Am. J. Hum. Genet. 55:907–915, 1994

Cerebrotendinous Xanthomatosis in the Israeli Druze: Molecular Genetics and Phenotypic Characteristics

Eran Leitersdorf,[1] Rifaat Safadi,[1] Vardiella Meiner,[1] Ayeleth Reshef,[1] Ingemar Björkhem,[3] Yechiel Friedlander,[2] Siman Morkos,[4] and Vladimir M. Berginer[5]

[1]Division of Medicine and The Center for Research, Prevention and Treatment of Atherosclerosis, Hadassah University Hospital, and [2]Department of Social Medicine, Faculty of Medicine, Hebrew University—Hadassah School of Public Health, Jerusalem; [3]Department of Clinical Chemistry, Huddinge University Hospital, Karolinska Institutet, Huddinge, Sweden; [4]Kupat Holim Klalit, Yarka, Western Galilee, Israel; and [5]Department of Neurology, Soroka Medical Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheba, Israel

Summary

Cerebrotendinous xanthomatosis (CTX) is an autosomal recessive lipid-storage disease caused by mutations in the sterol 27 hydroxylase gene (CYP27). Clinically, a multitude of neurological, skeletal, and vascular manifestations are usually present. Premature atherosclerosis has been reported in CTX and may be related to the metabolic derangement caused by the deficiency of the enzyme. A CYP27 nonsense mutation created by the deletion of cytosine$_{376}$ has been identified in four Israeli Druze CTX patients residing in the same village. Molecular screening for this mutation in families of two probands revealed a total of 10 homozygotes and 28 heterozygotes whose clinical and biochemical characteristics are described. Overall, except for tendon xanthomas, most of the clinical manifestations progress with age. The CYP27 mutation was associated with modest differences in the levels of plasma total cholesterol (TC) and LDL cholesterol (LDL-C). The distribution of plasma concentrations of TC and LDL-C in the CTX families was consistent with a polygenic model. A similar model that includes also the effects of the CYP27 genotypes was not better supported by the data. It may be concluded that, in CTX, the presence of a CYP27 mutation does not significantly affect the plasma concentrations of lipids and lipoproteins. Therefore, the reported increased prevalence of atherosclerosis in this disease must be related to other factors.

Introduction

Cerebrotendinous xanthomatosis (CTX) is an autosomal recessive lipid storage disease with multiorgan involvement. The clinical manifestations usually develop during the 2d decade of life and include diverse neurological manifestations with pyramidal weakness, cerebellar signs, peripheral neuropathy, and cognitive deterioration. Juvenile cataracts, recurrent bone fractures, tendon xanthomas, and atherosclerosis are also evident (Björkhem and Skrede 1989; Berginer et al. 1993).

CTX is caused by mutations in the sterol 27 hydroxylase gene (*CYP27*). Cloning of the cDNA for the human gene (Cali and Russell 1991) was followed by identification of two sporadic mutations (Cali et al. 1991). Determination of the gene structure facilitated the molecular identification of additional mutations (Leitersdorf et al. 1993; Meiner et al. 1994*a*, 1994*b*; Reshef et al., 1994). Although it is now evident that CTX is more common in distinct populations, the mechanisms that underlie its proliferation are unknown. It has been speculated that founder-gene effect may be responsible for its increased prevalence, but the presence, for example, of three different mutations causing CTX in the Jewish Moroccan population (Leitersdorf et al. 1993; Reshef et al. 1994) argues against the exclusivity of this possibility. It is therefore suggested that selective forces may be responsible for the proliferation of mutant genes at least in the Jewish Moroccan group.

Recently, CTX patients were clinically diagnosed in Yarka, a Druze village located in the Western Galilee region of northern Israel (Ben-Dror et al. 1988). The Druze is a small Middle Eastern Arab sect characterized by an eclectic system of doctrines and by a cohesion and loyalty among its members that have enabled them to maintain their identity and distinctive faith through almost 1,000 years (Goetz 1986). In this community consanguineous marriages are usually encouraged by tradition and therefore are very common. Molecular genetic analysis of CTX in this highly inbred group was the first objective of the current investigation.

Accelerated development of atherosclerosis may be one of the important clinical manifestations of CTX. In a review of 144 CTX cases, 10.4% had cardiovascular disease (Fujiyama et al. 1991). The mechanism underlying atherogenesis in CTX is far from understood. Plasma LDL cholesterol (LDL-C) levels were usually found to be normal or low in sporadic CTX cases (Björkhem and Skrede 1989). Interestingly, both the production rate and the fractional

Received April 26, 1994; accepted for publication July 7, 1994.

Address for address and reprints: Dr. Eran Leitersdorf, Director, Center for Research, Prevention and Treatment of Atherosclerosis, Division of Medicine, Hadassah University Hospital, 91120 Jerusalem, Israel.

0002-9297/94/5505-0008$02.00

catabolic rates of VLDL and LDL were found to be increased in a small number of CTX cases, possibly because of up-regulation of LDL receptors (Ballantyne et al. 1987; Tint et al. 1989). A similar metabolic response has been observed after interruption of the bile-acid enterohepatic circulation (Beil et al. 1982). In a recent study, Kuriyama et al. (1991) determined plasma lipids and lipoprotein concentrations in 8 clinically diagnosed CTX patients and in 12 controls who had other neurological disorders. Plasma LDL-C concentrations were found to be significantly ($P<.05$) lower in the CTX cases (89.9 mg/dl), as compared with those in controls (121.2 mg/dl). In addition, plasma HDL_2 levels were found to be higher, and plasma triglyceride levels lower, in the CTX cases. Four cases examined by Fujiyama and his co-workers had clinical and/or angiographic evidence for coronary-artery disease (CAD). These findings differ from those reported in a study by Shore et al. (1981), where low levels of plasma HDL were found in CTX and where a possible role in storage of sterols has been suggested. Fujiyama et al. (1991) concluded in their study that the lipoprotein profile of CTX cases is "anti atherogenic" and therefore that other parameters are likely to be responsible for atherogenesis in this disease.

All studies reported so far included only clinically diagnosed CTX cases, and therefore it was impossible to examine the impact that heterozygosity or homozygosity for a mutant *CYP27* allele has on plasma lipids and lipoproteins in CTX. The second objective of the current investigation was therefore focused on this issue, and the investigation used clinical, biochemical, and molecular data obtained from the highly inbred Druze population residing in a single village in Israel.

Patients and Methods

Patients

CTX index cases were from Druze families CTX 220 and CTX 221, which reside in the village of Yarka in the Galilee region of Israel. The diagnosis was verified according to the defined clinical and laboratory criteria (Björkhem and Skrede 1989; Berginer et al. 1993). Detailed interviews excluded possible relationship between the two large families, and further clinical evaluation, including complete physical examinations, of all available members of these two kindreds was performed. In addition, two recently diagnosed CTX patients, 222-1 and 223-1, and 34 Druze individuals who permanently reside in two local institutions for the handicapped (Beit Adnaan and Maon Dror) and who do not have a defined clinical diagnosis (such as the fragile X or Down syndrome) were examined. The study was approved by the Committee on Research Involving Human Subjects of The Hadassah University Hospital—Hadassah Medical School, Jerusalem, by the local authorities of the two institutions and by the Israeli Ministry of Labor.

Biochemical Analyses

Plasma total triglyceride (TG), total cholesterol (TC), and HDL cholesterol (HDL-C) levels were determined on fasting blood samples by using commercially available diagnostic kits (Boehringer Mannheim). Plasma LDL-C levels were calculated according to the Friedewald-Levy formula (Friedewald et al. 1972). Plasma cholestanol levels were determined by isotope-dilution mass spectrometry using deuterated cholestanol as an internal standard, a modification of the method described by Seyama et al. (1976).

RNA and DNA Mutation Analysis

Skin biopsy was obtained from a 41-year-old CTX patient (221 V-5) described elsewhere (Ben-Dror et al. 1988); fibroblast cultures were established; and total cellular RNA was extracted in 4 M guanidine thiocyanate (Chirgwin et al. 1979). The RNA was denatured in 3 M glyoxal, subjected to electrophoresis on 1.6% agarose gel (McMaster and Carmichael 1977), transferred to a nylon-based membrane (Biotrans nylon membrane; ICN), and hybridized with an [α-^{32}P]dCTP-labeled full-length human *CYP27* cDNA probe (Church and Gilbert 1984). Southern blot analysis was performed as described above, with genomic DNA extracted from blood leukocytes of the CTX patient and probed with an identical probe.

Analysis of the Sterol 27-Hydroxylase Gene Mutant Allele in CTX 221 V-5

The 5′ UTR, the splice junctions, and the coding region of the sterol 27-hydroxylase gene were amplified using PCR (Saiki et al. 1988) followed by SSCP analysis (Orita et al. 1989) as described elsewhere (Leitersdorf et al. 1993). In the analysis of exon 2, a band shift was detected in the mutant gene. This exon was reamplified using oligonucleotide primers 2aL (5′-*ATA*GGATCCTGGCCCAGTTATTCAGTTTTGATTG-3′) and 2bL (5′-*ATA*GCATGCGGGCCCTGTTCCAGTCCCTTCAGGC-3′). These primers are homologous to flanking intron sequences and include also 5′ extensions consisting of three irrelevant nucleotides (italicized) and six nucleotides (underlined) with the recognition sequence of *Bam*HI and *Sph*I restriction endonucleases, respectively. The PCR-amplified product was analyzed on a 1.5% agarose gel, and the appropriate band was excised, purified, digested with the respective restriction enzymes, and subcloned into the polylinker site of a bacteriophage M13 vector. Sequence analysis was performed using a Sequenase version II kit (United States Biochemicals) according to the dideoxynucleotide chain-termination method (Sanger et al. 1977).

Screening for the Druze Mutation

The newly characterized mutation abolishes the recognition sequence of the restriction endonuclease *Fnu*4HI, and therefore PCR and restriction analysis were used for

its detection. Exon 2 was amplified using oligonucleotides 2a and 2b (Leitersdorf et al. 1993). In the amplification reaction 10 μCi of [α-^{32}P]dCTP were included. After PCR, the DNA was digested by *Fnu*4HI, fractionated on a 6% polyacrylamide gel, and autoradiographed for 1 h at room temperature.

Statistical Analysis

The lipid and lipoprotein data were first adjusted for the effects of age and sex, throughout stepwise multiple regression. Each phenotype was modeled as a function of sex, age, age^2, age^3, sex × age, sex × age^2, and sex × age^3. Only significant terms were retained. The appropriate estimated partial regression coefficients were used to adjust the dependent variables for each individual. The association of the *CYP27* genotypes with cholestanol, lipid levels, and lipoprotein levels was tested by a one-way analysis of variance. This method was applied as a first approach to investigate the effects of the *CYP27* genotypes, although individuals within pedigrees could not be considered as independent.

The admixture analysis and the mixed model of segregation analysis (Morton and MacLean 1974; Lalouel et al. 1983) were then used to test for the involvement that major loci have in TC and LDL-C variability. This model incorporates the following independent sources of variation: a major locus with a large effect; polygenes, each with a small effect; and a random nontransmissible factor. Likelihoods of various genetic models were computed using PAP (Hasstedt and Cartwright 1981), and the maximum-likelihood parameter estimates were obtained with GEMINI (Lalouel 1979). For the mixed model, an approximation of the exact likelihood was employed. Different subhypotheses were tested with the likelihood-ratio criterion. The test of hypotheses done during this segregation analysis clearly indicated that no major gene is involved in the variability of these lipid variables. Therefore, for the present application, the data consisted of a quantitative trait; a two-allele measured marker, i.e., the *CYP27* genotypes; and polygenes. The objectives were to assess the separate effect of *CYP27* genetic polymorphism on TC and LDL-C levels and to estimate in these lipid variables the fraction of genetic variation that may be attributable to the genetic variation in *CYP27* genotypes.

Results

Blot analysis of RNA extracted from CTX 221 V-5 fibroblasts revealed the absence of 27-hydroxylase mRNA, and Southern blot analysis failed to show major gene rearrangement. SSCP analysis of the 5′ UTR of the gene and all exons and intronic flanking sequences revealed an abnormally migrating band only in the region of exon 2 (data not shown).

Sequence analysis of the *CYP27* mutant allele in CTX 221 V-5 revealed a deletion of cytosine$_{376}$ (fig. 1) that leads to a frameshift with an expected premature termination codon at residue number 109. This mutation abolishes a recognition site for *Fnu*4HI. PCR amplification followed by *Fnu*4HI restriction analysis (fig. 2) was then used for screening of genomic DNA samples obtained from the 67 study participants. A total of 10 homozygotes, 28 heterozygotes, and 29 normals were found. Although no CTX case was identified in the two institutions for the handicapped, one heterozygote was detected. The pedigree structures of CTX families 220 and 221 are shown in figure 3, which also includes the information obtained from the molecular screening. The clinical characteristics of the homozygotes are given in table 1, and the results of the lipid and lipoprotein analysis of all study participants are reported in the Appendix. We have not identified any individual with clinical characteristics of CTX who was not homozygous for the mutation.

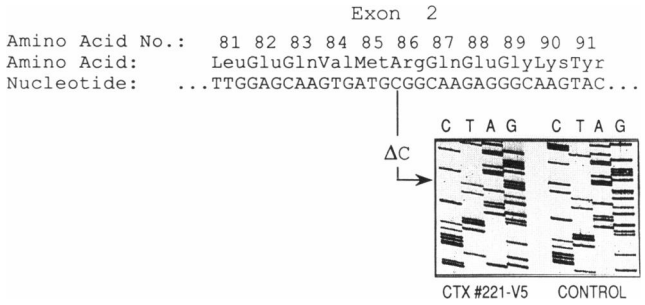

Figure 1 Sequence analysis of the *CYP27* mutation in CTX 221 V-5. Deletion of a cytosine is evident in exon 2 of the mutant gene.

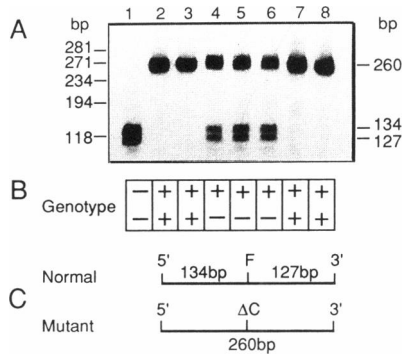

Figure 2 Detection of the Druze mutation. *A*, Results of the [α-^{32}P]dCTP-labeled PCR and *Fnu*4HI restriction analysis of a control DNA (lane 1) and DNA from individuals 221 V-5, 220 III-9, 220 III-4, 220 III-3, 220 IV-5, 220 IV-6, and 220 IV-7 (lanes 2–8). The position of migration of the molecular-weight standards (ϕX174 RF digested with *Hae*III) are shown on the left-hand side of the autoradiograph, and the calculated size for the different fragments on the right-hand side. *B*, Genotypes derived from the analysis shown in panel *A*. −/− = Normal; +/− = heterozygote for the mutation; and +/+ = homozygote for the mutation. *C*, Map of the PCR-amplified region, and fragments that result from *Fnu*4HI (F) restriction analysis.

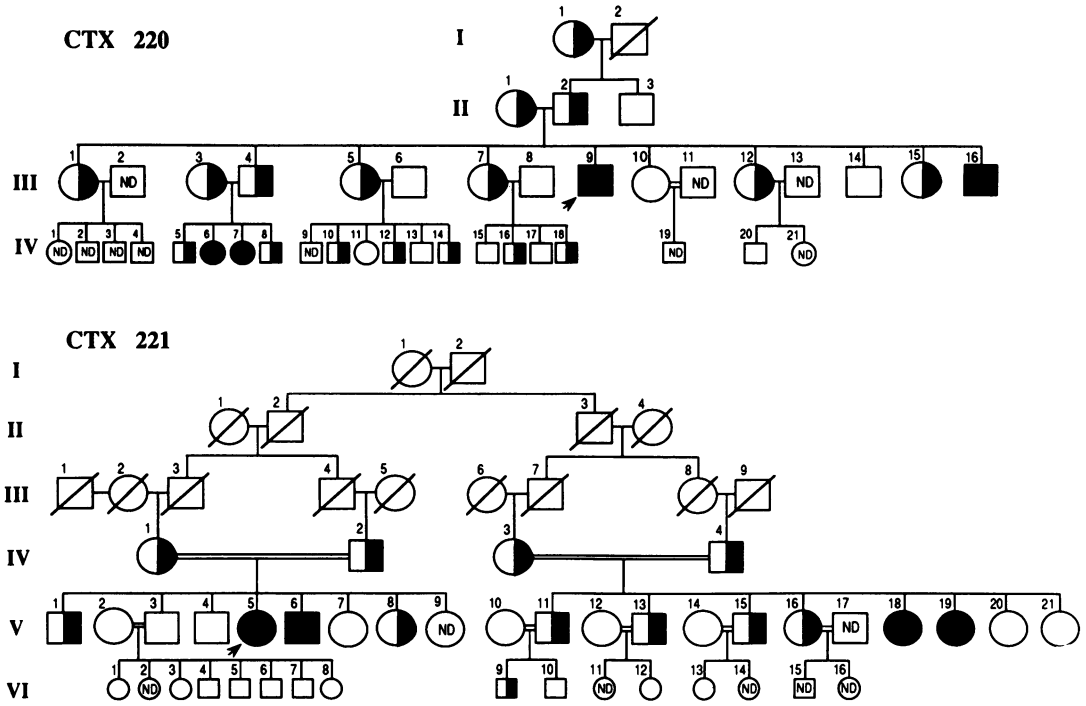

Figure 3 Pedigree structure of Druze CTX families 220 and 221. Index cases are indicated by arrows; blackened and half-blackened pedigree symbols indicate molecularly diagnosed homozygote and heterozygote cases, respectively. ND = molecularly uncharacterized individuals.

The cohort was in Hardy-Weinberg equilibrium with respect to the *CYP27* mutant allele ($\chi^2_{(1)}$=0.556; P=.46). Means and SDs of cholestanol, other lipids, and lipoprotein levels, by *CYP27* genotypes, are given in table 2. Mean cholestanol levels were ~33 mg/dl higher in the homozygotes (+/+) than those observed in the controls (−/−) and in the heterozygotes (+/−) ($P \leq .0001$). Interestingly, two asymptomatic individuals (V-10 and VI-10, mother and son) from CTX family 221 who had moderately elevated plasma cholestanol levels (18.7 μg/ml and 15.4 μg/ml, respectively) were found not to carry the mutant allele.

The controls had gender- and age-adjusted plasma cho-

Table 1

Clinical Data of 10 CTX Patients Homozygous for the Druze Mutation, Shown According to Age Stratification

	220 IV-7	220 IV-6	220 III-16	222-1	221 V-19	221 V-18	220 III-9	221 V-6	223-1	221 V-5
Background data:										
Gender	F	F	M	M	F	F	M	M	F	F
Age (years)	2	7	16	19	23	27	27	32	39	41
Physical findings:										
Pes cavus	−	−	+	+	+++	+++	+++	+++	+++	+++
Tendon xanthomas	−	−	−	−	+	++	++	+++	−	+++
Cataracts	+	++	++	++	++	++	++	++	++	++
Dementia	−	−	+	+	++	++	+++	+++	+++	+++
Pyramidal signs	−	−	+	+	+	+	++	++	+++	+++
Cerebellar signs	−	−	+	+	++	++	++	++	+++	+++
Convulsions	−	+	++	++	++	++	++	++	++	−
Neurological studies:										
EEG abnormality[a]	ND	+++	++	+++	+++	+++	+++	+++	+++	+++
Brain atrophy[b]	ND	++	++	ND	++	+++	+++	+++	ND	ND

NOTE.—(+++) to (−) = severe to absent: ND = not done.

[a] Irregular diffuse slow activity with periodical sharp-waves discharges.

[b] Confirmed by magnetic resonance imaging and/or computerized tomography.

Table 2

Mean ± SD Values of Plasma Cholestanol, Plasma Lipids, and Lipoprotein Levels, by CTX Genotype

Variable	*CYP27* Genotype[a]			*P*
	−/−	+/−	+/+	
No. of subjects	29	28	10	
Cholestanol (μg/ml)	4.2 ± 3.9	4.4 ± 1.8	37.7 ± 21.7	<.0001
TC (mg/dl)	180.4 ± 30.7	169.4 ± 23.5	166.9 ± 20.5	.207
LDL-C (mg/dl)	116.2 ± 23.1	104.5 ± 21.0	104.9 ± 15.6	.098
TG (mg/dl)	112.6 ± 73.6	120.8 ± 43.0	120.9 ± 50.1	.850
HDL-C (mg/dl)	41.9 ± 10.1	40.7 ± 8.8	37.8 ± 10.1	.515

NOTE.—Values were gender and age adjusted.

[a] −/− = Normal; +/− = heterozygote; and +/+ = homozygote for the Druze mutation.

lesterol levels 11.0 mg/dl higher than did the heterozygotes, and 13.5 mg/dl higher than did the homozygotes. Yet, these differences were not statistically significant. A borderline significant difference (P=.098) in mean plasma LDL-C was observed among the *CYP27* genotypes. Subjects with the −/− genotype had a higher mean LDL-C level than did subjects with either the +/− genotype or the +/+ genotype. Only small nonsignificant differences were observed in TG and HDL-C mean levels among the *CYP27* genotypes.

The results of measured genotype analysis are shown in table 3. For cholesterol, the best-fitting model with polygenes fitted the data better than did a sporadic model ($\chi^2_{(1)}$=4.6; P=.03). The model including the *CYP27* locus in addition to the polygenes was not significantly supported by the data. Polygenic effects were also significant for LDL-C, as indicated by comparison with the sporadic model ($\chi^2_{(1)}$=6.2; P=.01). Inclusion of the *CYP27* mutation into the model reduced the h^2 from .38 to .26, yet this model did not provide a better fit than did the model with polygenes alone ($\chi^2_{(2)}$=1.5; P=.55).

Discussion

A *CYP27* deletion mutation causing CTX in the Israeli Druze has been described. A deletion of cytosine in exon 2 results in a frameshift leading to a premature termination signal. The mutant allele is expected to result in a complete absence of sterol 27 hydroxylase activity in homozygotes and to only a partial defect in heterozygotes. The phenotypic characteristics, including the clinical manifestations and biochemical data of individuals heterozygote or homozygote for the mutant allele, are presented.

Table 3

Measured Genotype Analysis: Hypothesis Testing and Contribution of *CYP27* Genotypes to Variability of TC and LDL-C

	Model		
	Sporadic	Polygene	Polygene + *CYP27*
Frequency of allele (−)	.665	.665	.665
Total cholesterol (genotype-specific means):[a]			
−/−	174.0	175.1	178.6
+/−	...	...	173.1
+/+	...	...	168.2
σ	26.4	26.6	26.0
h^2	...	.361	.287
−2 Likelihood ($\log_e$)	753.0	748.4	747.2
LDL-(genotype-specific means):[a]			
−/−	109.8	111.9	115.1
+/−	...	...	107.9
+/+	...	...	107.9
σ	21.5	21.5	20.8
h^2	...	.384	.264
−2 Likelihood ($\log_e$)	725.0	718.8	717.3

[a] −/− = Normal; +/− = heterozygote; and +/+ = homozygote for the Druze mutation.

Diagnosis of CTX is usually based on the presence of a multitude of clinical characteristics and the demonstration of increased plasma concentrations of the bile alcohols and cholestanol (Björkhem and Skrede 1989; Berginer et al. 1993). Although the latter is important for the diagnosis of CTX, increased levels of this steroid were demonstrated in primary biliary cirrhosis (Huijghebaert et al. 1989) and cholestasis (Kibe et al. 1980). Here we show that, although, in general, plasma cholestanol concentrations are elevated in homozygotes, noncarriers in CTX families (221 V-10 and 221 VI-10) may have moderately elevated levels. This finding is important, since, in the absence of molecular diagnosis, these individuals may have been suspected of being CTX cases. The possibility that they may carry another *CYP27* mutant allele has been ruled out by SSCP analysis of all exons and the 5′ region of the *CYP27* gene. It is therefore suggested that the "gold standard" for the diagnosis of CTX should be the verification of homozygosity or compound heterozygosity for *CYP27* mutant allele(s).

Here we show that, in homozygotes, plasma cholestanol concentrations may vary up to sevenfold (10.8–72.6 μg/ml). This finding is of special interest, since all these CTX cases carry an identical nonsense mutation and are from the same origin group. It may therefore be suggested that the regulation of the metabolic pathway that leads to formation or metabolism of cholestanol may differ between individuals and may reflect other, yet unidentified genetic and/or environmental mechanisms. Further research into this complex scientific issue is now feasible, especially in populations where the *CYP27* mutations have already been identified (Leitersdorf et al. 1993; Meiner et al. 1994*a*; Reshef et al. 1994), and a large number of cases may be investigated.

In the current investigation, 28 heterozygotes were identified. The possibility that the heterozygote state may be biochemically manifested is intriguing. Here we show that age-adjusted mean plasma cholestanol concentrations in heterozygotes is identical to the levels found in noncarrier members of their families. If it is assumed that heterozygotes have half the level of sterol 27-hydroxylase activity, the metabolic pathway that leads to the formation of cholestanol may only become significant at much lower levels.

Age- and gender-adjusted plasma TC and LDL-C levels were found to be somewhat lower in heterozygotes and homozygotes, as compared with those in controls, yet these differences did not reach statistical significance. Admixture and segregation analyses revealed that the data fitted a simple polygenic model without any involvement of a major gene. Incorporation of the information regarding the *CYP27* genotypes did not lead to a better-fitted model. This may suggest that the increased synthesis of VLDL and LDL in CTX may be totally offset by the increased catabolism through the LDL pathway.

On the basis of our results, it is evident that the increased prevalence of atherosclerosis in CTX cannot be explained simply by alteration of the plasma lipid and lipoprotein profile. Other mechanisms, which may be related either to bile alcohols or to the lack of sterol 27 hydroxylase activity in the cellular constituents of the atheroma, must be more important. Recently, normal human macrophages were shown to contain a high level of sterol 27-hydroxylase activity (Björkhem et al., in press). Evidence has been obtained that this enzyme is able to metabolize cholesterol into 27-hydroxycholesterol and 3β-hydroxy-5-cholestanoic acid, which are transported out from the macrophage. This may be a defense mechanism for the macrophage when it is exposed to excess cholesterol. A lack of this mechanism in patients with CTX may be part of the explanation for an early development of atherosclerosis. Additional investigations are needed, however, to establish its relative importance.

The mutation described here accounts for a wide spectrum of clinical manifestations in the CTX patients (table 1). It is evident that, in general, the clinical manifestations progress with age. An interesting finding is that, although usually xanthomas also progress with age, a 38-year-old patient (CTX 223-1) has no tendon xanthomas, albeit prominent neurological involvement. A similar finding has recently been reported in a CTX case of Jewish Moroccan origin (Leitersdorf et al. 1993). It is evident that additional genetic and/or environmental factors may contribute to xanthoma formation. It should be emphasized that, in contradistinction to the Jewish Moroccan case, here the lipid profile of patient 223-1 did not differ significantly from that of other affected individuals who harbor identical mutant alleles and who have xanthomatosis.

In newly discovered CTX families, the slowly progressive nature of the clinical findings may lead to late diagnosis and delayed treatment (Berginer et al. 1993). The current investigation includes the youngest CTX patient yet described, a 2-year-old physically healthy (except for a mild cataract) baby (CTX 220 IV-7). It is generally believed that the most important clinical manifestations of CTX are those resulting from cholestanol deposition. The deficiency of sterol 27-hydroxylase activity may, however, be responsible for clinical symptoms not related to the accumulation of cholestanol. Therefore, treatment with CDCA aimed at the normalization of plasma cholestanol (Berginer et al. 1984) in this young girl may allow us in the future to differentiate between cholestanol-related and cholestanol-unrelated clinical manifestations. If the premature atherosclerosis in CTX is related to the lack of sterol 27-hydroxylase in macrophages, treatment with CDCA should have little or no effect on its development.

The availability of a rapid screening method for the mutation described here facilitates possible genetic screening in the Druze population. The Druze sect was founded in the 11th century and remained isolated because of religious restrictions prohibiting outbreeding. Although sev-

eral genetic markers that were examined among the Druze showed significant deviations from Hardy-Weinberg distribution, possibly because of increased homozygosity related to a high degree of consanguinity (Nevo 1988), our investigation did not reveal any deviation. Other recessive disorders, including Krabbe disease, another rare progressive autosomal recessive neurodegenerative disorder, were found to be prevalent in a large Druze kindred in Israel, possibly as a result of a similar mechanism (Zlotogora et al. 1985). Surprisingly, we have not identified CTX among 34 Druze individuals who permanently reside in two local institutions for the handicapped. This finding is difficult to explain, especially since the disease causes severe dementia and physical handicap, which are usually extremely difficult to treat adequately at home.

In conclusion, the current investigation of a relatively large number of individuals heterozygote or homozygote for a *CYP27* deletion mutation enabled detailed analysis of the impact of this mutation on plasma lipid and lipoprotein concentrations. Apart from the increased plasma levels of cholestanol, the parameters studied here are not affected by the presence of the mutant allele. Further investigations on atherogenesis in CTX should therefore focus on other possible mechanisms.

Acknowledgments

Shoshi Ben David, Eti Butbul, Osnat Eliav, and Liat Triger are thanked for excellent technical assistance. This research was supported by grant 88-0186 from the United States—Israel Binational Science Foundation (BSF), Jerusalem; by the Sarah Mayer Research Foundation; and by the Swedish Medical Research Council.

Appendix

Table A

Plasma Lipid and Lipoprotein Concentrations in CTX Families 220 and 221 and in Two Sporadic Cases

Patient Identification	Age (years)	Gender	Genotype[a]	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Cholestanol (μg/ml)
220 I-1	97.0	F	+/−	222	125	48	149	7.14
220 II-1	52.0	F	+/−	236	112	56	158	4.40
220 II-2	54.0	M	+/−	216	216	32	144	6.68
220 II-3	46.0	M	−/−	213	126	36	152	3.40
220 III-1	34.0	F	+/−	158	56	39	108	2.02
220 III-3	27.0	F	+/−	207	143	58	120	6.08
220 III-4	33.0	M	+/−	227	154	32	164	4.88
220 III-5	31.0	F	+/−	193	122	37	132	3.50
220 III-6	38.0	M	−/−	236	354	32	133	3.68
220 III-7	32.0	F	+/−	185	68	53	118	3.36
220 III-8	34.0	M	−/−	187	91	45	124	6.28
220 III-9	27.0	M	+/+	175	115	39	113	10.80
220 III-10	20.0	F	−/−	193	206	34	118	4.32
220 III-12	24.0	F	+/−	174	109	51	101	2.40
220 III-14	22.0	M	−/−	195	64	44	138	5.24
220 III-15	18.0	F	+/−	123	64	44	66	2.60
220 III-16	16.0	M	+/+	156	155	39	86	12.10
220 IV-5	8.0	M	+/−	166	40	44	114	4.88
220 IV-6	7.0	F	+/+	146	43	52	85	36.20
220 IV-7	2.0	F	+/+	156	72	46	96	26.90
220 IV-8	.1	M	+/−	159	160	31	96	ND
220 IV-9	14.0	M	ND	136	55	43	82	3.56
220 IV-10	9.0	M	+/−	160	52	54	96	5.70
220 IV-11	7.0	F	−/−	208	44	45	154	5.90
220 IV-12	6.0	M	+/−	161	103	33	107	5.62
220 IV-13	4.0	M	−/−	162	50	54	98	3.28
220 IV-14	2.0	M	+/−	179	106	40	118	8.00
220 IV-15	8.0	M	−/−	192	44	66	117	3.32
220 IV-16	6.0	M	+/−	211	59	57	142	4.92
220 IV-17	5.0	M	−/−	143	52	32	101	4.94
220 IV-18	4.0	M	+/−	155	49	46	99	3.62
220 IV-20	2.0	M	−/−	184	99	44	120	3.76

Table A (continued)

Patient Identification	Age (years)	Gender	Genotype[a]	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Cholestanol (μg/ml)
221 IV-1	62.0	F	+/−	206	272	29	123	3.74
221 IV-2	65.0	M	+/−	183	69	39	130	3.02
221 IV-3	57.0	F	+/−	211	134	29	155	3.28
221 IV-4	52.0	M	+/−	240	215	26	171	2.92
221 V-1	44.0	M	+/−	185	150	32	123	2.84
221 V-2	45.0	F	−/−	240	162	30	178	2.46
221 V-3	45.0	M	−/−	221	235	30	144	4.00
221 V-4	37.0	M	−/−	161	89	42	101	ND
221 V-5	41.0	F	+/+	186	157	29	126	25.10
221 V-6	32.0	M	+/+	199	129	32	141	23.60
221 V-7	30.0	F	−/−	175	136	35	113	2.54
221 V-8	27.0	F	+/−	171	73	50	106	3.84
221 V-10	28.0	F	−/−	243	337	48	128	18.70
221 V-11	31.0	M	+/−	198	128	29	143	3.92
221 V-12	24.0	F	−/−	143	49	40	93	4.80
221 V-13	29.0	M	+/−	227	202	30	157	5.86
221 V-14	21.0	F	−/−	118	49	33	75	2.90
221 V-15	26.0	M	+/−	138	160	31	75	2.18
221 V-16	23.0	F	+/−	188	172	44	110	3.88
221 V-18	27.0	F	+/+	210	67	65	132	54.80
221 V-19	23.0	F	+/+	176	50	52	114	50.30
221 V-20	21.0	F	−/−	178	63	48	117	3.14
221 V-21	20.0	F	−/−	158	134	37	94	1.76
221 VI-1	27.0	F	−/−	131	46	50	72	1.42
221 VI-3	22.5	F	−/−	147	100	43	84	2.32
221 VI-4	18.0	M	−/−	123	135	28	86	1.34
221 VI-5	18.0	M	−/−	156	130	41	89	2.72
221 VI-6	16.0	M	−/−	126	70	40	72	1.76
221 VI-7	15.0	M	−/−	124	93	33	72	1.96
221 VI-8	13.0	F	−/−	127	193	33	55	1.72
221 VI-9	6.0	M	+/−	193	82	42	135	9.00
221 VI-10	3.5	M	−/−	147	87	36	94	15.40
221 VI-12	4.5	F	−/−	92	58	28	52	3.90
221 VI-13	2.5	F	−/−	105	80	30	59	1.60
222-1	19.0	M	+/+	115	71	37	64	72.60
223-1	39.0	F	+/+	218	157	53	134	65.10

[a] −/− = normal; +/− = heterozygote; +/+ = homozygote for the Druze mutation; and ND = not done.

References

Ballantyne CM, Vega GL, East C, Richards G, Grundy SM (1987) Low density lipoprotein metabolism in cerebrotendinous xanthomatosis. Metabolism 36:270–276

Beil U, Crouse JR, Einarsson K, Grundy SM (1982) Effects of interruption of the enterohepatic circulation of bile acids on the transport of very low density lipoprotein triglycerides. Metabolism 31:438–444

Ben-Dror G, Geler A, Berginer VM, Manelis Y (1988) Cerebrotendinous xanthomatosis in a Druze family from Western Galilee. Harefuah 115:224–226

Berginer VM, Salen G, Shefer S (1984) Long term treatment of cerebrotendinous xanthomatosis with chenodeoxycholic acid. N Engl J Med 311:1649–1652

——— (1993) Cerebrotendinous xanthomatosis. In: Rosenberg RN, Prusiner SB, DiMauro S, Barchi RL, Kunkel LM (eds) Molecular and genetic basis of neurological disease. Butterworth Heinemann, Boston, pp 295–305

Björkhem I, Andersson O, Diczfalusy U, Sevastik B, Duan C, Xiu RJ, Lund E. Atherosclerosis and sterol 27-hydroxylase: evidence for a role of this enzyme in elimination of cholesterol from human macrophages. Proc Natl Acad Sci USA (in press)

Björkhem I, Skrede S (1989) Cerebrotendinous xanthomatosis and phytosterolemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease. McGraw-Hill, New York, pp 1283–1293

Cali JJ, Hsieh CL, Francke U, Russell DW (1991) Mutations in the bile acid biosynthetic enzyme sterol 27-hydroxylase underlie cerebrotendinous xanthomatosis. J Biol Chem 266: 7779–7783

Cali JJ, Russell DW (1991) Characterization of the human sterol

27-hydroxylase: a mitochondrial P-450 that catalyzes multiple oxidation reactions in bile acid biosynthesis. J Biol Chem 266: 7774–7778

Chirgwin MJ, Przybyla EA, McDonald JR, Rutter JW (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294–5299

Church GM, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci USA 81:1991–1995

Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 18:499–502

Fujiyama J, Kuriyama M, Arima S, Shibata Y, Nagata K, Takenaga S, Tanaka H, et al (1991) Atherogenic risk factors in cerebrotendinous xanthomatosis. Clin Chim Acta 200:1–11

Goetz PW (ed) (1986) Druze. In: The new encyclopedia britannica. Vol 4. Encyclopedia Britannica, Chicago

Hasstedt SJ, Cartwright PE (1981) PAP: pedigree analysis package. Tech rep no 13, Department of Biophysics and Computing, University of Utah, Salt Lake City

Huijghebaert SW, Karlaganis G, Renner EL, Preisig R (1989) Increased urinary excretion of bile alcohol glucoronides in patients with primary biliary cirrhosis. J Lipid Res 30:1673–1679

Kibe A, Nakai S, Kuramoto T, Hoshita T (1980) Occurrence of bile alcohols in the bile of a patient with cholestasis. J Lipid Res 21:594–599

Kuriyama M, Fujiyama J, Yoshidome H, Takenaga S, Matsumuro K, Kasama T, Fukuda K, et al (1991) Cerebrotendinous xanthomatosis: clinical and biochemical evaluation of eight patients and review of the literature. J Neurol Sci 102:225–232

Lalouel JM (1979) GEMINI—a computer program for optimization of general nonlinear functions. Tech rep no 14, Department of Medical Biophysics and Computing, University of Utah, Salt Lake City

Lalouel JM, Rao DC, Morton NE, Elston RC (1983) A unified model for complex segregation analysis. Am J Hum Genet 35: 816–826

Leitersdorf E, Reshef A, Meiner V, Levitzki R, Pressman Schwartz S, Dann EJ, Berkman N, et al (1993) Frameshift and splice-junction mutations in the sterol 27-hydroxylase gene cause cerebrotendinous xanthomatosis in Jews of Moroccan origin. J Clin Invest 91:2488–2496

McMaster KG, Carmichael GG (1977) Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Proc Natl Acad Sci USA 74:4835–4838

Meiner V, Marais DA, Reshef A, Björkhem I, Leitersdorf E (1994*a*) Premature termination codon at the sterol 27-hydroxylase gene causes cerebrotendinous xanthomatosis in an Afrikaner family. Hum Mol Genet 3:193–194

Meiner V, Meiner Z, Reshef A, Marais DA, Björkhem I, Leitersdorf E (1994*b*) Cerebrotendinous xanthomatosis: molecular diagnosis enables pre-symptomatic detection of a treatable disease. Neurology 44:288–290

Morton NE, MacLean CT (1974) Analysis of family resemblance. III. Complex segregation of quantitative traits. Am J Hum Genet 26:489–503

Nevo S (1988) Genetic blood markers in Arab Druze of Israel. Am J Phys Anthropol 77:183–190

Orita M, Suzuki Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879

Reshef A, Meiner V, Berginer VM, Leitersdorf E (1994) Molecular genetics of cerebrotendinous xanthomatosis in Jews of North African origin. J Lipid Res 35:478–483

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, et al (1988) Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239: 487–491

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74: 5463–5467

Seyama Y, Ichikawa K, Yamakawa T (1976) Quantitative determination of cholestanol in plasma with mass fragmentography: biochemical diagnosis of cerebrotendinous xanthomatosis. J Biochem 80:223–228

Shore V, Salen G, Cheng FW, Forte T, Shefer S, Tint GS (1981) Abnormal high density lipoproteins in cerebrotendinous xanthomatosis. J Clin Invest 68:1295–1304

Tint GS, Ginsberg H, Salen G, Le NA, Shefer S (1989) Chenodeoxycholic acid normalizes elevated lipoprotein secretion and catabolism in cerebrotendinous xanthomatosis. J Lipid Res 30:633–640

Zlotogora J, Regev R, Zeigler M, Iancu TC, Bach G (1985) Krabbe disease: Increased incidence in a highly inbred community. Am J Med Genet 21:765–770