been mostly explored using cultured fibroblasts, prompting the examination of the transcripts from blood leucocytes as a simple and rapid technique.**

***Methods*—An alteration in *CYP27* of the proband was searched for by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis and subsequent sequencing. Samples of RNA were subjected to reverse transcription PCR (RT-PCR) and the product of the proband was amplified with nested primers and sequenced.**

***Results*—A homozygous G to A transition at the 5' end of intron 7 was detected in the patient. In RT-PCR analysis, only a truncated transcript was detected in the patient, whereas both normal and truncated transcripts were detected in the siblings. The sequencing of the patient's cDNA fragment disclosed a direct conjuction of exon 6 and exon 8.**

***Conclusion*—The mutation at splice donor site and the truncation of mRNA were identical with those of a recently reported Italian patient, although different in symptomatology. The application of blood leucocytes can be a simple technique on analysing a constructive abnormality of *CYP27* mRNA.**

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Keywords: CYP27; cerebrotendinous xanthomatosis; leucocyte; phenotypic heterogeneity

Department of Dynamic Pathology
K Shiga
R Fukuyama
S Fushiki

Department of Neurology
K Shiga
K Nakajima

First Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan
S Kimura

Division of Internal Medicine, Sonobe Tan-ikai Hospital, Kyoto, Japan
S Kimura

Correspondence to:
Dr Shinji Fushiki, Department of Dynamic Pathology, Research Institute for Neurological Diseases and Geriatrics, Kyoto Prefectural University of Medicine, Kawaramachi Hirokoji, Kamigyo-ku, Kyoto 602–8566, Japan. Telephone 0081 75 251 5769; fax 0081 75 251 5849; email: sfushiki@koto.kpu-m.ac.jp

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Cerebrotendinous xanthomatosis (CTX) is an autosomal recessive neurometabolic disease presenting xanthomatosis, juvenile atherosclerosis, and progressive neurological deficits including pyramidal tract signs, cerebellar ataxia, and mental retardation.[1] The pathogenesis is linked to genetic alterations in the mitochondrial sterol 27-hydroxylase gene (*CYP27*) that disrupt hydroxylation of the side chain of cholesterol in bile acid systhesis.[2 3] To date 25 different mutations in *CYP27* have been reported; however no hot spot has been identified. We here examined a Japanese family with CTX for a sequence alteration in *CYP27*. An anticipated splicing defect led us to examine the size and the sequence of its transcript. Although sterol 27-hydroxylase mRNA is detected in various tissues,[4] its expression in leucocytes was not known and abnormality of the *CYP27* transcript has been mainly analysed using cultured fibroblasts.[5] Instead, we here examined *CYP27* transcript using blood leucocytes to establish a simple and rapid technique.

Materials and methods

THE PATIENT AND THE FAMILY

The proband was a 39 year old Japanese man who manifested tendon xanthomatosis, gait ataxia, and progressive mental retardation. The patient's IQ was 52.1 on the Kohs block design test,[6] equivalent to the intelligence of a person aged 8 years and 4 months. Brain MRI showed cortical atrophy. The serum cholestanol concentration was 37.5 μg/ml (control value; 2.35 (SD 0.73) μg/ml). Clinical and laboratory profiles satisfied the diagnostic criteria for CTX.[7] His parents, who are first cousins, his brother and sister, and normal subjects were included for the study.

POLYMERASE CHAIN REACTION (PCR) SINGLE STRANDED CONFORMATIONAL POLYMORPHISM (SSCP) ANALYSIS, DNA SEQUENCING, AND RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) ANALYSIS

Genomic DNA was extracted by standard methods from blood samples. Each exon with its 5'- and 3'- flanking intronic sequences was amplified by PCR with nine sets of primers. The primers for exon 1 to 6 were synthesised as previously reported[5] and primers for exon 7 to 9 were designed based on published sequence data.[2 8] Before electrophoresis, 3 μl of each PCR product were denatured in 20 μl 96% formamide at 80°C for 5 minutes. The reaction solutions were electrophoresed on 10% or 12.5% polyacrylamide gels containing 10%

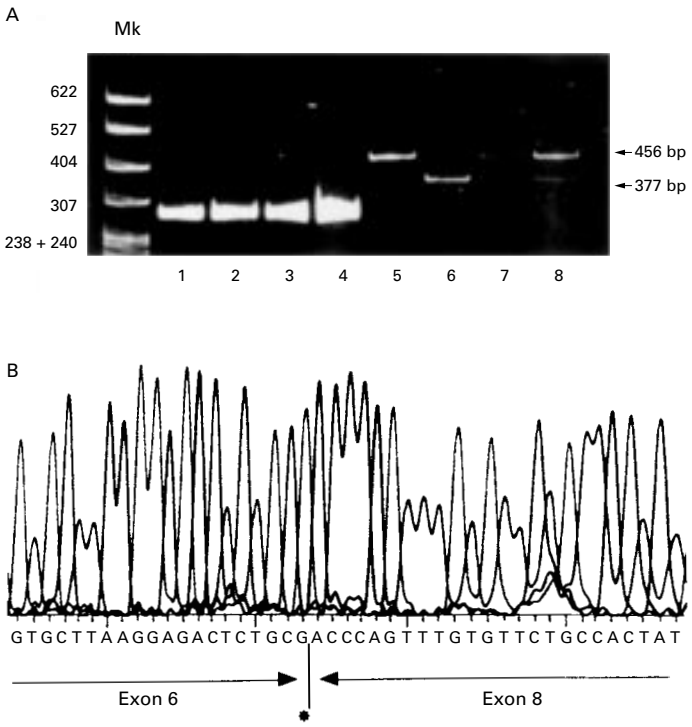

*Figure 2 (A) RT-PCR analysis of β actin gene (298 bp) (lanes 1–4) and CYP27 (lanes 5–8). Lanes 1 and 5; normal control. Lanes 2 and 6; the proband. Lanes 3 and 7; the brother. Lanes 4 and 8; the sister. Mk=size marker. Numbers at the left are sizes of each band in bp. (B) Sequencing results of the patient's transcript. * shows a position where the 3'-end of exon 6 and 5'-end of exon 8 were conjoined.*

glycerol at 10°C. The fixed gels were silver stained. The putative mutant DNA fragment was purified and sequenced. We digested exon 7 containing PCR products of all family members with *Nla III* and analysed the restriction fragment length polymorphism (RFLP).

Reverse transcription PCR analysis of the transcript of CYP27

Total RNA were extracted by standard methods from blood samples. RNA isolated from a liver (a gift from Dr. Muramatsu) was used as a positive control as *CYP27* is highly expressed in liver.[4] The RNA samples were reverse transcribed into cDNA (Reverse Transcription System, Promega, Madison, WI, USA), which were then amplified with the following primers (forward; 5'-AAGCTGTGCTTAAGGAGACT-3', reverse; 5'-AGCAAGGCGGAGACTCAGCT-3'). The transcript from normal subjects would cover a 456 bp cDNA fragment within exon 6 through 9.

Nested PCR and sequencing of the proband transcript

We designed another set of nested primers (forward; 5'-GTGCTTAAGGAGACTCTGCG-3', reverse; 5'-TTGCGAGGAGTAGCTG CATC-3') located within the first RT-PCR product. Nested PCR amplification was performed in a total volume of 25 µl containing 0.5 µl RT-PCR product, 2 µM of each primer, 50 µM dNTP, and 1U Taq DNA polymerase (*Ex* Taq,

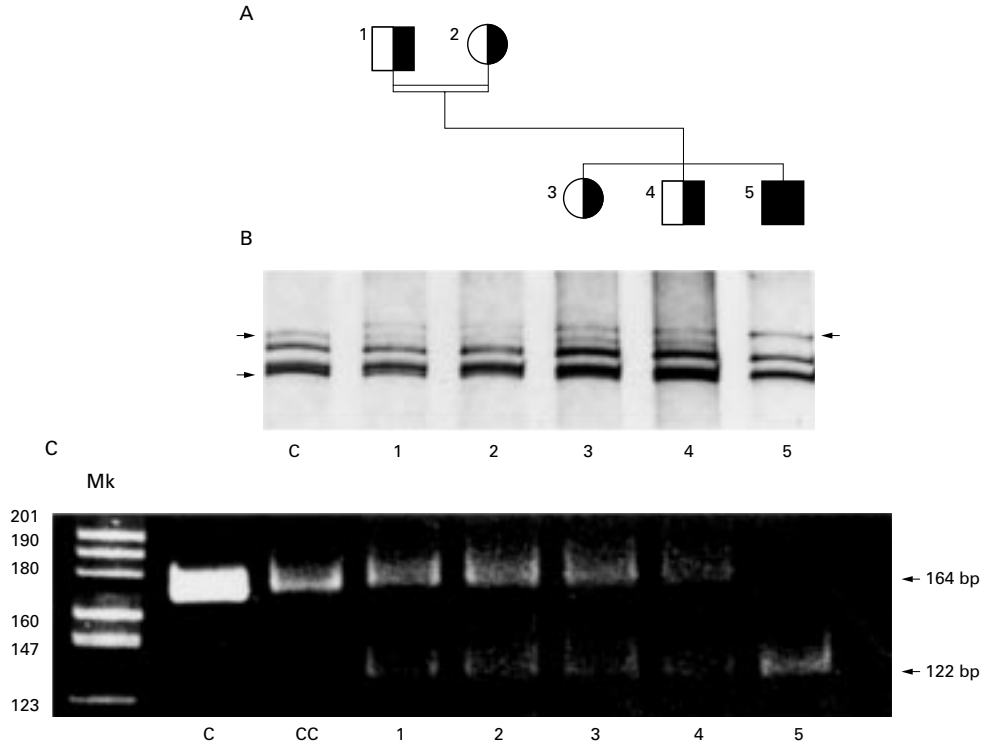

Figure 1 Pedigree structure (A), single strand conformational polymorphism (SSCP) analysis (B), and restriction fragment length polymorphism (RFLP) analysis (C) of the family. Numbers in (A) correspond to those in (B) and (C). (A) The proband is marked with a dark box. Half darkened symbols designate heterozygotes. (B) PCR-SSCP analysis of exon 7 containing DNA fragments. Lane C; normal control; lanes 1–5; members of the family. Small arrows on the left indicate the normal bands and an arrowhead on the right shows the mutant band. (C) RFLP analysis with Nla III of exon 7 containing PCR products . Lane C; normal control DNA before digestion. Lane CC and 1–5, control and the family members' DNA after digestion, respectively. Numbers at the left indicate DNA sizes in bp (MspI digested pBR322). Mk=size marker.

TaKaRa, Tokyo, Japan) with a denaturation step for 5 minutes at 95°C followed by 15 cycles of 95°C for 30 seconds, 58°C for 40 seconds, and 72°C for 30 seconds. The product was then purified and sequenced.

Results

PCR-SSCP analysis on the DNA fragments including exons 1–6, 8, and 9 of the proband showed the same band patterns as the control (data not shown), whereas the exon 7 containing fragment of the patient showed a different migration pattern on gels compared with the control (fig 1 B, lane C and 5). The denatured DNA fragments of his parents and siblings showed both wild type and mutant bands (fig 1 B, lanes 1–4). The sequencing of exon 7 containing PCR product of the patient disclosed a G to A transition at the first nucleotide of the intron 7 (data not shown). This mutation generates a new restriction site for *Nla III*. The PCR products from controls were not digested with *Nla III* (fig 1 C lane CC), with which the patient's fragment was completely digested (fig 1 C lane 5) and digestion of parents' and siblings' DNA showed two bands. In RT-PCR analysis, a cDNA fragment, 456 bp in size, was detected in control leucocytes (fig 2 A, lane 5), although fainter than that in liver (data not shown). A truncated transcript, 377 bp in size (fig 2 A, lane 6), was amplified from the proband RNA, whereas both normal and mutant transcripts were detected in the siblings (fig 2 A, lanes 7 and 8). The sequencing of the patient's transcript resulted in a direct conjunction of exon 6 and exon 8, showing a skipping of exon 7(fig 2 B).

Discussion

A homozygous G to A transition at the first nucleotide of intron 7 was found in this patient. The result of PCR-RFLP analysis indicates that the parents and the siblings are heterozygous for this mutation, confirming the autosomal recessive trait. The sequence GT, the first two nucleotides at a splice donor site, is highly conserved in most eukaryotes[9] and a mutation at this critical position disrupts normal splicing.[10] We here confirmed a skipping of exon 7 in the patient's transcript. A conjunction of exon 6 and exon 8 results in a shift in the reading frame, generating a new sequence of 28 amino acids preceding a premature termination codon in exon 8.[11] This truncation of *CYP27* mRNA results in lack of a heme-binding domain, which is required for the hydroxylase activity. Thus this mutation presumably leads to a disrupted bile acid synthesis. The same mutation has been reported in an Italian patient[11]; his intelligence, however, remained normal, whereas this Japanese counterpart had a progressive mental retardation. The difference in intelligence among another mutation (Arg441Trp) was also reported in Japanese patients with CTX.[12 13] It has recently been shown that *cyp27* deficient mice in which enzymatic activity of sterol 27-hydroxylase is lacking showed no CTX pathology in the CNS.[14] The phenotypic heterogeneity in the same mutations and the lack of CNS pathology in knock out mice suggest that, in addition to the genetic alteration in *CYP27*, other genetic and environmental factors may play a crucial part in the CNS pathogenesis of CTX. To date an expression of *CYP27* has been demonstrated in various tissues including liver and duodenum in rabbits, and has been mostly explored in cultured fibroblasts in humans.[4] We here first demonstrated the expression of *CYP27* in leucocytes, implying that mRNA analysis using blood samples is effective for a rapid diagnosis of CTX.

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