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Succinyl CoA: 3-Oxoacid CoA Transferase (SCOT): Human cDNA Cloning, Human Chromosomal Mapping to 5p13, and Mutation Detection in a SCOT-Deficient Patient

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Summary

Succinyl CoA: 3-oxoacid CoA transferase (SCOT; E.C.2.8.3.5) mediates the rate-determining step of ketolysis in extrahepatic tissues, the esterification of acetoacetate to CoA for use in energy production. Hereditary SCOT deficiency in humans causes episodes of severe ketoacidosis. We obtained human-heart SCOT cDNA clones spanning the entire 1,560-nt coding sequence. Sequence alignment of the human SCOT peptides with other known CoA transferases revealed several conserved regions of potential functional importance. A single ~3.2-kb SCOT mRNA is present in human tissues (heart > leukocytes ≫ fibroblasts), but no signal is detectable in the human hepatoma cell line HepG2. We mapped the human SCOT locus (OXCT) to the cytogenetic band 5p13 by in situ hybridization. From fibroblasts of a patient with hereditary SCOT deficiency, we amplified and cloned cDNA fragments containing the entire SCOT coding sequence. We found a homozygous C-to-G transversion at nt 848, which changes the Ser 283 codon to a stop codon. This mutation (S283X) is incompatible with normal enzyme function and represents the first documentation of a pathogenic mutation in SCOT deficiency.

Introduction

Ketone bodies are major vectors of energy transfer from liver to extrahepatic tissues (Mitchell et al. 1995). They are especially important for the brain, which has no other substantial lipid-derived source of energy. Ketoacidosis, a marked increase in ketone body concentration, is a medical emergency that occurs commonly in diabetes and other conditions.

Succinyl CoA: 3-oxoacid CoA transferase (SCOT; E.C.2.8.3.5) catalyzes the reversible transfer of a CoA moiety from succinyl CoA to the ketone body acetoacetate, the first step of ketone-body utilization. Tissue SCOT activity levels correlate well with ketolytic capacity (Williamson et al. 1971; Williamson 1992).

Six patients have been clinically described with autosomal recessive deficiency of SCOT (Tildon and Cornblath 1972; Spence et al. 1973; Middleton et al. 1987; Saudubray et al. 1987; Perez-Cerda et al. 1992; Sakazaki et al. 1995). All patients in whom it was evaluated had sustained hyperketonemia and episodes of severe ketoacidosis. Despite the apparent rarity of SCOT deficiency, ketosis is a common pediatric problem for which the diagnosis of SCOT deficiency is frequently considered. Furthermore, the clinical severity of SCOT deficiency has varied among reported patients, and the true incidence and clinical spectrum of SCOT deficiency are unknown.

SCOT is a mitochondrial matrix homodimer in pig heart and sheep kidney (Sharp and Edward 1978). Elegant studies of the SCOT reaction (Hersh and Jencks 1967*a*, 1967*b*; White and Jencks 1976*a*, 1976*b*; White et al. 1976; Pickart and Jencks 1979; Moore and Jencks 1982; Fierke and Jencks 1986) have demonstrated ping-pong kinetics and interactions of SCOT with two parts of the CoA molecule: formation of a thioester with the sulfhydryl group of CoA and noncovalent interaction with the ADP moiety of CoA. Rochet and Bridger (1994) recently identified the epsilon carboxyl group of Glu 344 as the site of thioester formation with CoA, but little is known about other structure-function relationships in the SCOT peptide.

SCOT cDNAs have been cloned from pig heart (Lin and Bridger 1992) and rat brain (Ganapathi et al. 1987),

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although only the pig SCOT cDNA sequence has been published. To aid the molecular diagnosis of SCOT deficiency and to delineate conserved regions in SCOT presumably important for enzyme function, we determined the complete coding sequence of the human-heart SCOT cDNA and compared it with the sequence of porcine SCOT, other known CoA transferases, and a SCOT-like cDNA from *Caenorhabditis elegans*. We performed chromosomal mapping of the human SCOT locus and have defined a causal mutation in a SCOT-deficient patient.

Material and Methods

Plasmids, Probes, and Sequencing

The plasmids pBlueScript KS (+) (Stratagene) and pGEM-T (Promega) were used for cloning. Probes were radiolabeled by random oligonucleotide priming (Feinberg and Vogelstein 1983). Dideoxy sequencing was performed with modified T7 DNA polymerase (Sequenase, United States Biochemical) according to the manufacturer's instructions. All sequences presented in this article were obtained at least twice, once on each strand.

Human SCOT cDNA Oligonucleotides

The sequence, position and orientation (sense, *s*, or antisense, *as*), of the oligonucleotides used in this article are as follows. For oligonucleotides of degenerate sequence, the possible nucleotides at a given position are indicated in lowercase letters separated by a diagonal (/). SCOT-42, 5′-GAAGATGGCGGCTCTCAAA-3′ (−4 to +15, s); SCOT-8, 5′-GGNTGc/tGCNGGNTAc/tTT341-3′ (78–96, s); SCOT-29, 5′-CGCGGATCCGGCACCATCAGGGATGTCTTTTACA-3′ (147–172, as); SCOT-30, 5′-TAGCCCAAAACCACCAACCAAAACC-3′ (174–199, as); SCOT-6, 5′-AGCTCCACTTCTAATTCACCAG-3′ (384–406, as); SCOT-7, 5′-GTGTAAAATGCAGGAACTCCAG-3′ (residues 450–472, as); SCOT-78, 5′- AGACATCCATATTCCTCAG-3′ (771–790, s); SCOT-24, 5′-AGCCTGGTACAAATATCCATA-3′ (1566–1586, as); SCOT-25, 5′- CAATTATGATTATTGATGTCC-3′ (1626–1646, as); SCOT-3, 5′-TTTCAGACTTTATGCAGCCA-3′ (1685–1704, s); and SCOT-4, 5′-TTATCTCATTTGTTAAGGCTGA-3′ (1768–1787, s).

cDNA-Library Screening

We screened a random-primed human-heart cDNA library in λ ZAP phage (Stratagene, 936208) using as probe an *Eco*RI fragment spanning residues 203–1770 of a pig-heart SCOT cDNA (Lin and Bridger 1992), kindly supplied by W. Bridger. Duplicate filters were hybridized as described by Wang et al. (1993) and washed twice at room temperature for 10 min and twice at 65°C for 15 min in 1% SDS/2 × SSC, then twice in 0.2 × SSC at room temperature for 5 min (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate). Pure hybridizing plaques were isolated and plasmid DNA was prepared as described by the manufacturer.

RNA Isolation

We prepared whole-cell or whole-tissue RNA by the ultracentrifugation of guanidinum isothiocyanate lysates through a cesium-chloride cushion as described by Sambrook et al. (1989). Human-heart RNA was prepared from a specimen of infundibular myocardium discarded following surgical correction of subvalvular pulmonary stenosis. Porcine myocardial RNA was obtained from a newborn piglet.

Northern Analysis

This was performed as described by Wang et al. (1993) except that filters were washed twice at room temperature for 15 min with 0.1 × SSC/0.1% SDS solution, then twice at 50°C for 15 min in the same solution. As probe for both human and porcine RNA blots, we used the 1.3-kb human SCOT cDNA subclone, hSCOT-G, which spans residues 490–1770.

Reverse Transcription and cDNA Amplification of the Human SCOT cDNA

We synthesized human-heart cDNAs using a protocol based on that of Frohman et al. (1988). Five micrograms of whole tissue RNA from human heart; mouse Moloney leukemia virus reverse transcriptase (BRL), 25 U; dNTPs, each 20 mM; Inhibit-ace (5′-3′, Inc.), 1 U; Tris HCl pH 8.4, 20 mM; KCl, 50 mM; $MgCl_2$, 2.5 mM and SCOT-7, 25 pmol, were incubated in a 20-μl volume for 50 min at 42°C. The reaction was stopped by heating at 95°C for 7 min. The amplification mixture contained 1 μl of the cDNA synthesis reaction; dNTPs, each 50 μM; SCOT-6 and SCOT-8, each 0.35 μg; Tris HCl pH 8.3, 20 mM; KCl, 100 mM; $MgCl_2$, 2.5 mM and 2.5 U of *Taq* polymerase in a total volume of 50 μl. Thirty cycles of amplification were performed (94° C, 45 s; 50° C, 45 s; 72° C, 1 min) followed by a 5-min extension at 72°C. The amplified fragment was cloned and sequenced.

RACE Amplification of Human SCOT cDNA

5′ RACE amplification (Frohman et al. 1988) was performed with the 5′ AmpliFinder RACE kit (Clontech). For first-strand synthesis, we used 5 μg of human-heart RNA and primed with SCOT-30. The 5′ ends of the human SCOT cDNA were amplified according to the manufacturer's instructions, using the nested primer, SCOT-29.

3′ RACE amplification was performed as described by Boukaftane et al. (1994), with two exceptions. First, as a gene-specific primer, we used SCOT-3, and SCOT-4 as a nested primer. Second, the annealing temperature for the amplification reaction was 55°C.

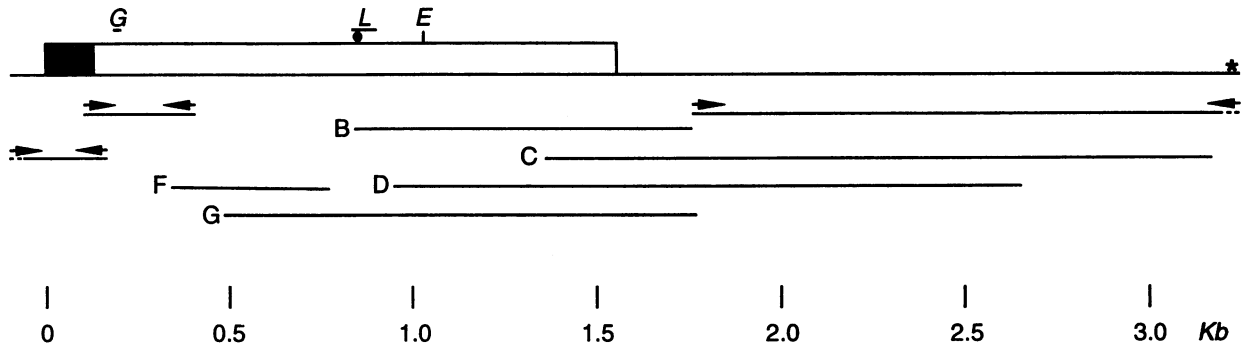

Figure 1 Human SCOT cDNA clones. The 1,560-nt coding region of human SCOT is indicated by the box, with the filled region corresponding to the mitochondrial leader (residues 1–117). The italicized letters above the cDNA indicate the locations of the following: G = putative CoA binding glycine-rich region; L = linker region absent in bacterial CoA transferases; and E = CoA-binding Glu 344 residue. The solid circle (●) shows the location of the Ser 283 codon, and the asterisk (*) corresponds to the begining of the poly (A) tract at residue 3222 of the human SCOT cDNA. The lower panel depicts the human SCOT cDNA subclones. Clones B, C, D, F, and G were obtained by library screening; the others, flanked by arrows, were obtained by RT-PCR (solid lines) or RACE-PCR (indicated by dashed lines at one end).

Sequence Comparisons of SCOT and Other CoA Transferases

We aligned the deduced sequences of human and porcine SCOT with those of the SCOT-like peptide deduced from genomic sequencing of *C. elegans* chromosome III (Wilson et al. 1994) and of three bacterial CoA transferases: β-ketoadipate: succinyl CoA transferases (E.C.2.8.3.6) from *Acinetobacter calcoaceticus* (Cat I and Cat J) and *Pseudomonis putida* (Pca I and Pca J), and acetoacetyl CoA: acetate/butyrate: CoA transferase (E.C.2.8.3.9) from *Clostridium acetobutylicum* (Ctf A and Ctf B). Alignments were obtained with the Geneworks 2.1 package and refined manually.

Chromosome Mapping of the Human SCOT Locus

In situ hybridization was performed as described by Wang et al. (1993). As a probe, we used the tritiated human SCOT cDNA subclone, SCOT G (fig. 1). The position of silver grains directly over or touching well-banded metaphase chromosomes were placed on an International System for Cytogenetic Nomenclature idiogram.

cDNA Synthesis and Amplification from Patient Samples

Fibroblasts from the patient (cell line 9677), her father (10422), and her mother (10423) were cultured in Eagle's minimal essential medium containing 10% FCS. We purified fibroblast genomic DNA with a Sepa Gene kit (Sanko Junyaku) and total RNA with a ISOGEN kit (NIPPON GENE) according to the manufacturer's recommendations.

First-strand cDNA synthesis was performed using 5 μg of total RNA and 0.5 μg of SCOT-25 and 200 U of M-MLV reverse transcriptase (GIBCO BRL) in a 20-μl volume at 37°C for 60 min as recommended by the manufacturer. One microliter of this cDNA solution was used as an amplification template with 30 pmol each of SCOT-42 and SCOT-24 and TaKaRa *Taq*, 2.5 U (Takara Shuzo). Forty amplification cycles (94°C, 1 min; 56°C, 2 min; 72°C, 3 min) were performed. As a control we also performed cDNA synthesis of mitochondrial acetoacetyl-CoA thiolase (T2), priming with 0.5 μg of the primer 135 and then amplifying with primers 60 and 135 as described previously (Fukao et al. 1992). Amplified fragments were isolated following electrophoresis in 1.1% agarose, purified using a GENECLEAN II kit (BIO 101), subcloned into the pMOSBlue T vector (Amersham), sequenced using a PRISM Ready Reaction Terminator Cycle Sequencing kit (Applied Biosystems), and analyzed on an ABI 373A DNA sequencer (Applied Biosystems).

Mutation Detection in cDNA and Genomic DNA of a SCOT-Deficient Patient

We amplified cDNA fragments by using SCOT-78 and a modified antisense primer, SCOT-86, 5′-CATCTCCCTCTTTCCGaATT-3′, that spans positions 868–849 and contains a single mismatch (shown in lowercase) that creates an *Eco*RI site in fragments containing the S283X mutation but not in those of normal sequence. Amplification was for 40 cycles, as follows: 94°C, 1 min; 50°C, 2 min; and 72°C, 2 min. We also amplified genomic DNA with these primers, performing 40 cycles (94°C, 1 min; 50°C, 2 min; and 72°C, 3 min). From the amplified genomic fragment (see Results and Discussion) we designed an intronic primer, SCOT-85, 5′-TAACAATGGGAGAGAGCTCA-3′, positioned 89–108 nt upstream of the acceptor splice junction. With this primer and the above mismatched primer, we amplified the region of the S285X mutation from genomic DNA, using the following conditions for 40 cycles: 94°C, 1 min; 50°C, 2 min; and 72°C, 2 min.

Results and Discussion

Cloning and Analysis of Human-Heart SCOT cDNA

We identified five human SCOT cDNA clones among 500,000 plaques screened in the human-heart cDNA library (fig. 1). Together, these clones span residues 313–3216. To determine the extremities of the SCOT mRNA, we amplified and sequenced the products of primer extension. The human SCOT cDNA (fig. 1) extends 5′ to residue −98, contains a 1,560-bp open reading frame and a 1,662-bp 3′ UTR ending in a 17-residue polyadenylate tract. A polyadenylation consensus sequence AAUAAA (Wahle and Keller 1992) is located 16 nt upstream of the poly(A) tract. Since the N-terminus of mature porcine SCOT is at Thr 40 (Lin and Bridger 1992), we predict that mature human SCOT spans Thr 40 to Asn 520 and has a molecular mass of 52,096 daltons, and the mitochondrial leader of 4,068 daltons. During the course of our work, three SCOT subclones were submitted to GenBank as expressed sequence tags: clone a02012t (GenBank accession no. T19124, residues −50 to 251 of the human SCOT cDNA); yf76g04.r1 (GenBank R13381, residues 1401 to 1800) and EST110696 (GenBank H34111, residues 774 to 1114).

The residues surrounding the translation initiation codon of human SCOT, CGAAGATGG differ somewhat from the Kozak translation initiation site consensus CAGCCATGG, but contain a purine in the −3 position and a guanidine at +4, which are the most conserved nucleotides of the motif (Kozak 1987). The mitochondrial leader of human SCOT contains a predominance of dibasic residues (6/39, 15.4%) and no acidic amino acids, as do most mitochondrial leaders (von Heijne 1986). The presence of Phe and Ser residues, respectively, 8 and 5 positions before the cleavage site at position 39, suggests that the SCOT precursor may be sequentially cleaved by processing peptidase and mitochondrial intermediate peptidase (Kalousek et al. 1992) on mitochondrial entry.

Northern Analysis

We analyzed RNA from four different human sources: heart and the hepatoma-derived cell line HepG2 (fig. 2), as well as lymphoblasts and fibroblasts (not shown). A single ~3.2-kb message was present in heart and lymphoblasts. This was present but barely detectable in fibroblasts and undetectable in HepG2 RNA. In mammalian tissues, SCOT activity is highest in heart, followed in order by kidney, brain, and muscle, whereas in liver SCOT is undetectable (Williamson et al. 1971; Williamson, 1992). Our observations suggest that in humans the tissue-specific differences of SCOT activity correlate with the levels of the SCOT mRNA.

We observed that pig-heart SCOT mRNA is ~3.9 kb long (not shown). In contrast, the reported pig SCOT cDNA is only 1,851 nt long. In comparison with the human SCOT cDNA, the reported pig-heart SCOT cDNA lacks ~2 kb from the 3′ UTR (Lin and Bridger 1992) and has no polyadenylation consensus site. Therefore, the published sequence of the pig SCOT cDNA may be truncated at the 3′ end.

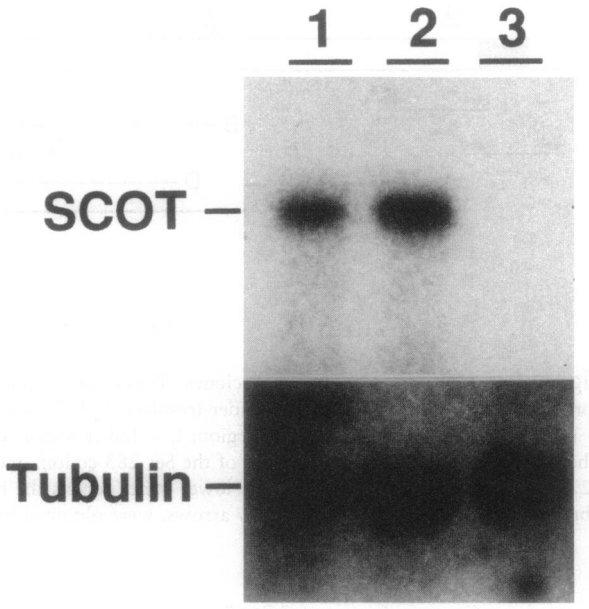

Figure 2 Northern blot analysis of human SCOT. Filters with total RNA from human heart, 5 μg (lane 1) and 10 μg (lane 2), and the human hepatoma derived cell line HepG2, 5 μg (lane 3), were probed with the radiolabeled SCOT cDNA (*upper panel*), stripped, and reprobed with an alpha tubulin cDNA (*lower panel*).

Sequence Comparison of SCOT with Other CoA Transferases

The coding regions of the human and pig SCOT cDNAs are identical at 1407/1560 residues (90.1%), and the amino acid identity is 483/520 (92.8%), similar to the conservation of other metabolic enzymes among mammals (Mitchell et al. 1993; Boukaftane et al. 1994; Doolittle et al. 1996).

The deduced sequence of the SCOT-like peptide from *C. elegans* is shown in figure 3 and is similar to mammalian SCOT in the region of the mature peptide, 315/483 residues (65.2%) being identical. The N-terminus of the *C. elegans* SCOT peptide contains six dibasic residues, consistent with a mitochondrial leader, but differs greatly from mammalian SCOT leaders and contains an in-frame methionine codon that potentially could initiate translation, immediately before the cleavage point of the mitochondrial leaders. Little is known about the presence of ketone body metabolism in *C. elegans*. The substrates and localization of the SCOT-like peptide will have to be determined directly.

We aligned the sequences of the mammalian SCOT peptides with those of three other CoA transferases of bacterial origin (fig. 3). Bacterial CoA transferases exist

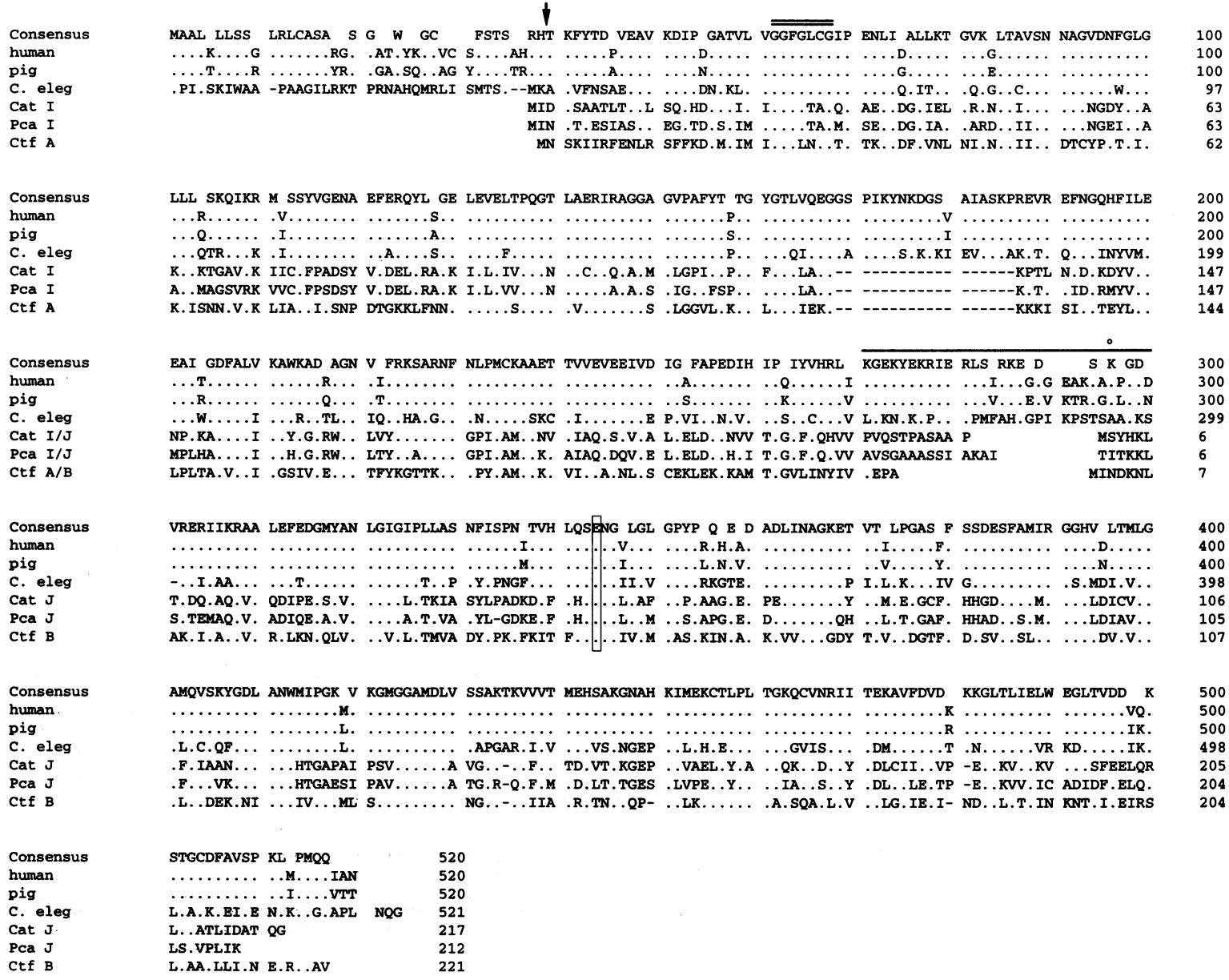

Figure 3 Peptide sequence comparison of known CoA transferases. The consensus sequence indicates residues identical in human and pig SCOT. Below this are aligned the sequences of human and pig SCOT, of the SCOT-like *C. elegans* peptide, and of the bacterial CoA transferases described in Material and Methods. In these sequences, only residues differing from the mammalian consensus are specified, and the positions of identical residues are indicated by periods (.). Deletions with respect to the mammalian consensus are indicated by a dash (–). Insertions with respect to mammalian SCOT sequences (not shown) are as follows: Thr-Glu in the *C. elegans* SCOT-like peptide between mammalian SCOT residues 176 and 177; Arg-Gln in all Cat I and Pca I between positions 116 and 117; and Gly (Pca J) and Asp (Cat J) between residues 418–419. Other landmarks include the cleavage site of the mitochondrial leader (*vertical arrow*), the conserved glycine cluster (*double overscore*), the linker region (*single overscore*) containing the proteolytic cleavage site (*open circle*), and the active-site glutamate (*boxed*).

Figure 4 SCOT gene mapping by in situ hybridization. The SCOT locus, OXCT, maps to chromosome 5p13.

as hetero-oligomers of two subunits. The N-terminal subunit of each aligns to within two residues of the first residue of mature mammalian SCOT and terminates at residue 274 (CtfA) to 284 (Pca I). The alignment of the other subunits of the bacterial CoA transferases begins at residues 294–295 of mammalian SCOT. Between residues 38 and 231 of human SCOT, the sequence identity with each of the three bacterial CoA transferases varies from 40% to 43%; from human SCOT residues 294–508, it is 43.9%–53.7%.

The sequence alignment in figure 3 highlights regions of SCOT with proved or potential functional importance. The second-longest region of amino acid identity common to all CoA transferases is a tetrapeptide, SENG, at residues 343–346 of the human SCOT cDNA, which includes the CoA–binding Glu 344 residue (Rochet and Bridger 1994). The longest conserved region, the hexapeptide GGAMDL at human SCOT residues 424–429, has no known functional correlates. The glycines clustered at residues 62, 63, and 68 are perfectly conserved in all CoA transferases. A similar region is also present in the βαβ region of the ADP-binding fold of enzymes with NAD or FAD cofactors (Wierenga et al. 1986), suggesting that this region may interact with the adenine moiety of CoA (Parales and Harwood 1992).

In contrast, residues 280–300 of mammalian SCOT are poorly conserved or absent in the bacterial CoA transferases. This region presumably serves to link the two catalytically active domains. Of note, this region contains the Ala 295–Lys 296 dipeptide known to be cleaved during proteolytic digestion of purified SCOT in vitro, a reaction that does not abolish SCOT enzyme activity (Lin and Bridger 1992).

Chromosomal Mapping of the Human SCOT Gene

Analysis of 200 silver grains (fig. 4) following in situ hybridization revealed that 29 (14.5%) clustered on the short arm of chromosome 5 in the region 5p12-13 ($P < .0001$), suggesting that the human SCOT locus (designated OXCT, for 3-oxoacid-CoA-transferase) maps to this region.

Description of the SCOT-Deficient Patient

The early course of the patient has been described before (Perez-Cerda et al. 1992). In brief, she was born to first-cousin parents of Spanish descent. She developed ketoacidosis at 36 h of life and has had several other ketotic episodes associated with fasting, infections, stress, or prolonged physical exertion. When reevaluated at the age of 6 years 10 mo, her growth, intelligence, and physical examination including fundoscopy were normal, as were cerebral magnetic resonance imaging and electroencephalography. A metabolic profile obtained after a 9-h fast revealed the following values of circulating metabolites: 3-hydroxybutyrate, 1.6 mM (normal <0.4 mM); acetoacetate, 0.73 mM (normal, <0.3 mM), with normal values of plasma glucose, total carnitine, ammonia, lactate, and pyruvate. Urinary organic acids at this time (expressed as mmol/mol creatinine) showed elevated levels of 3-hydroxybutyrate (3,479; normal <100), acetoacetate (791; normal <2), and 3-hydroxyisovalerate (205; normal <46). Cur-

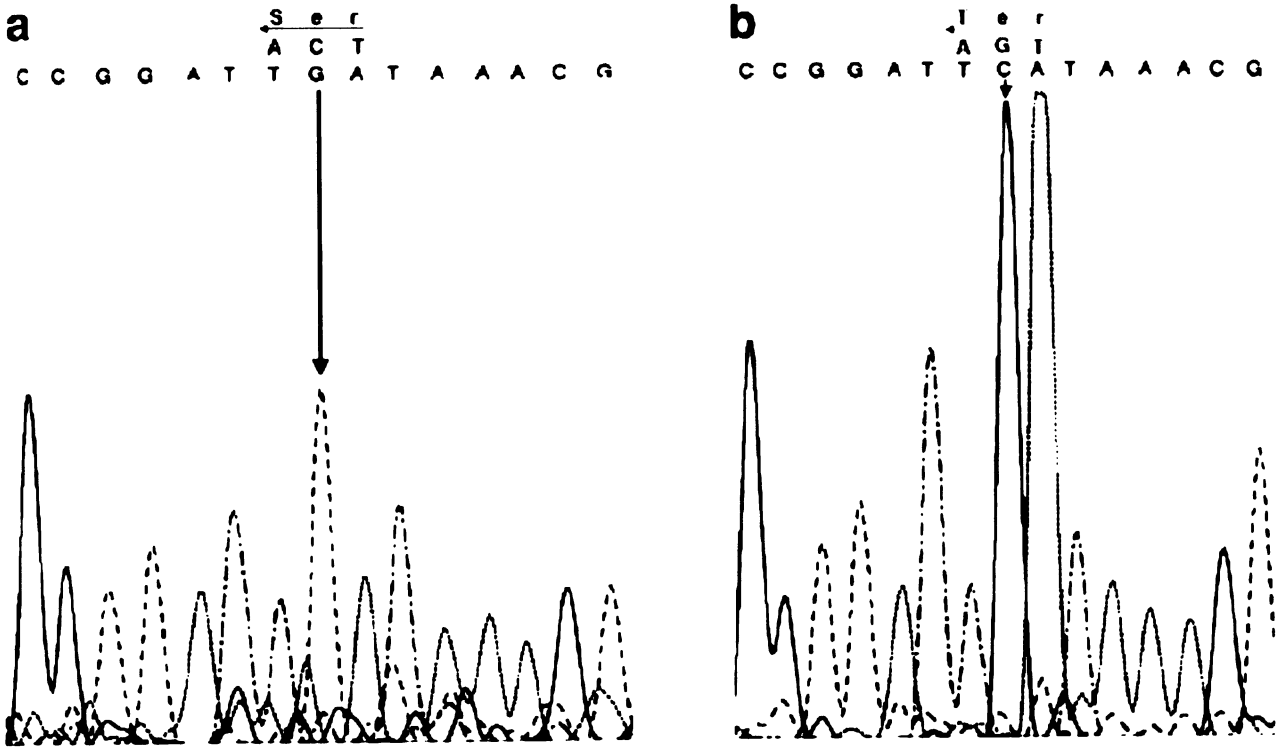

Figure 5 Sequences of (*a*) normal and (*b*) mutant amplified genomic DNAs. The sequence is derived from the noncoding strand.

rently, her only treatment is avoidance of fasting for longer than 6 h.

Molecular Analysis of SCOT Deficiency

A full-length SCOT cDNA was amplified from the fibroblast RNA of the SCOT-deficient patient and her parents. The yield of SCOT cDNA from the patient was much lower than from the parents. In contrast, a control cDNA (3-oxothiolase) was amplified with equal efficiency from samples of the patient and her parents. Two SCOT cDNA clones from the patient contained a C-to-G transversion at position 848, which substitutes a stop codon (TGA) for the normal Ser 283 codon (TCA) and hence is designated S283X (fig. 5).

We amplified cDNA fragments from the patient and her parents, using a modified oligonucleotide, which creates an *Eco*RI site in products containing the S283X mutation but not in fragments of normal sequence. cDNA amplified from the patient was completely digested with *Eco*RI (data not shown), suggesting that her SCOT cDNA was homogeneous for the S283X mutation. In contrast, digestion of cDNAs from the parents indicated that only a small fraction of their mRNA contained S283X (not shown).

These results, as well as the history of parental consanguinity, suggested that the parents are each S283X heterozygotes but that the S283X-containing SCOT transcripts are present in smaller amounts than normal SCOT mRNA. This is consistent with the presence of a premature termination mutation, which in other systems (Brody et al. 1992*a;* Fukao et al. 1994) has been shown to reduce mRNA stability. By extension, it also suggests that S283 is not located in the final two exons of the SCOT gene, since such mRNAs tend to be normally stable (Baserga and Benz 1988; Urlaub et al. 1989; Cheng et al. 1990; Brody et al. 1992*b*).

We studied genomic DNA in order to conclusively demonstrate segregation of S283X in this family. Since the genomic structure of the human SCOT gene is unknown, we attempted to amplify genomic DNA using the same oligonucleotides as for cDNA amplification. This yielded a 1.7-kb fragment that contains an intron between cDNA positions 840 and 841 (fig. 6*a*). The donor and acceptor sequences score 94 and 81, respectively, on the scale of Shapiro and Senapathy (1987), suggesting they could function efficiently in splicing. To amplify genomic DNA from the patient and her parents, we used a sense intronic primer and the mismatched exonic oligonucleotide described above in order to obtain a 146-bp fragment, which we then digested with *Eco*RI (fig. 6*b*). The patient's fragment was completely digested, whereas about half of that amplified from each parent was cleaved, proving the patient to be a S283X homozygote and her parents to be S283X heterozygotes.

The deduced sequence of the S283X-containing SCOT cDNA predicts the mutant peptide to be trun-

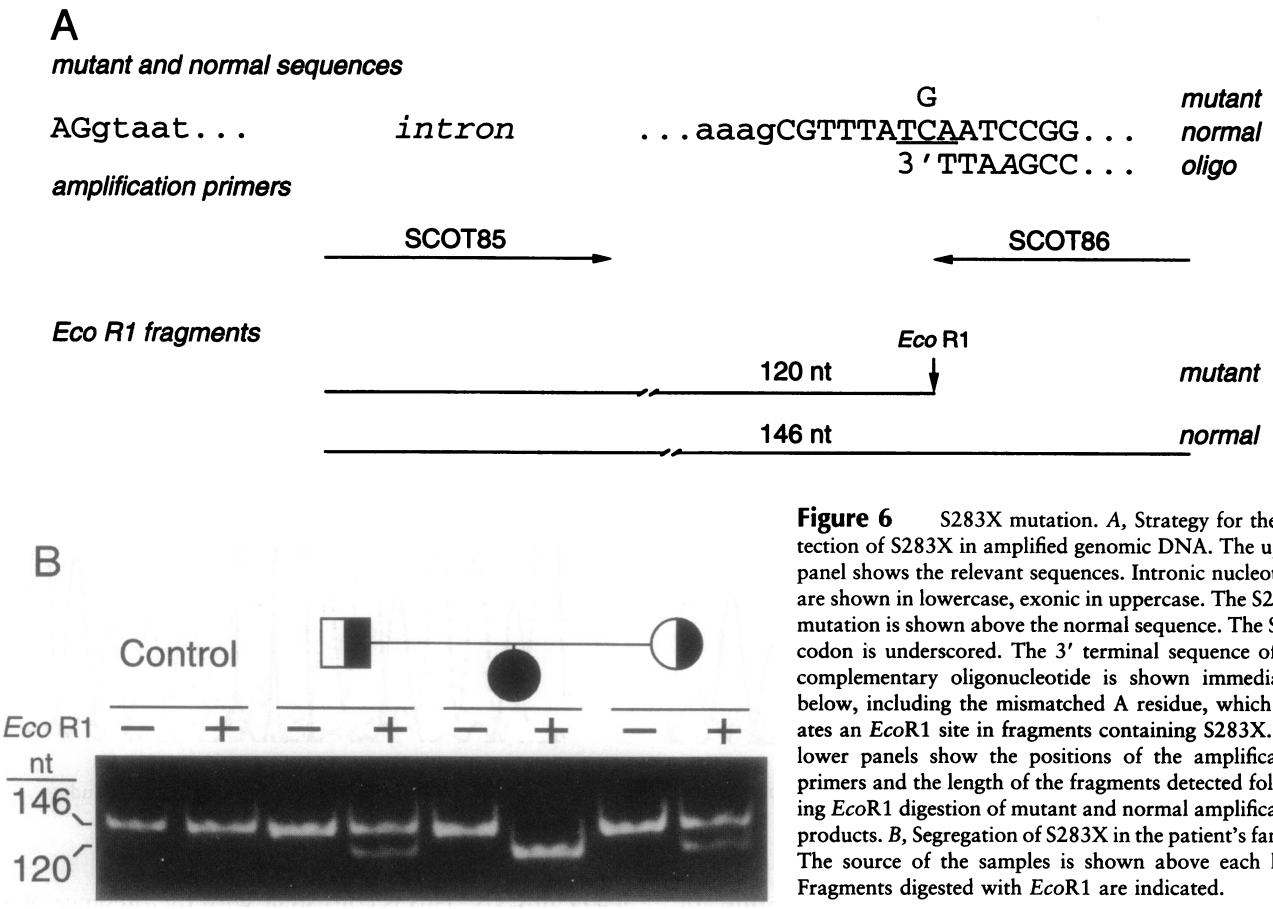

Figure 6 S283X mutation. *A*, Strategy for the detection of S283X in amplified genomic DNA. The upper panel shows the relevant sequences. Intronic nucleotides are shown in lowercase, exonic in uppercase. The S283X mutation is shown above the normal sequence. The S283 codon is underscored. The 3′ terminal sequence of the complementary oligonucleotide is shown immediately below, including the mismatched A residue, which creates an *Eco*R1 site in fragments containing S283X. The lower panels show the positions of the amplification primers and the length of the fragments detected following *Eco*R1 digestion of mutant and normal amplification products. *B*, Segregation of S283X in the patient's family. The source of the samples is shown above each lane. Fragments digested with *Eco*R1 are indicated.

cated and to lack 237 carboxy-terminal amino acids including the catalytically essential Glu 344 residue (fig. 3). This strongly suggests that the S283X SCOT peptide would be devoid of SCOT activity even if it were stable.

It is interesting that assay of SCOT in cells from this patient revealed a residual SCOT activity 23% of control (Peréz-Cerdá et al. 1992). Such a high level of residual activity is unusual for a recessively inherited enzymopathy, but similar results have been reported in other SCOT-deficient patients (Middleton et al. 1987; Saudubray et al. 1987). In the case of the patient reported here, there are at least two possible explanations for the finding of residual activity. First, the exon containing S283X may be skipped, and the resulting mutant SCOT peptide might contain a deletion that allows some residual catalytic activity. Although we can not formally eliminate this possibility, we feel it is unlikely, since no truncated fragments were observed following amplification of SCOT cDNA from the patient's cells. Although alternate splicing may occur at low frequency, it is unlikely that such a low abundance of truncated mRNA would generate a substantial amount of peptide. A second explanation, more probable in our opinion, is that substantial background activity is present in the SCOT assay due to spontaneous hydration of the substrate acetoacetyl-CoA and/or to the presence of 3-oxothiolases and possibly of acetyl CoA deacylase in fibroblasts, which can also use acetoacetyl CoA as a substrate and which are only partially inhibited with available agents. Background activity is proportionally higher in tissues with a low level of SCOT activity such as fibroblasts.

This observation suggests that rigorous determination of residual SCOT activity in SCOT-deficient patients may be best performed with mutation detection and expression studies of the mutant peptide. This will be essential for testing of missense mutations in SCOT, because the biological effect of these mutations is otherwise difficult to assess. We have discovered one such mutation (Kassovska-Bratinova et al. 1995), which changes the relatively conserved C456 codon (fig. 3) to a phenylalanine codon, and are currently studying this in an expression system.

Although the patient reported here has a marked tendency to develop ketosis, she has had a mild course in comparison with others who experienced fatal ketoacidotic episodes. The demonstration of a null mutant in this patient suggests that epigenic or environmental factors are important determinants of clinical severity in hereditary SCOT deficiency and possibly in other ketotic states such as uncontrolled diabetes.

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