Two new mutations in the sterol 27-hydroxylase gene in two families lead to cerebrotendinous xanthomatosis

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Abstract This report concerns two new mutations in the sterol 27-hydroxylase gene in two patients with cerebrotendinous xanthomatosis (CTX). In a Surinam-Creole patient (patient A), a G deletion on position cDNA 546/547 in exon 3 led to a frameshift and the introduction of a premature termination codon. In a Dutch patient (patient B), a C→T transition at position 496 in exon 3 also led to a premature termination codon. Patient A was homozygous for the mutation, whereas patient B was compound heterozygous, a C→T transition also being found in exon 6 at position 1204. The two new mutations were confirmed by restriction analysis with the restriction enzymes FokI and MaeI, respectively.

Introduction Cerebrotendinous xanthomatosis (CTX; McKusick 213700), first described by van Bogaert et al. in 1937, is a rare, autosomal recessive, lipid-storage disease characterised by abnormal bile acid synthesis caused by a deficiency of the mitochondrial enzyme sterol 27-hydroxylase (CYP 27; Oftebro et al. 1981). This deficiency leads to reduced synthesis of cholic acid and almost no chenodeoxycholic acid (CDCA) is produced. The absence of the negative feedback mechanism of CDCA on 7a-hydroxylase, the rate-limiting enzyme in bile acid synthesis, leads to an excessive production of cholestanol, which accumulates in many tissues (Bjorkhem et al. 1995). In CTX, bile alcohols are produced via 24- and 25-hydroxylation of 5β-cholestan-3α,7α,12α-triol. The biochemical diagnosis is made by determination of bile alcohols in urine and by the determination of the serum cholestanol/cholesterol ratio (CCR; Wolthers et al. 1983).

The clinical characteristics of CTX are premature bilateral cataract, formation of tendon xanthomas and neurological and neuropsychiatric abnormalities, i.e. pyramidal and cerebellar signs, peripheral neuropathy and dementia (Björkhem and Boberg 1995). Although the disease manifests itself in childhood in many patients, the diagnosis of CTX is rarely made before the age of 20. Since early treatment with chenodeoxycholic acid is able to stabilise or even reverse the disease in its initial stages (Berginer et al. 1984, 1994), CTX should be considered as a diagnosis as early as possible.

Andersson et al. (1989) have described the cDNA that encodes rabbit mitochondrial CYP 27, a member of the mitochondrial cytochrome P450 enzyme family. The cDNA for the human CYP 27 has been cloned (Cali et al. 1991) and its gene localised on chromosome 2 (q33-qter) by Cali and Russell (1991). The structure of the CYP 27 gene has been established by Leitersdorf et al. (1993).

To date, 14 different mutations have been described in the CYP 27 gene in CTX patients. This report describes two new mutations found in the CYP 27 gene in two patients with CTX.

Patients

Patient A
This 35-year-old woman presented with Achilles tendon xanthomas at the age of 17. She subsequently developed visual impairment caused by bilateral cataract. Her cognitive function gradually deteriorated; psychiatric disturbances and epilepsy developed. The diagnosis of CTX was made at the age of 33 years by determination of a high concentration of bile alcohols (5β-cholestan-3α,7α,12α,23,25 pentol and 5β-cholestan-3α,7α,12α,24,25 pentol) in her urine. She had four healthy brothers.

Patient B
This 56-year-old woman was diagnosed as having CTX, after 16 years of progressive spastic tetraparesis and severe mental deterio-
ration. No xanthomomas were present. She was operated on for a bilaterial cataract at the age of 34. The urinary bile acid concentration was indicative of CTX and the diagnosis was confirmed by determination of the serum level of cholesterol, which was 70.5 \( \mu \text{mol/l} \) (normal range 3.3–12.5 \( \mu \text{mol/l} \)), and CCR, which was 1.57% (normal range: 0.08%–0.21%). The patient had four brothers, only two of whom could be examined; they did not have CTX.

### Materials and methods

The CCR in serum was measured according to Wolthers et al. (1983), by using capillary gas chromatography on a CP wax 52 CB column (25 m, 0.2 mm internal diameter; Chrompack, Middelburg, The Netherlands). The urinary excretion of bile alcohols was also measured by means of capillary gas chromatography, mainly according to Wolthers et al. (1983).

The CYP 27 gene was amplified in four fragments (exons 1, 2, 3–5 and exons 6–9), by the polymerase chain reaction (PCR) from genomic DNA of leukocytes. Exons 3–9 with their intron boundaries were subsequently amplified separately, with the two PCR fragments 3–5 and 6–9 as a template. Primers used for PCR amplification and sequence analysis of exon 3 were: forward primer 5'-GTGAGCTACTTCTGTGCTGCCCTCTCGC-3'; reverse primer 5'-GAGCACAACCTCTCCTGGACCCATT-3' (Leenders et al. 1993). The amplifiers thus obtained were subjected to single-strand conformation polymorphism (SSCP) analysis by using the Pharmacia Phast System. Running conditions for patient A were: 12.5% polyacrylamide gel, 4°C, 400 V, 5.0 mA, 1.0 W, 325 Vh. Running conditions for patient B were: 20% polyacrylamide gel, 20°C, 400 V, 5.0 mA, 1.0 W, 300 Vh.

Cycle sequencing of the coding and the non-coding strands of exon 3 was carried out by the Taq Dye Deoxy Terminator method using an ABI DNA sequencer (Applied Biosystem type 377). The cDNA sequence and the amino acids were numbered according to Cali and Russell (1991).

The amplifiers of exon 3 of patient A and of her mother were digested with the restriction enzyme FokI, which spliced the normal exon. The amplifier of exon 3 of patient B was digested with the restriction enzyme Mael, which spliced the mutant exon.

### Results

**Patient A**

SSCP analysis of the amplifier of exon 3 showed bands not present in the controls. In the SSCP of the mother, we found the same pattern, combined with that of a normal control amplifier (Fig 1A). The father was unknown.

Direct sequencing of exon 3 of patient A demonstrated a G deletion at position 546 or 547 (wildtype nucleotide sequence: cDNA 541–551 TATACGAGATGC; mutant nucleotide sequence: cDNA 541–550 TATAGCAGATGC). This deletion leads to a frameshift and a premature termination codon TGA at position 563–565 (wildtype nucleotide sequence). We were able to confirm the mutation by means of restriction enzyme analysis with FokI, which spliced the normal exon (Fig 1B). The patient was homozygous for this mutation. The mutation was not found in 59 controls.

**Patient B**

SSCP analysis of the amplifier of exon 3 showed one abnormal band combined with those of a normal control amplifier (Fig 2A). Direct sequencing of the exon 3 demonstrated a C→T transition on position 496 (wildtype nucleotide sequence: cDNA 487–498 CAGCTGCGCCAG; mutant nucleotide sequence: cDNA 487–498 CAGCTGCGCCTAG). This transition changes codon 126 into a premature termination codon. We were able to confirm the mutation by means of restriction enzyme analysis with Mael, which spliced the mutant exon (Fig 2B). The mutation was not found in 59 controls. In this patient, a second mutation, viz. a C→T transition, was found in exon 6 at position 1204 by means of SSCP analysis, direct sequencing and restriction analysis with the enzyme AcI (data not shown).
To date, 14 mutations have been reported in the CYP 27 gene. Eight missense mutations (Kim et al. 1994; Cali et al. 1994; Reshef et al. 1994; Nakashima et al. 1994; Watts et al. 1996; Okuyama et al. 1996), 2 deletions and one insertion leading to a frameshift (Leitersdorf et al. 1993, 1994; Segev et al. 1995), one splice acceptor site mutation (Leitersdorf et al. 1993), one premature termination codon (Meiner et al. 1994) and one partial deletion of the gene (Garuti et al. 1996). Of these 14 mutations, one is in exon 1, three in exon 2, one in exon 4 and one in intron 4, two in exon 5, one in exon 6, one in exon 7 and three in exon 8. The partial deletion of the gene spans the region from intron 6 to the 3' flanking region eliminating exons 7–9 (Garuti et al. 1996).

In this study, two clinically-established CTX cases were investigated for mutations in the CYP 27 gene. DNA from both patients was PCR-amplified with primers targeted at exons 1–9 of this gene. SSCP analysis of exon 3 of patient A and of exons 3 and 6 of patient B showed an abnormal migration pattern. Direct sequencing of these exons demonstrated three mutations.

In exon 3 of patient A, there was a (cDNA) 546/547 G deletion leading to a frameshift with the introduction of 5 different amino acids (Met-Leu-Ser-Met-Arg) and a premature termination codon in exon 3. The patient was homozygous for this mutation.

In exon 3 of patient B, a 496 C→T transition changed codon 126 into a premature termination codon. In this patient, a second mutation, viz. a 1204 C→T transition, was found in exon 6. This missense mutation has been described by Cali et al. (1991). Patient B is therefore probably a compound heterozygote for these mutations.

The conserved parts of the gene are located in the regions of exon 6–8. The expressed enzymes in both mutations therefore presumably lack a stretch of amino acids essential for the function of the enzyme.

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References