Mutational Analysis of a Patient with Concomitant Cerebrotendinous Xanthomatosis and Smith-Lemli-Opitz Syndrome

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Abstract

Cerebrotendinous Xanthomatosis (CTX) is a rare recessively inherited disorder of bile acid synthesis caused by mutations in the sterol 27-hydroxylase gene (CYP27) located on human chromosome 2. The disease is characterized by tendon xanthomatosis, juvenile cataracts and progressive neurological dysfunction. Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder of cholesterol metabolism caused by mutations in the gene for Δ^7-dehydrocholesterol reductase in chromosome 11. It is characterized by congenital malformations, mental retardation, dysmorphism of multiple organs, and delayed neuropsycomotor development. A patient was discovered with clinical history and signs consistent with CTX. Elevated levels of cholestanol were found using biochemical analysis, which confirmed the CTX disease. Elevated levels of 7-DHC consistent with SLOS were also found. To elucidate the molecular basis for this unusual biochemistry, we screened the CYP27 and DHCR7 genes for mutations. Genomic DNA was extracted from the patient's blood and used to perform PCR amplification and sequencing of exons 1-9 and 3-9 for CTX and SLOS respectively, intron-exon boundaries. For CTX, we identified a previously reported 2 bp deletion in exon 6 (Δ2bpC1201) and a novel mutation G276C in exon 1 that affects the splice site. Although no mutations were observed in DHCR7 gene, several polymorphisms were found in exon 6 at C703T (D146D), exon 9 at C1423T (D386D) and T1537C (G424G). Thus we have a genetic and biochemical configuration of CTX in our patient. There were no mutations identified in the DHCR7 gene. Our interpretation for the elevated 7DHC of presentation is that the upregulation of cholesterol biosynthesis resulted in an excess production of the precursors.

Introduction

Cerebrotendinous Xanthomatosis (CTX) is a rare, recessively inherited disorder of bile acid synthesis. CTX was first described in 1937 in individuals with neurological dysfunction and tendon xanthomas. Later studies demonstrated that cholesterol and the 5α-reduced form of cholesterol, cholestanol accumulate in neural and other tissues of CTX subjects [1]. CTX is caused by mutations of the mitochondrial enzyme sterol 27-hydroxylase (CYP27), located on human chromosome 2 [1, 2]. Clinical features include tendon xanthomas, juvenile cataracts, and nervous system dysfunction, i.e. mental retardation, behavioral and psychiatric problems, pyramidal tract paresis, cerebellar ataxia, and peripheral neuropathy. Other common features include osteoporosis, bone fractures, chronic diarrhea in children and premature atherosclerosis [2]. Long term bile acid therapy with Chenodeoxycholic acid (CDCA), the most deficient biliary bile acid, decreased cholestanol levels and improved neurologic function in most CTX subjects. More than 250 incidents of CTX have been discovered throughout the world [2]. About 37 different mutations of the

CYP27 gene have been identified in CTX patients around the world [3].

Smith-Lemli-Opitz Syndrome (SLOS) is an autosomal recessive disorder of cholesterol metabolism caused by mutations in the gene for Δ^7-dehydrocholesterol reductase (DHCR7) in chromosome 11[4-6]. SLOS was described and diagnosed solely based on clinical characteristics, but now is regularly confirmed by detection of elevated serum levels of 7–dehydrocholesterol (7DHC). Serum 7DHC levels range from 10-fold to more than 2,000-fold greater than normal [7]. Today, the biochemical diagnosis is based on the measurement of plasma 7DHC or the activity of Δ^7-sterol-reductase in fibroblast cultures using UV spectrophotometry. SLOS is frequently associated with low plasma cholesterol levels. However, 10% of the affected patients show normal cholesterol levels [8]. Clinical features include congenital malformations, mental retardation, dysmorphism of multiple organs, delayed neuropsycomotor development, and polydactyly [9]. The estimated incidence of SLOS is 1 in 20,000-40,000 births, more prevalent in American Caucasians. 19 different mutations of the DHCR7 gene have been reported in SLOS patients from the US and Europe [10].

Patients and Methods

Our patient of interest (Patient 1) is a female born on February 28, 1994. She displays clinical history and signs consistent with CTX. Biochemical analysis shows elevated levels of cholestanol confirming the CTX disease. Interestingly, we also found elevated levels of 7DHC consistent with SLOS suggesting this patient may have a second diagnosis. Our aim is to perform mutational analysis of this patient for the SLOS. I predict that our patient will display mutations for both diseases, though the implication of this possibility is rare.

Amplification--To begin, genomic DNA was extracted from the patient's blood. PCR amplification and sequencing were performed on Exons 1-9 and 3-9 for CTX and SLOS respectively. The reaction mixture contained: 20ng DNA, 10ng of each primer, 10x NH1.5 Buffer, .2 u Taq DNA polymerase (New England BioLabs), and distilled H_20 for a final volume of 50 µl. PCR was performed on Hybaid Multiblock System. Table 1-2 show primers used for each exon. Not every exon could be amplified under the same PCR conditions. Table 3. summarizes PCR conditions used to amplify each exon.

Table 1. Primers for CYP27 gene amplification

Exon	Forward Primer	Reverse Primer
1	ACTCAGCACTCGACCCAAAGGTGCA	CCACTCCCATCCCCAGGACGCGATG
2	TGGCCCAGTTATTCAGTTTTGATTG	GGGCCCTGTTCCAGTCCCTTCAGGC
3	GCTTATCTTTGTCGTGTTCCTCTGC	GAGCACAACCTCTCCCTGACCCATT
4	TCTGCCTCCTGTGATGGCCTCTGTG	GCTCATGCACAGACCTGGAGTCACC
5	GCTCTTGGTCCTTGGAGATCATGAC	ACTGGTTACGGTTGGGAGCTGGGGG
6-8	TTCCTAGAATCGCCTCACCTGATCT	CAGGCTCAGAGAAGGCAGTG
8-9	CCAGTTTGTGTTCTGCCAC	CCCAGCAAGGCGGAGACTCA

Table 2. Intronic primers for DHCR7 exon amplification

Exon	Forward Primer	Start Site	Reverse Primer	Start Site	Product Size
3	GGTGGATGCAACAGGGAAAGGTGG	IVS2 -70	AGGCTGGAAAGCTCTGAG	IVS3 +62	240
4	CCCAGTGTGACTGCCTGCATCCG	IVS3 -32	ACGCTCCCCACCTGCTGTGTCCC	IVS4 +47	302
5	CTGCTATTCGTCCCCCTTTGCAGG	IVS4 -67	GTCTTAGGGACAAAGCAGCGCTGG	IVS5 +92	250
6	AAGCATGCTTCAGCCCAGCCAAGC	IVS5 -76	CTTTCTACATCAGGCTGGACCCGC	IVS6 +38	328
7	TGGGCTCTCGCTAAGTAAGGTGGC	IVS6 -80	CATCGGCGTTTCACCCTCTCCAGC	IVS7 +35	321
8	TGTGATTTCCCCGAGGTCCATGGG	IVS7 -71	GCTTAGCATGTGTCTGCCAAATGC	IVS8 +55	258
9	CAAAGCACCGCTTGACCCCTTCCC	IVS8 -37	CCTGGCAGAACACGCTCTTG	5'UTR +54	556

Table 3. PCR Conditions

Exon	Denaturation	Amplification x35 cycles			Extension
SLO 3	94°C 4 min	94° 45sec	65°C 45sec	72°C 1min	72°C 10min
SLO 4-8	94°C 4 min	94°C 50sec	65°C 45sec	72°C 2min	72°C 10min
SLO 9	94°C 4 min	94°C 1min	65°C 45sec	72°C 3min	72°C 10min
CTX 1	95°C 5min	95°C 45sec	65°C 1min	72°C 1min	72°C 10min
CTX 4	95°C 5min	95°C 45sec	65°C 30sec	72°C 1min	72°C 10min
CTX 5	95°C 5min	95°C 45sec	68°C 3min	72°C 1min	72°C 10min
CTX 2,3 6-9	95°C 5min	95°C 45sec	65°C 1min	72°C 2min	72°C 10min

Aliquots of PCR products were separated and analyzed by gel electrophoresis through .7% agarose gel.

Sequencing—PCR products were purified with QIAquick columns or excised out of gel and purified with Qiagen Purification Kit. Purified products were sequenced using Beckman Coulter's CEQ Dye Terminator Cycle Sequencing. Sequences were then analyzed for mutations of their respective gene.

Cloning-- To confirm a deletion mutation on CYP27, exon 6 was cloned using Invitrogen's TOPO TA Cloning Kit for Sequencing. Invitrogen TOPO®-Cloning Reaction: Mix together PCR Products and cloning vector. Transform into TOP10 *E.Coli* cells. Select and Analyze colonies. Isolate plasmid DNA and sequence.

Results

No mutations were found in the patient's DHCR7 gene to confirm SLOS. However, several polymorphisms were found shown in Table 4.

Table 4. Polymorphisms found on DHCR7 gene

Nucleotide Change	Effect on Coding Sequence	Exon
703C>T	D146D	6
1423C>T	D386D	9
1537T>C	G424G	9

Mutations were found on the patient's CYP27 gene which confirms the diagnosis of CTX. Table 5 summarizes these mutations.

Table 5. Mutations found on CYP27 gene

Nucleotide Change	Effect on Coding Sequence	Exon
275 + 1G>C	Splicing site mutation	1
Δ2bpC1201	Frameshift mutation	6

Fig. 1. 276 + 1 G>C splicing site mutation on Exon 1 of CYP27 gene

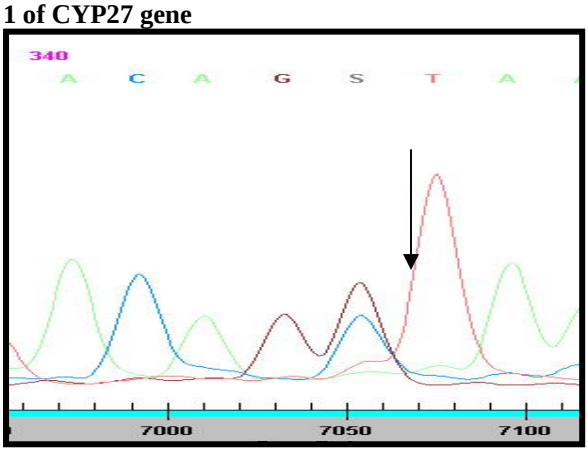


Fig. 2 Normal Allele

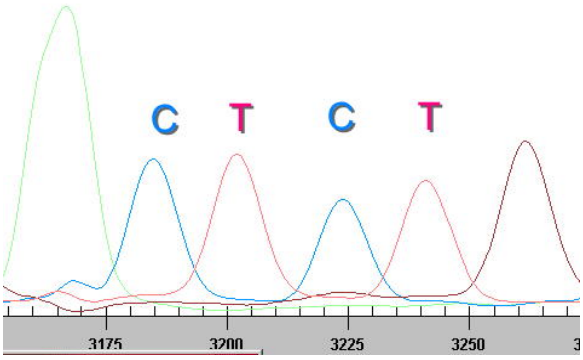
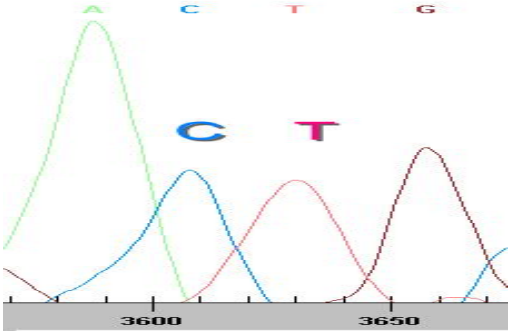


Fig. 3 2bp deletion 1200 frame shift mutation on CYP27 Exon 6



Because no mutations were found to confirm SLOS in our patient, we decided to test her 7DHC levels against those of other patients before and after treatment with CDCA. Table 6 shows results of these analyses.

Table 6. Re-analysis of Patient 7DHC levels

Patient	Date	Treatment	Cholesterol (mg/dl)	7DHC (mg/dl)	8DHC (mg/dl)
CTX A	1/21/04	Pre		1.2	2.9
	5/25/04	CDCA		0.09	0.41
CTX B	1/25/04	Pre		1.60	3.60
	5/25/04	CDCA		0.10	0.44
Our Proband	?	Pre	117	2.50	3.60
	5/18/04	?	146	1.90	3.60
Controls (Children)				0.036+0.020 n=313	0.060+0.037 n=30

Discussion

From mutational analysis, we were able to establish a genetic and biochemical configuration of CTX in our patient. No mutations were found on the DHCR7 gene. According to Krakowiak et al., to date, all individuals with SLOS have been shown to have mutations in the sterol Δ^7-reductase gene [7]. We were also able to rule out the possibility of the patient having SLOS despite the elevated levels of 7DHC. According to studies, the most predictive biochemical value in SLOS is the 7DHC/cholesterol ratio in plasma [9]. Patients with a plasma 7DHC/cholesterol ratio between 0.5 and 1.0 have moderate SLOS. Patients with a ratio <.05 have moderate SLOS while a ratio>1.0 is associated with severe SLOS [7]. Our studies are in agreement with, P.E. Jira et al., this biochemical ratio, although a useful tool for the prognosis of SLOS, cannot predict severity accurately [9]. From Table 6, all three patients display elevated levels of 7DHC that decreases upon treatment. Our patient has a slightly higher level of 7DHC that also decreases after treatment. Therefore, our findings show that it is possible to have elevated levels of 7DHC without having SLOS. In *The Smith-Lemli-Opitz Syndrome,* Kelley et al. discusses causes for increased 7DHC levels. In some conditions, like CTX, 7DHC may be increased 10 to 20-fold [11]. Therefore, it is important to note the presence of similarly increased levels of other sterol precursors and cholestanol to provide a correct and accurate diagnosis.

Conclusion

Mutational analysis confirms the CTX disorder and rules out SLOS in our patient. Elevated levels of 7DHC in our patient demonstrates that cholesterol biosynthesis can be highly upregulated in CTX and may lead to elevations of cholesterol precursors in the blood.

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