Molecular genetics of cerebrotendinous xanthomatosis in Jews of North African origin

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Abstract Cerebrotendinous xanthomatosis (CTX) is an autosomal recessive sterol storage disease characterized by the accumulation of a bile alcohol, cholestanol, in diverse tissues. The disorder is manifested by extensive nervous system involvement, juvenile cataracts, tendon xanthomas, and premature atherosclerosis and is caused by sterol 27-hydroxylase (EC 1.14.13.15) mutations. Recently, two mutations were shown to cause CTX in four Jewish families of Moroccan origin. An additional mutant allele, found in a Jewish family of Algerian origin is characterized here. Sequence analysis revealed a C to T transition at cDNA position 1037 which predicted a threonine to methionine substitution at residue 306 (designated T306M). It is highly suggestive, but not definitive, that this transition is the mutation causing CTX in this family. A search for additional cases from Jewish families of North African extraction identified five new families including 10 cases. The three sterol 27-hydroxylase gene mutations account for all 10 CTX families and their presence may suggest the existence of positive selective forces that lead to an increased prevalence of this relatively rare disease in Jews from North Africa.—**Reshef, A., V. Meiner, V. M. Berginer, and E. Leitersdorf.** Molecular genetics of cerebrotendinous xanthomatosis in Jews of North African origin. *J. Lipid Res.* 1994. **35:** 478–483.

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Cerebrotendinous xanthomatosis (CTX) is an autosomal recessive lipid storage disease manifested clinically by tendon xanthomas, juvenile cataracts, and progressive neurological dysfunction (1). The latter includes pyramidal dysfunction, cerebellar ataxia, and peripheral neuropathy (2). These symptoms often develop during the second and third decades of life and become more severe with increasing age leading to profound incapacitation. Epileptic seizures may develop and psychiatric manifestations may precede the onset of other neurological characteristics (2). The slowly progressive nature of the disease and the non-uniformity of the clinical manifestations even within each CTX family may preclude clinical diagnosis at an early stage.

The major symptoms in CTX are due to the generalized accumulation of cholestanol, a 5α-dihydro derivative of cholesterol, in diverse tissues (2). It has been shown that deficient activity of the mitochondrial sterol 27-hydroxylase results in depleted bile acid stores leading to increased cholestanol synthesis via alternative pathways (1). Specific treatment with chenodeoxycholic acid (CDCA) may prevent disease progression concomitantly with lowering cholestanol levels and is sometimes associated with reversal of some of the neurological disabilities (3).

CTX cases reported thus far originate from Japan, the United States, Israel, and The Netherlands (1). Several CTX families were reported in Jews originating from Morocco and therefore a founder mechanism was suggested (4). Following the molecular cloning of the human sterol 27-hydroxylase complementary DNA (5) and characterization of the gene structure (6), two distinct mutations were identified in Jews of Moroccan origin (6). An extended search for CTX in Sephardic Jews of North African origin revealed the presence of six families with 13 cases and a novel sterol 27-hydroxylase gene mutation reported here.

METHODS

Analysis of the sterol 27-hydroxylase gene mutant allele in a Jewish family of Algerian origin

The structure of pedigree CTX #202 has been previously reported (7). Skin biopsies were obtained from CTX patient #202-4, fibroblast cultures were established, and total cellular RNA was extracted in 4 M guanidine thiocyanate. The RNA was denatured in 3 M glyoxal, subjected to electrophoresis on 1.6% agarose gel (8), transferred to a nylon-based membrane (Biotrans Nylon Membrane, ICN), and hybridized with an [α-^{32}P]dCTP-

Abbreviations: CTX, cerebrotendinous xanthomatosis; CDCA, chenodeoxycholic acid; PCR, polymerase chain reaction; SSCP, single-strand conformational polymorphism.

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labeled full-length human sterol 27-hydroxylase cDNA probe (9). Southern blotting analysis was performed as described (10) using genomic DNA that had been extracted from blood leukocytes obtained from CTX patient #202-4 and a normal control, digested with *Bam*HI restriction endonuclease, and probed with an identical probe (**Fig. 1**). To identify the location of the mutation, PCR amplification (11) of genomic DNA obtained from peripheral blood leukocytes and SSCP (single-strand conformational polymorphism) analysis (12) were used. All exons and the 5' flanking region of the sterol 27-hydroxylase gene were amplified as described (6). The PCR reactions included 10 μCi of [α-^{32}P]dCTP. The PCR products of the 5' region of the gene and exons 6–9 were digested with *Hae*II and *Ava*II restriction endonucleases, respectively, prior to analysis on a 6% nondenaturing polyacrylamide gel containing 10% (V/V) glycerol. Following the identification of an abnormally migrating band in exon 5 it was re-amplified using flanking oligonucleotides 5a (5'-GCTCTTGGTCCTTGGAGA TCATGAC-3') and 5b (5'-ACTGGTTACGGTTGGGAG CTGGGGG-3') (**Fig. 2**). The PCR product was subjected to electrophoresis on a low gelling SeaPlaque^{R} agarose (FMC, Bio Products). The band corresponding to exon 5 was excised and both strands were directly sequenced using oligonucleotide primers 5a and 5b and a Sequenase^{R} version 2 kit (United States Biochemicals, La Jolla, CA).

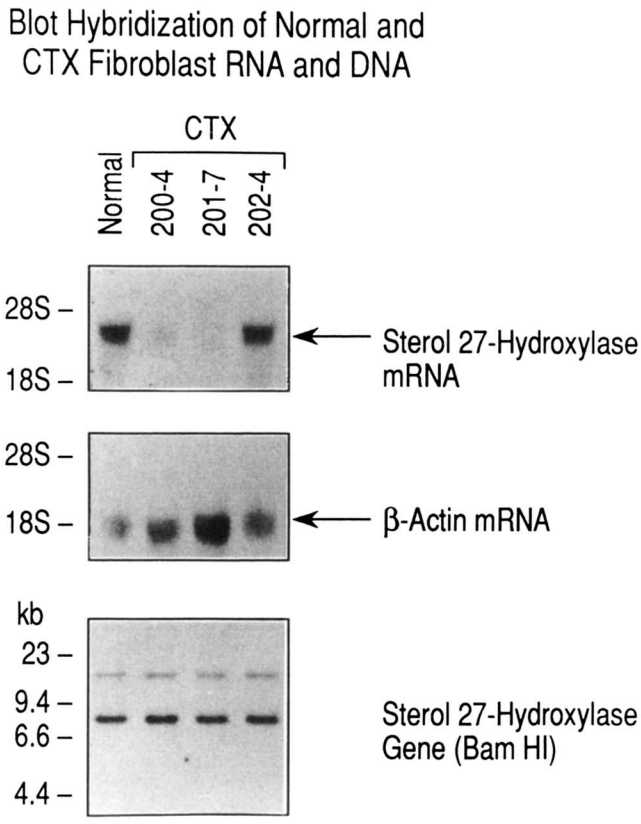


Fig. 1. Blot hybridization of CTX fibroblast RNA and DNA. Total cellular RNA was prepared from fibroblast cultures and genomic DNA from blood leukocytes. RNA and Southern blotting analysis were performed and the nylon membranes were probed with a full-length ^{32}P-labeled human sterol 27-hydroxylase cDNA probe. For comparative analysis, the RNA blot was also probed with a β actin cDNA probe. Lane 1, control DNA; lane 2, CTX #200-4 (CTX case of Jewish-Kurdish origin); lane 3, CTX #201-7 (reference 6)); lane 4, CTX #202-4.

Recruitment of CTX families of North African extraction

After the identification of three mutations causing CTX in Jews of North African origin, a search for additional cases was conducted through individual patient files from the Departments of Ophthalmology and Human Genetics of the Hadassah University Hospital in Jerusalem. Inclusion criteria consisted of known CTX clinical features and characteristic Jewish-Sephardic surnames. Detailed interviews of the families provided demographic and genealogical data. For each patient and family member a fasting blood sample was collected in 0.15% (W/V) EDTA for biochemical analysis and genomic DNA extraction.

Screening for sterol 27-hydroxylase gene mutations

As the three known mutations are mapped to exon 4, intron 4, and exon 5, screening of new CTX families was carried out by PCR-SSCP analysis using oligonucleotides that flank these regions. For the detection of exon 4 mutation (deletion of thymidine) oligonucleotides 4a and 4b were used as described (6); for intron 4 (guanosine to adenosine transition at the 3' splice acceptor site) and exon 5 (reported here) mutations oligonucleotides 5a and 5b (shown above) were used. For each PCR-SSCP analysis a positive control carrying the corresponding mutation was included. Positive identification of a mutant allele required complete homology to the positive control. Compound heterozygotes were identified according to the presence of a combined band pattern comprised of that of the positive control and the normal control for each mutant allele. Verification of the existence of two mutant alleles in a compound heterozygote was also done by PCR and restriction analysis (6).

Biochemical analysis

Plasma total triglyceride, cholesterol, and HDL-cholesterol levels were determined on fasting blood samples using commercially available diagnostic kits (Boehringer Mannheim, Germany). Plasma LDL-cholesterol levels were calculated according to the Friedewald, Levy, and Fredrickson formula (13). Plasma cholestanol levels were determined by the gas chromatographic method (14).

RESULTS

The clinical manifestations of affected members from families CTX #201, CTX #203, CTX #204, and CTX

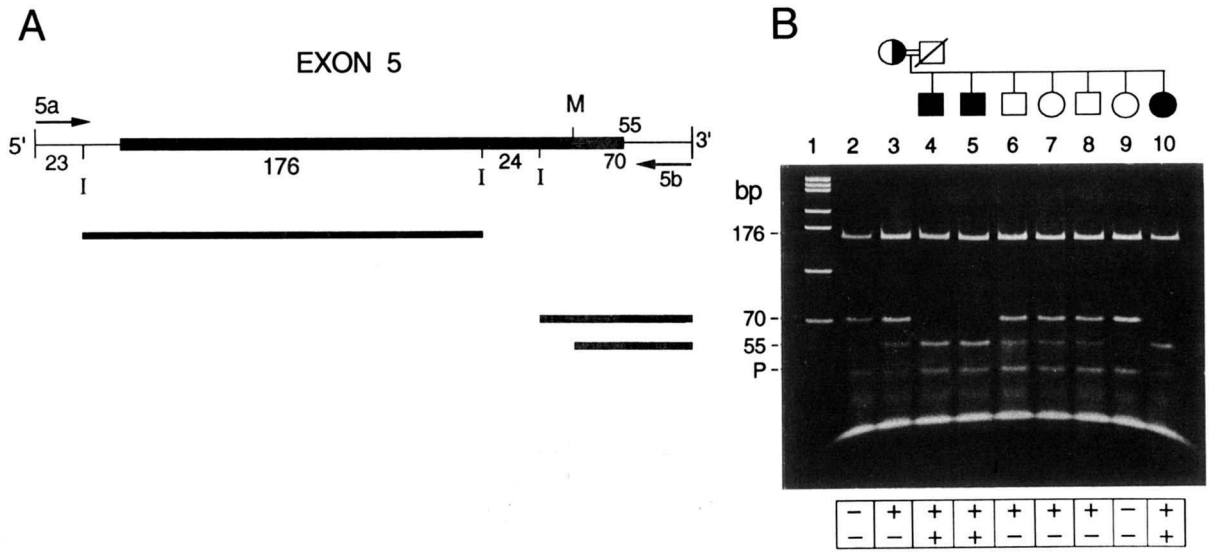


Fig. 2. Detection of the sterol 27-hydroxylase mutation in family CTX #202. A: Oligonucleotides 5a and 5b, homologous to intron sequences flanking exon 5 are shown. The invariable *Nla*III sites (I) are shown below, the *Nla*III site created by the mutation (M) is shown above the map of exon 5, and the exact fragment sizes are indicated. B: The structure of pedigree CTX #202 is shown. Full darkened pedigree symbols indicate clinically diagnosed CTX patients. The mother who is an obligate heterozygote has a half darkened pedigree symbol. The results of the *Nla*III-digested PCR fragments, size fractionated on a 6% polyacrylamide gel and stained with ethidium bromide, for a control DNA sample and for each individual are shown. Lane 1: Phi-X DNA digested with *Hae*III; lane 2: control DNA; lanes 3–10: all available individuals in family CTX #202 [lane 4 (index case), CTX #202-4; lane 5, CTX #202-5; lane 10, CTX #202-10]. The calculated fragment sizes (bp) for the PCR products are shown. Fragment P is a PCR artifact. The interpretation of the results (genotypes) is shown below the photograph of the ethidium stained gel: −/− = normal; +/− = heterozygote; +/+ = homozygote.

#206 were previously described (6). The clinical characteristics of the three CTX patients in family #202 are shown in **Table 1.** It is evident that although the three patients have an identical mutation and typical clinical characteristics of the disease, some manifestations differ. The lack of tendon xanthomas and cataracts in CTX patient #202-10, who is 18 years old, is unusual. In addition, plasma LDL cholesterol levels that are usually low in CTX are normal or elevated in these three patients. RNA and Southern blotting analysis revealed a normal sized mRNA and no gene rearrangement (Fig. 1). Screening the nine exons of the sterol 27-hydroxylase gene using SSCP revealed a band shift in exon 5. Sequence analysis revealed a C to T transition at cDNA position 1037 which predicted a threonine to methionine substitution at residue 306 (designated T306M) of the sterol 27-hydroxylase (data not shown). The mutation creates an *Nla*III restriction site. To verify the existence of the mutation in members of family CTX #202, PCR amplification and restriction analysis was performed (Fig. 2). The mother and three children were proven to be heterozygous and the three CTX patients homozygous for the mutation. This mutation was not detected in CTX cases of other origins including a Caucasian American, French, Druze, Japanese, Dutch, and South African analyzed in our laboratory.

Screening of ten affected members from five additional Jewish families of North African extraction suspected of having CTX was carried out. Each family was traced back to its origin (**Fig. 3**). PCR-SSCP analysis revealed that each one of the mutant alleles corresponded to one of the three known mutations. Using the combined information including the place of origin and the molecular characterization of distinct mutations, a map was designed showing the clusters of mutant alleles in North Africa (Fig. 3). Seventy eight percent (11/14) of the mutant alleles shown in the map originate from Morocco and the rest from Algeria and Tunisia combined. This finding is in accordance with the relative number of individuals originating from these regions that currently live in Israel [499.9 thousand Moroccans (79.8%) and 126.5 thousand Algerians and Tunisians combined (20.2%)] (15).

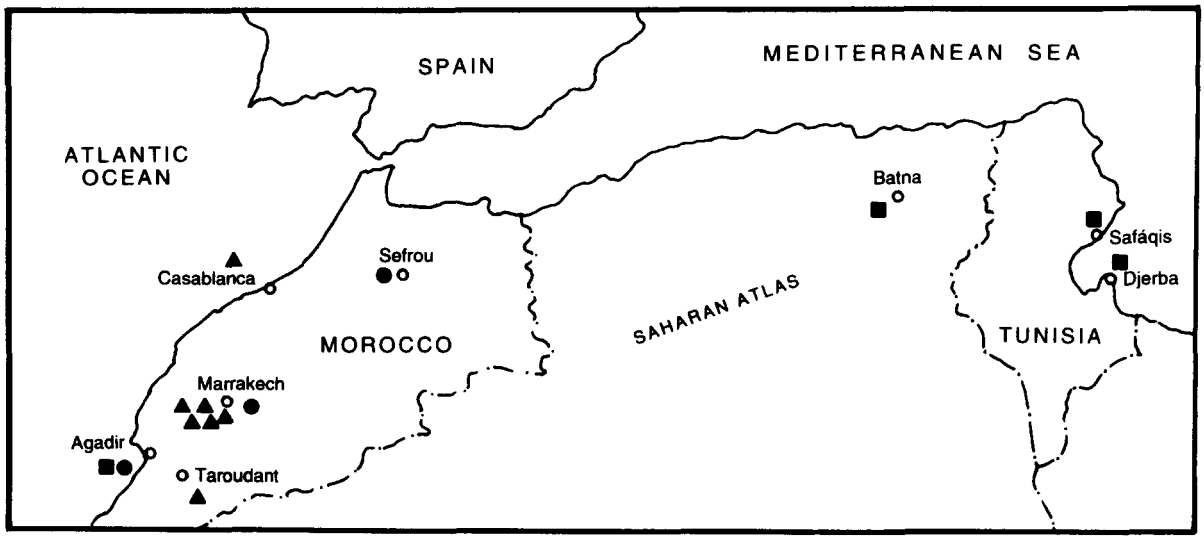
DISCUSSION

Here we show that three distinct sterol 27-hydroxylase gene mutations cause CTX in Jews of North African ori-

TABLE 1. Clinical and laboratory data of the CTX patients in family #202

Variable	#202-4	#202-5	#202-10
Background data			
Sex	M	M	F
Age (years)	35	32	18
Physical findings			
Myopathic facial expression	+ + +	+ + +	+ +
Pes cavus	+ + +	+ + +	+ +
Tendon xanthomas	+ + +	+	–
Cataracts	+ + +	+ + +	–
Dementia	+ +	+ +	+ +
Pyramidal signs	+ + +	+ + +	+
Cerebellar signs	+ +	+ + +	–
Convulsions	–	–	+ +
Neurological studies			
EEG abnormality[a]	+ + +	+ + +	+ + +
Diffuse brain atrophy[b]	+ + +	+ + +	+
Plasma lipids and lipoproteins			
Cholesterol mg/dl	260	196	212
Cholestanol mg/dl[c]	2.8	3.2	3.0
Triglyceride mg/dl	226	96	72
HDL-cholesterol mg/dl	42	32	38
LDL-cholesterol mg/dl[d]	173	145	160

Values (+ + +) to (–) denote severe to absent.
[a]Irregular diffuse slow activity with periodical sharp waves discharges.
[b]Confirmed by magnetic resonance imaging and computerized tomography.
[c]Normal level < 1 mg/dl.
[d]Calculated as described (13).



Family number (cases)	Consanguinity	Origin	Mutant allele(s)
201 (2)	+	Taroudant	▲
202 (3)	+	Batna	■
203 (1)	+	Agadir	●
204 (1)	–	Marrakech	▲●
206 (1)	+	Sefrou	●
210 (2)	+	Casablanca	▲
211 (2)	+	Marrakech	▲
212 (3)	–	Agadir, Marrakech	■▲
213 (2)	–	Marrakech	▲▲
214 (1)	–	Djerba/Safáqis	■■

▲ = 3' - SPLICE JUNCTION MUTATION - INTRON 4
■ = MISSENSE MUTATION (T306M) - EXON 5
● = DELETION OF THYMIDINE - EXON 4

Fig. 3. Distribution of mutant sterol 27-hydroxylase alleles in North Africa. The demographic characteristics of 10 Jewish CTX families originating from North Africa are presented. The exact location of each one of the three mutant alleles is shown on the map. For each consanguineous family, only a single mutant allele is counted, therefore a total of 14 independent alleles are shown for the 10 CTX families.

gin. The relative frequency of these three mutations as estimated from the 10 CTX families reported here suggests that the intron 4 3' splice-junction mutation is the most common (7/14, 50% of the mutant alleles). In addition, it is evident that these mutant alleles are not evenly distributed in North Africa and there is a predilection for Morocco (11/14, 79%).

We describe a newly recognized missense mutation in the sterol 27-hydroxylase gene that results in a methionine for threonine substitution. This mutation is the only one identified after SSCP analysis of the gene and has been shown to co-segregate with the CTX phenotype in three unrelated families. The mechanism by which this amino acid change may account for abnormal enzymatic activity is unclear as no information is yet available on the tertiary structure of the protein. Two functional domains of the protein, including the heme and the ferredoxin binding sites are known (16). It remains to be shown whether there are additional functional domains or whether the amino acid substitution described here interacts with the known domains at the tertiary structure level. Definitive proof that the mutation described here is responsible for the CTX phenotype awaits in vitro expression studies.

The phenotypic expression of mutations in the sterol 27-hydroxylase gene vary between families and even within families some of the clinical manifestations differ (6). Here we show that the missense mutation (T306M) in exon 5 follows a similar intra-familial heterogeneity as demonstrated in Table 1. In accordance with this pattern, CTX patient #202-5 (age 32 years) has less prominent tendon xanthomas as compared to his sibling CTX #202-4 (age 35 years). The paucity of symptoms in the youngest affected individual (CTX #202-10) could reflect an earlier stage of the disease. Plasma LDL cholesterol concentrations were shown to be low in CTX probably due to up-regulation of LDL receptors (17). Here we demonstrate normal or elevated cholesterol levels in our three patients. Recently, in several newly diagnosed CTX families, no correlation could be demonstrated between plasma LDL cholesterol concentrations and the CTX genotypes (data not shown). This finding may be related to other major genes or environmental factors that control plasma LDL cholesterol.

The results of the molecular analysis of sterol 27-hydroxylase gene mutations causing CTX in Jews from North Africa may shed more light on possible mechanisms related to the increased prevalence of the disease in this subset of the Jewish Sephardic population. Founder mechanisms with or without genetic drift and selective processes possibly determine the frequency of any genetic disease in distinct populations. The founder mechanism operates when an isolated community that carries, by chance, an increased proportion of a specific mutation expands rapidly. Genetic drift is another chance phenomenon that occurs in the initial population when in some large families a larger proportion of individuals than expected carry the mutant gene. Although founder populations usually remain confined to a specific geographical location (18) in some cases satellite distribution of a founder mutation had been demonstrated (19). In the North African Jews five different genotypes including two compound heterozygotes are responsible for the CTX phenotype. We show that a single mutant allele (intron 4-3' splice-junction) accounts for 50% of the mutant chromosomes and is found in Morocco. The second most common mutation (T306M) accounts for 29% (4/14) and is found mainly in Tunisia and Algeria but also in Morocco. This observation may be compatible with a founder mechanism with a unique mutation in each one of these relatively isolated Jewish populations. The relative frequency of the three mutations in compound heterozygotes should reflect the prevalence of these mutant alleles in the North African population. Although the possibility of a founder mechanism is intriguing, the possibility of selection should not be ruled out especially because three mutations were found. The increased frequency of these mutant chromosomes may be maintained by compensating advantage to heterozygotes.

A tendency for the aggregation of inherited disorders of a similar nature is also possible. In accordance with this notion, congenital adrenal hyperplasia caused by mutations in the steroid 11β-hydroxylase gene is also relatively frequent among Jews originating from North Africa (20). This enzyme shares some molecular characteristics with the sterol 27-hydroxylase, as both are cytochrome P450 genes (21, 22).

It cannot be ruled out that the observed increased frequency of CTX in Jews originating from Morocco as compared to Algeria and Tunisia merely reflects the relative representation of these Jewish communities in the Israeli population today (15). It is therefore possible that CTX is common in both populations and it remains to be shown whether it is confined only to Jews or may be found in other ethnic groups currently residing in North Africa.

It has recently been suggested that molecular analysis of gene haplotypes may provide additional insight as to the mechanisms of proliferation of mutant genes in isolated populations (23). When several mutations are associated with a distinct gene haplotype, selection is an attractive explanation. When the mutations are associated with diverse haplotypes, other mechanisms including the founder effect are suggested. Molecular characterization of polymorphic sites in the sterol 27-hydroxylase gene are currently underway and may provide additional insight as to the mechanism of proliferation of mutant sterol 27-hydroxylase alleles in the Jewish population originating from North Africa.

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