Report

Mutations in the Gene Encoding Peroxisomal Sterol Carrier Protein X (SCPx) Cause Leukencephalopathy with Dystonia and Motor Neuropathy

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In this report, we describe the first known patient with a deficiency of sterol carrier protein X (SCPx), a peroxisomal enzyme with thiolase activity, which is required for the breakdown of branched-chain fatty acids. The patient presented with torticollis and dystonic head tremor as well as slight cerebellar signs with intention tremor, nystagmus, hyposmia, and azoospermia. Magnetic resonance imaging showed leukencephalopathy and involvement of the thalamus and pons. Metabolite analyses of plasma revealed an accumulation of the branched-chain fatty acid pristanic acid, and abnormal bile alcohol glucuronides were excreted in urine. In cultured skin fibroblasts, the thiolytic activity of SCPx was deficient, and no SCPx protein could be detected by western blotting. Mutation analysis revealed a homozygous 1-nucleotide insertion, 545_546insA, leading to a frameshift and premature stop codon (I184fsX7).

To date, several peroxisomal fatty-acid oxidation defects due to deficiency of a single enzyme and/or protein in the peroxisome have been identified, including X-linked adrenoleukodystrophy (MIM 300100), Refsum disease (MIM 266500), and α-methylacyl-CoA racemase (AMACR) deficiency (MIM 604489). Peroxisomes play an important role in the breakdown of very-long-chain fatty acids (VLCFAs), polyunsaturated fatty acids, and branched-chain fatty acids, like phytanic and pristanic acid, but also have a role in the formation of primary C_{24}-bile acids by β-oxidation of the bile-acid intermediates di- and trihydroxycholestanoic acids (DHCA and THCA).[1] Peroxisomes in mammals contain two sets of β-oxidation enzymes, which differ in substrate specificity[1] (fig. 1). Straight-chain acyl-CoA oxidase (SCOX) is responsible for the initial oxidation of VLCFAs, whereas branched-chain acyl-CoA oxidase (BCOX) oxidizes the branched-chain acyl-CoAs pristanoyl-CoA, DHC-CoA, and THC-CoA. The enoyl-CoA esters of both straight- and branched-chain fatty acids are then hydrated and subsequently dehydrogenated by the same enzyme: D-bifunctional protein (DBP). The second multifunctional protein in the peroxisome, L-bifunctional protein, is involved in the degradation of long-chain dicarboxylic acids, but these acids are also handled by DBP.[2] The last step of the β-oxidation process, thiolytic cleavage, is performed by sterol carrier protein X (SCPx) in the case of branched-chain substrates, whereas straight-chain substrates are most likely handled by both SCPx and the classic 3-ketoacyl-CoA thiolase.[1] In addition to the β-oxidation enzymes, AMACR is required for β-oxidation of branched-chain substrates. Since the peroxisomal β-oxidation system is stereospecific and only accepts *S* isomers as substrate—and pristanic acid naturally occurs as a mixture of two different diastereomers—(2*R*)-pristanic acid must first be converted to its *S* isomer before it can be degraded via β-oxidation (fig. 1). AMACR is responsible for this conversion and also converts (25*R*)-DHCA and (25*R*)-THCA, formed exclusively from cholesterol, into their respective *S* isomers. Consequently, patients with a deficiency of AMACR accumulate pristanic acid, DHCA, and THCA.

In addition to patients with an isolated deficiency of SCOX (MIM 264470) or DBP (MIM 261515),[1] a patient with a tentative peroxisomal 3-ketoacyl-CoA thiolase deficiency has been described, but reinvestigation of this patient revealed the true defect to be at the level of DBP.[3] All patients with a peroxisomal fatty-acid ox-

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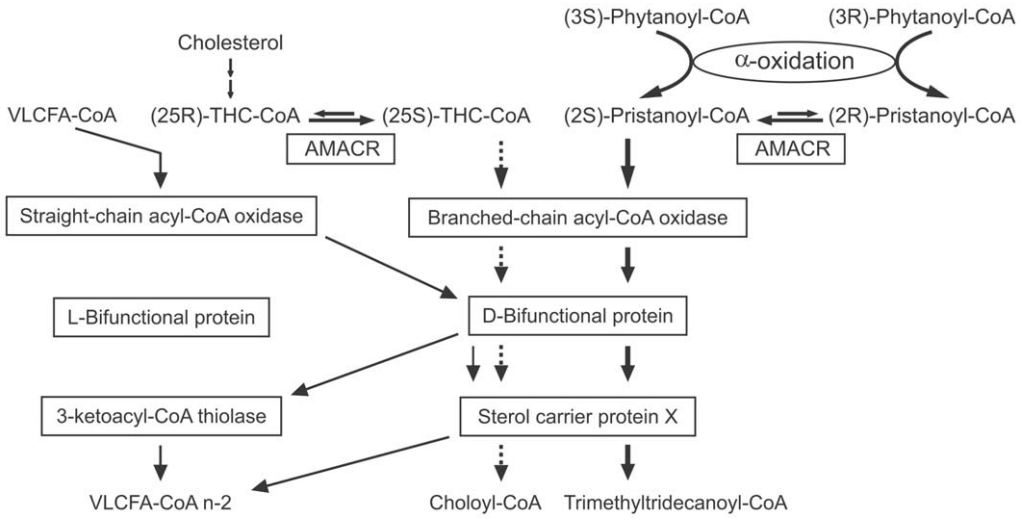

Figure 1 Schematic representation of the fatty-acid β-oxidation machinery in human peroxisomes catalyzing the oxidation of VLCFA-CoAs and branched-chain fatty acyl-CoAs (pristanoyl-CoA and THC-CoA). Because BCOX can handle only *S* isomers, (2*R*)-pristanoyl-CoA and (25*R*)-THC-CoA first have to be converted to their *S* isomers by AMACR. Subsequently, the branched-chain fatty acyl-CoAs are oxidized by BCOX, DBP, and SCPx, whereas oxidation of VLCFA-CoAs involves SCOX, DBP, and both 3-ketoacyl-CoA thiolase and SCPx. "VLCFA-CoA n-2" represents VLCFA-CoA shortened by two carbon atoms after one β-oxidation cycle.

idation defect present with neurological symptoms. In most of the disorders, the neurological symptoms are present from birth or childhood, but, in the majority of patients with Refsum disease and AMACR deficiency, these symptoms develop only after adolescence.

The patient we describe here, a 45-year-old white man, was admitted to the hospital with a 28-year history of dystonic head tremor and spasmodic torticollis. The patient reported that he had noticed a stutter for the first time when he was a 7-year-old boy. At age 17 years, he observed a spasmodic torticollis to the left side, with a dystonic head tremor in stressful situations. At age 29 years, he consulted a urologist for a fertility checkup, and hypergonadotrophic hypogonadism and azoospermia were diagnosed. The 44-year-old brother of the patient was fertile and did not have any neurological symptoms, whereas the 41-year-old brother was reported to have similar neurological complaints as the patient. According to his mother, he is infertile and stutters; however, he declined to undergo neurological examination or further diagnostic tests.

During the past several years, the dystonic symptoms in the patient had worsened, and, when he was age 44 years, additional clinical and laboratory investigations were performed. Cranial magnetic resonance imaging (MRI) showed bilateral hyperintense signals in the thalamus, butterfly-like lesions in the pons, and lesions in the occipital region, without gadolinium enhancement (fig. 2). Neurological examination revealed hyposmia, pathological saccadic eye movements, and a slight hypoacusis, but it was otherwise a normal cranial nerve examination. There were brisk deep-tendon reflexes of the upper extremities but diminished reflexes of the lower extremities, plantar sole responses, no pareses, normal superficial sensation (pin-prick, light touch, and temperature), and a reduced vibration sense of 4/8 at the lateral malleoli. There were signs of slight cerebellar ataxia with a left-sided intention tremor, balance and gait impairment, and a slight left-sided rebound phenomenon. Ophthalmologic investigation revealed no abnormalities.

A complete blood count, coagulation analysis, and serum electrolytes and liver enzyme levels were normal. Serum cholesterol was slightly elevated at 5.7 mmol/liter. Copper and ceruloplasmin levels in serum and urine were within the normal range. A lumbar puncture yielded clear, colorless cerebrospinal fluid (CSF) at normal pressure. The CSF contained 1 cell/μl, and the protein, lactate, and glucose concentrations were normal. Because of the absence of pleiocytosis and the negative results for oligoclonal bands, borrelia serology, and virological examination of CSF, an inflammatory central nervous process was considered to be very unlikely.

Nerve conduction studies of the lower extremities showed a predominantly motor and slight sensory neuropathy, with conduction blocks in the tibial nerves, reduced motor action potentials in the left peroneal nerve, and a reduced amplitude of the left sural nerve (table 1). Nerve conduction velocities of the right median nerve displayed a reduced conduction velocity, normal F-wave latencies, and normal motor action potentials. The motor-evoked potentials of the abductor digiti minimi mus-

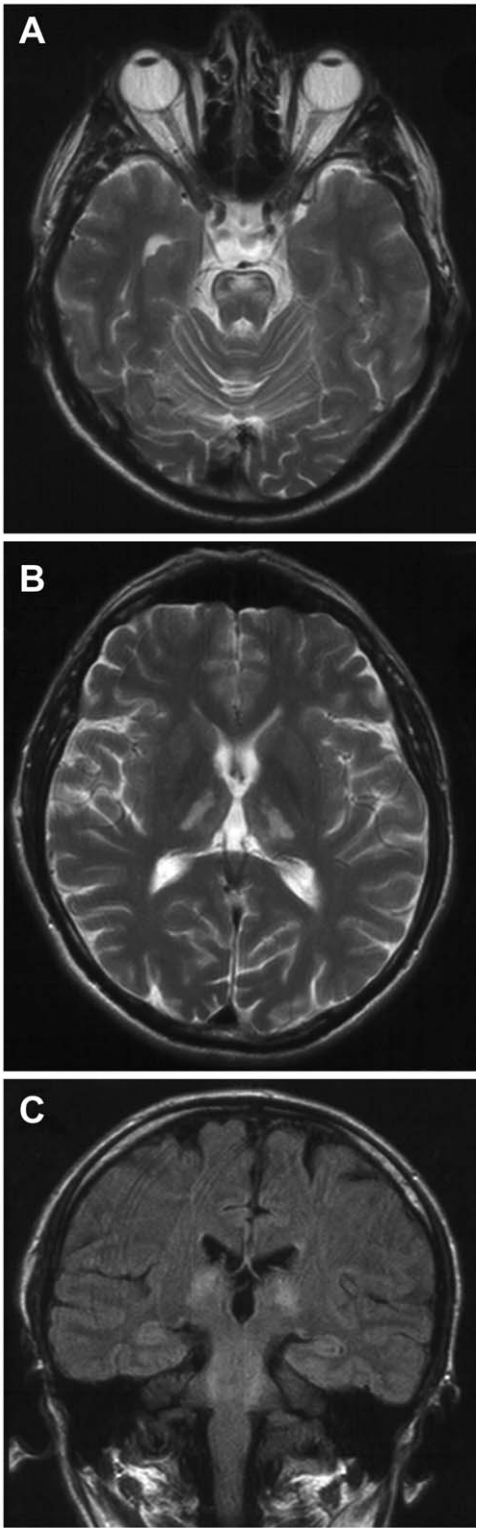

Figure 2 *A*, Axial T2-weighted MRI showing bilateral pons lesions. *B*, Axial T2-weighted MRI showing bilateral intense signals in the thalamus. *C*, Coronal T1-weighted MRI showing bilateral involvement of the pons and thalamus.

cle were prolonged and revealed double-sided lesions of the pyramidal tract to the upper extremity. After cortical stimulation, motor-evoked potentials of the tibial anterior muscle were abolished, also suggesting bilateral pyramidal lesions to the legs. The sensory-evoked potentials showed bilateral lesions of the posterior funiculus of the spinal cord. The bilateral latencies of peak V of the acoustic-evoked potentials and the interpeak latency between peaks I and V and between peaks III and V were prolonged, corresponding to a bilateral pontine lesion (table 2). The blink reflex, with a reproducible prolonged R2c latency after left stimulation, was compatible with a medullary lesion. Nystagmography showed a spontaneous nystagmus to the left side, which could be suppressed by fixation; square wave jerks and slowed saccades could be detected, indicating vestibulo-cerebellar lesions. Extracranial and transcranial Doppler sonography showed normal results.

Because of a clinical suspicion of AMACR deficiency, the plasma levels of the branched-chain fatty acids pristanic acid and phytanic acid were measured. The level of pristanic acid was markedly increased (39.8 μmol/liter; control range 0–3.1 μmol/liter), and the level of phytanic acid was slightly increased (10.1 μmol/liter; control range 0–9 μmol/liter), whereas the level of the straight-chain fatty acid C26:0 was just above the highest value of the normal range (table 3). Traces of the bile acid intermediates DHCA and THCA were detectable in both plasma and urine. Subsequent investigations in cultured skin fibroblasts of the patient revealed a strongly reduced β-oxidation activity with pristanic acid as substrate (131 pmol/h/mg; control range 691–2,178 pmol/h/mg), normal C26:0 β-oxidation activity (1,048 pmol/h/mg; control range 1,025–2,994 pmol/h/mg), and normal C26:0 level (0.31 μmol/g; control range 0.18–0.38 μmol/g). The biochemical abnormalities were indicative of AMACR deficiency, but, surprisingly, direct measurement of AMACR activity in fibroblasts showed no abnormalities. Because of the reduced pristanic acid β-oxidation activity, we measured the activity of all the other enzymes involved in the peroxisomal breakdown of branched-chain fatty acids. BCOX activity was normal, and DBP activity was even increased, but the activity of SCPx, measured as described elsewhere,[4] was completely deficient in fibroblasts of the patient.

SCPx is a 58-kDa protein that consists of an amino-terminal thiolase domain and a carboxy-terminal sterol carrier protein 2 (SCP2) domain.[5,6] After import into peroxisomes, the domains are cleaved, giving rise to a 46-kDa thiolase and a 13-kDa SCP2.[4,7] Immunoblot analysis with an antibody against SCPx revealed the absence of both the full-length 58-kDa SCPx and the 46-kDa thiolase domain of SCPx in fibroblasts of the patient (fig. 3*A*). Because SCP2 also has its own promoter,[5] immunoblot analysis with an antibody against SCP2

Table 1

Electrophysiological Findings in the SCPx-Deficient Patient

A. Nerve Conduction Studies

	Patient			Normal Range		
	Amplitude	NCV (m/s)	F-Wave (ms)	Amplitude	NCV (m/s)	F-Wave (ms)
Tibial nerve right	9.6/3.9[a] mV	44	56.5	>2.6 mV	>40	<51
Tibial nerve left	6.0/2.4[a]mV	45	60.7	>2.6 mV	>40	<51
Peroneal nerve right	2.9/2.9[a]mV	46	ND	>2.7 mV	>40	
Peroneal nerve left	2.2/1.3[a]mV	44	ND	>2.7 mV	>40	
Median nerve right	7 mV	43	25	>5 mV	>50	<26
Sural nerve right	4 μV	42	NA	>4 μV	>41	NA
Sural nerve left	2.4 μV	44	NA	>4 μV	>41	NA

B. Sensory-Evoked Potentials

	Patient			Normal Range		
	P40 (ms)	ERB (ms)	N20 (ms)	P40 (ms)	ERB (ms)	N20 (ms)
Tibial nerve right	NPR	NA	NA	<45.7	NA	NA
Tibial nerve left	NPR	NA	NA	<45.7	NA	NA
Median nerve right	NA	11	24.6	NA	<12.1	<22
Median nerve left	NA	11	24	NA	<12.1	<22

C. Motor-Evoked Potentials

	Patient			Normal Range		
	Cortical (ms)	IF (ms)	F-Wave (ms)	Cortical (ms)	IF (ms)	F-Wave (ms)
TA muscle right	NPR	19	60	<33.6	<17.5	<51
TA muscle left	NPR	18.5	62	<33.6	<17.5	<51
ADM muscle right	23.1	15.3	27.2	<22.2	<15.4	<29
ADM muscle left	23.4	14.4	28	<22.2	<15.4	<29

D. Blink Reflex

	Patient			Normal Range		
	R1	R2	R2c	R1	R2	R2c
Right	11.5	35.6	37.1	<12.2	<39.1	<40.1
Left	11.0	38.1	42.8	<12.2	<39.1	<40.1

Note.—ADM = abductor digiti minimi; IF = intervertebral foramen; NA = not applicable; NCV = nerve conduction velocities; ND = not done; NPR = no potential recordable; TA = tibial anterior.

[a] Shown as distal stimulation/proximal stimulation.

showed the normal presence of the 13-kDa SCP2. The classic peroxisomal 3-ketoacyl-CoA thiolase was also normally present in fibroblasts of the patient, as shown by immunoblot analysis. To confirm the SCPx deficiency at the molecular level, we amplified the SCPx cDNA in three overlapping fragments, followed by sequencing, and found a homozygous 1-nt insertion (of an adenine) at position 545 (545_546insA) (fig. 3*B*) leading to a frameshift and premature stop codon (I184fsX7). Homozygosity was confirmed at the genomic level, and the patient's mother was heterozygous at the genomic level. No biochemical abnormalities were found in plasma or urine from the patient's mother.

Table 2

Acoustic-Evoked Potentials in the SCPx-Deficient Patient

Acoustic-Evoked Potentials	Latency (ms)			Interpeak Latency (ms)		
	I	III	V	I-III	III-V	I-V
Right	1.45	4.05	6.70	2.61	2.64	5.25
Left	1.46	4.12	6.65	2.66	2.52	5.19
Normal range	<1.86	<4.16	<6.23	<2.72	<2.31	<4.67

Because of the elevated pristanic acid levels, the patient began a phytanic acid–restricted diet. After 9 mo of the diet, the phytanic acid levels in plasma were within the normal range and the pristanic acid levels had decreased to 6.2 μmol/liter. Another analysis, performed

Table 3

Biochemical Data on Plasma and Fibroblasts from the Patient and Control Subjects

Sample and Biochemical Measure	Patient	Control Subjects	
		Mean ± SD	Range
Plasma (μmol/liter):			
Phytanic acid	10.1	2.8 ± 2.1	0–9
Pristanic acid	39.8	.3 ± .4	0–3.1
DHCA	.1	.003 ± .014	0–.13
THCA	.1	.007 ± .019	0–.09
C26:0	1.34	.77 ± .19	.46–1.31
Fibroblasts:			
C26:0[a]	.31	.29 ± .06	.18–.38
C26:0 β-oxidation[b]	1,048	1,552 ± 399	1,025–2,994
Pristanic acid β-oxidation[b]	131	1,450 ± 393	691–2,178
Phytanic acid α-oxidation[b]	46	62 ± 23	32–173
AMACR activity[c]	167	99 ± 28	45–149
SCOX activity[c]	293	90 ± 28	49–151
BCOX activity[c]	211	178 ± 79	87–340
DBP-hydratase activity[c]	433	278 ± 88	121–600
DBP-dehydrogenase activity[c]	217	93 ± 53	25–261
SCPx activity[c]	ND[d]	86 ± 28	49–139

[a] μmol/g.
[b] pmol/h/mg.
[c] pmol/min/mg.
[d] Not detectable.

after 14 mo of the diet, showed that the pristanic acid levels had stabilized at a marginally elevated level (8.1 μmol/liter). The levels of C26:0 were completely normal on both occasions (0.54 and 0.68 μmol/liter, respectively). Since the beginning of the diet, no progression of symptoms has been observed. A follow-up cranial MRI showed no increase of the leukoencephalopathy.

Mice with a targeted disruption of the gene encoding SCPx develop ataxia, reduced muscle tone, and peripheral neuropathy (uncoordinated movements, unsteady gait, and trembling) when fed a phytol-enriched diet[8] and thus closely resemble their human counterpart. The present patient also shows striking similarities to AMACR-deficient patients, both at the clinical and at the biochemical level. The most prominent clinical symptom of AMACR deficiency is adult-onset sensory motor neuropathy,[9–11] which was also present in our patient. Similar to a recently described AMACR-deficient patient, our patient showed high signals in the thalamus and pons on MRI. These gray and white matter abnormalities are present not only in SCPx and AMACR deficiencies but also in DBP deficiency.[12] Remarkably, the SCPx-deficient patient does not have retinitis pigmentosa, which is frequently observed in AMACR deficiency and Refsum disease.[9–11,13]

Just like AMACR-deficient patients, our patient accumulated pristanic acid. However, only trace amounts of the bile acid intermediates DHCA and THCA were detectable in plasma and urine. Bile and serum of SCPx knockout mice contain increased levels of 3α,7α,12α-trihydroxy-27-nor-5β-cholestane-24-one, which is an alternative metabolite of the substrate of SCPx, 3α,7α,12α-trihydroxy-24-keto-cholestanoyl-CoA, and is formed by decarboxylation.[14] Interestingly, tandem mass spectrometric analysis of urine from our patient consistently showed large peaks, with m/z 611 and 627,

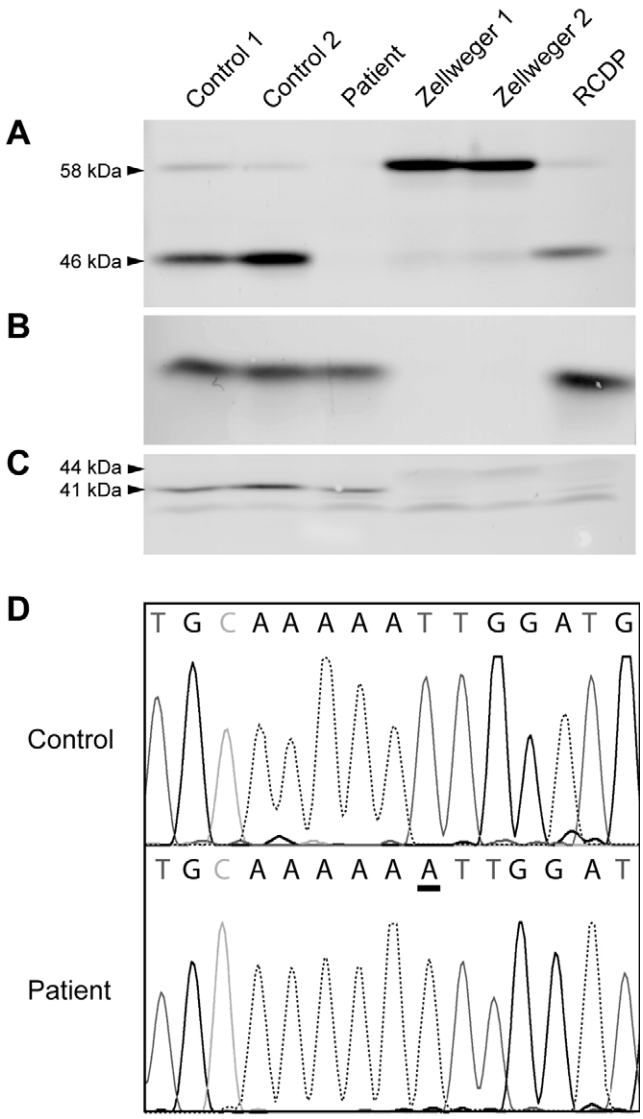

Figure 3 *A–C,* Immunoblot analysis of fibroblasts from two control subjects, the SCPx-deficient patient, two patients with Zellweger syndrome, and one patient with RCDP type 1. *A,* Antibody was used against SCPx. The arrowheads indicate the 58-kDa full-length protein and the 46-kDa thiolase domain of SCPx. *B,* Antibody was used against SCP2 (13 kDa). *C,* Antibody was used against peroxisomal 3-ketoacyl-CoA thiolase. The arrowheads indicate the 44-kDa precursor form and the 41-kDa mature form of 3-ketoacyl-CoA thiolase. *D,* Automated sequence analysis revealed a homozygous insertion of an adenine (545_546insA) at both the cDNA and the genomic level, leading to a frameshift and premature stop codon (I184fsX7).

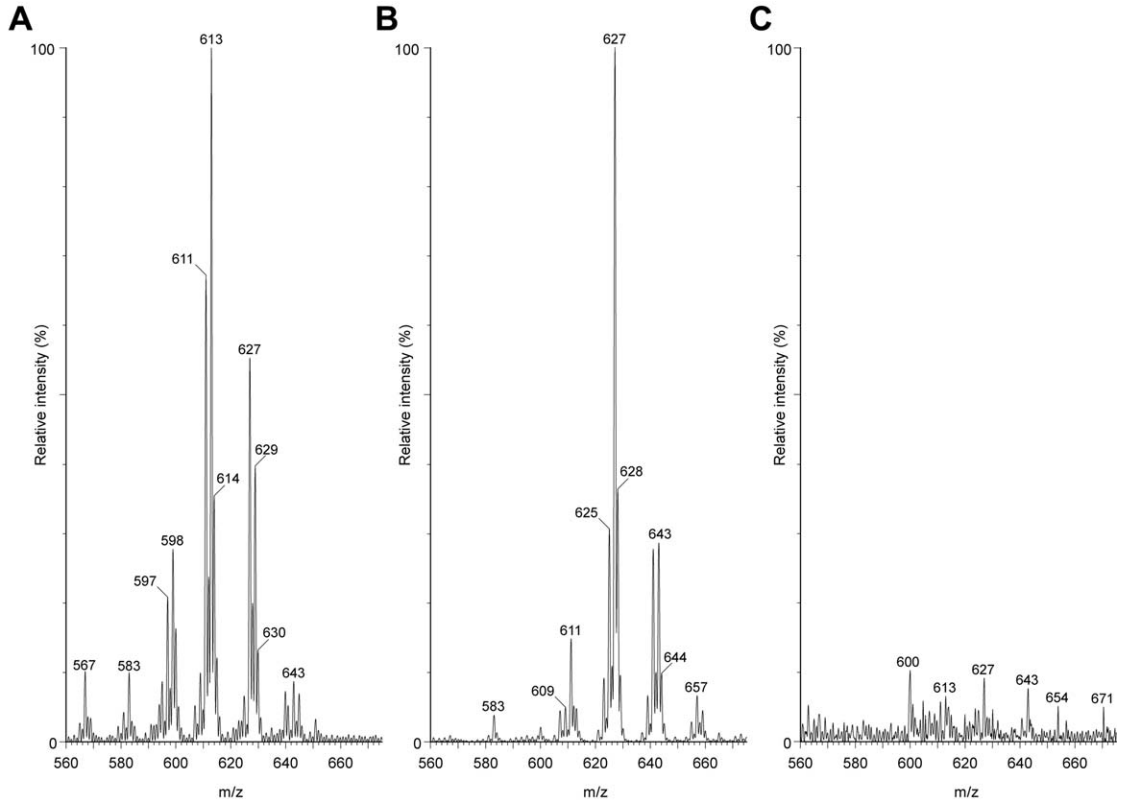

Figure 4 Negative-ion electrospray tandem mass spectrometric analysis of urine bile acids and bile alcohols from the SCPx-deficient patient (*A*), a patient with CTX (*B*), and a control subject (*C*). Abnormal glucuronic acid conjugates of bile alcohols were detected in urine from the SCPx-deficient patient and the patient with CTX. We postulated the identity of the metabolites as follows: m/z 597, 27-nor-5β-cholestanetetrol glucuronide; m/z 611, pentahydroxy-27-nor-5β-cholestane-24-one or 5β-cholestanetetrol glucuronides; m/z 613, 27-nor-5β-cholestanepentol glucuronide; m/z 627, hexahydroxy-27-nor-5β-cholestane-24-one or 5β-cholestanepentol glucuronides; m/z 629, 27-nor-5β-cholestanehexol glucuronide; and m/z 643, heptahydroxy-27-nor-5β-cholestane-24-one or 5β-cholestanehexol glucuronides.

which most likely correspond to pentahydroxy-27-nor-5β-cholestane-24-one and hexahydroxy-27-nor-5β-cholestane-24-one glucuronides, respectively (fig. 4). In addition, compounds with m/z 613 and 629 were abundantly present, which most likely correspond to 27-nor-5β-cholestanepentol and 27-nor-5β-cholestanehexol glucuronides, respectively (fig. 4). This may be considered indirect evidence of the inability to metabolize 3α,7α,12α-trihydroxy-24-keto-cholestanoyl-CoA and is in full agreement with the findings in SCPx knockout mice.[14] Furthermore, the presence of large amounts of bile alcohols in the patient's urine suggests that the block in bile acid biosynthesis at the level of SCPx may initiate an alternative pathway for bile acid biosynthesis not requiring a peroxisomal β-oxidation step—that is, the microsomal 25-hydroxylase pathway.[15] Metabolites of this pathway, bile alcohols, are also found in patients with cerebrotendinous xanthomatosis (CTX [MIM 213700])[16] (fig. 4), who have a deficiency of the mitochondrial 27-hydroxylase, which is a step upstream of SCPx in bile acid biosynthesis. In plasma of the SCPx-deficient patient, 3α,7α,12α-trihydroxy-27-nor-5β-cholestane-24-one could not be detected, most likely because this compound is not ionized efficiently and therefore cannot be detected using our tandem mass spectrometric method.

In vitro studies have shown that both SCPx and the classic 3-ketoacyl-CoA thiolase can handle VLCFAs as substrates.[1] Patients with rhizomelic chondrodysplasia punctata (RCDP) type 1 (MIM 215100) lack 3-ketoacyl-CoA thiolase in their peroxisomes because of a defect in *PEX7,* the gene encoding the peroxisome-targeting signal 2 receptor.[17,18] Although 3-ketoacyl-CoA thiolase is mistargeted in RCDP type 1, no abnormality in VLCFA β-oxidation has been observed in patients,[19,20] which suggests that SCPx can take over its function for the straight-chain fatty acyl-CoAs. The fact that our SCPx-deficient patient had normal levels of VLCFAs suggests that the classic 3-ketoacyl-CoA thiolase also can maintain normal VLCFA β-oxidation activity in the absence of SCPx.

In conclusion, we have identified the first known pa-

tient with SCPx deficiency. This patient presented with dystonia, a predominant motor neuropathy, advanced leukoencephalopathy with cerebellar signs, and involvement of the pyramidal tract and the posterior columns. Our findings imply that the group of single peroxisomal β-oxidation enzyme deficiencies extends to AMACR, SCOX, DBP, and SCPx deficiencies. The elucidation of the true defect in this case has increased our knowledge of the peroxisomal β-oxidation system and its substrates and of the clinical consequences of metabolic derangements associated with defects in the peroxisomal β-oxidation system.

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Web Resources

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for X-linked adrenoleukodystrophy; Refsum disease; AMACR, SCOX, and DBP deficiencies; CTX; and RCDP type 1)

References

1. Wanders RJA, Barth PG, Heymans HSA (2001) Single peroxisomal enzyme deficiencies. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The molecular and metabolic bases of inherited disease. McGraw-Hill, New York, pp 3219–3256
2. Ferdinandusse S, Denis S, Van Roermund CWT, Wanders RJA, Dacremont G (2004) Identification of the peroxisomal β-oxidation enzymes involved in the degradation of long-chain dicarboxylic acids. J Lipid Res 45:1104–1111
3. Ferdinandusse S, van Grunsven EG, Oostheim W, Denis S, Hogenhout EM, IJlst L, van Roermund CWT, Waterham HR, Goldfischer S, Wanders RJA (2002) Reinvestigation of peroxisomal 3-ketoacyl-CoA thiolase deficiency: identification of the true defect at the level of D-bifunctional protein. Am J Hum Genet 70:1589–1593
4. Ferdinandusse S, Denis S, van Berkel E, Dacremont G, Wanders RJ (2000) Peroxisomal fatty acid oxidation disorders and 58 kDa sterol carrier protein X (SCPx): activity measurements in liver and fibroblasts using a newly developed method. J Lipid Res 41:336–342
5. Ohba T, Rennert H, Pfeifer SM, He Z, Yamamoto R, Holt JA, Billheimer JT, Strauss JF 3rd (1994) The structure of the human sterol carrier protein X/sterol carrier protein 2 gene (SCP2). Genomics 24:370–374
6. Ossendorp BC, Van Heusden GP, De Beer AL, Bos K, Schouten GL, Wirtz KW (1991) Identification of the cDNA clone which encodes the 58-kDa protein containing the amino acid sequence of rat liver non-specific lipid-transfer protein (sterol-carrier protein 2): homology with rat peroxisomal and mitochondrial 3-oxoacyl-CoA thiolases. Eur J Biochem 201:233–239
7. Wirtz KW (1997) Phospholipid transfer proteins revisited. Biochem J 324:353–360
8. Seedorf U, Raabe M, Ellinghaus P, Kannenberg F, Fobker M, Engel T, Denis S, Wouters F, Wirtz KW, Wanders RJ, Maeda N, Assmann G (1998) Defective peroxisomal catabolism of branched fatty acyl coenzyme A in mice lacking the sterol carrier protein-2/sterol carrier protein-x gene function. Genes Dev 12:1189–1201
9. Clarke CE, Alger S, Preece MA, Burdon MA, Chavda S, Denis S, Ferdinandusse S, Wanders RJ (2004) Tremor and deep white matter changes in α-methylacyl-CoA racemase deficiency. Neurology 63:188–189
10. Ferdinandusse S, Denis S, Clayton PT, Graham A, Rees JE, Allen JT, McLean BN, Brown AY, Vreken P, Waterham HR, Wanders RJA (2000) Mutations in the gene encoding peroxisomal α-methylacyl-CoA racemase cause adult-onset sensory motor neuropathy. Nat Genet 24:188–191
11. McLean BN, Allen J, Ferdinandusse S, Wanders RJ (2002) A new defect of peroxisomal function involving pristanic acid: a case report. J Neurol Neurosurg Psychiatry 72:396–399
12. Ferdinandusse S, Denis S, Mooyer PAW, Dekker C, Duran M, Soorani-Lunsing RJ, Boltshauser E, Macaya A, Gartner J, Majoie CBLM, Barth PG, Wanders RJA, Poll-The BT (2006) Clinical and biochemical spectrum of D-bifunctional protein deficiency. Ann Neurol 59:92–104
13. Wierzbicki AS, Lloyd MD, Schofield CJ, Feher MD, Gibberd FB (2002) Refsum's disease: a peroxisomal disorder affecting phytanic acid alpha-oxidation. J Neurochem 80:727–735
14. Kannenberg F, Ellinghaus P, Assmann G, Seedorf U (1999) Aberrant oxidation of the cholesterol side chain in bile acid synthesis of sterol carrier protein-2/sterol carrier protein-x knockout mice. J Biol Chem 274:35455–35460
15. Duane WC, Pooler PA, Hamilton JN (1988) Bile acid synthesis in man: in vivo activity of the 25-hydroxylation pathway. J Clin Invest 82:82–85
16. Honda A, Salen G, Matsuzaki Y, Batta AK, Xu G, Hirayama T, Tint GS, Doy M, Shefer S (2005) Disrupted coordinate regulation of farnesoid X receptor target genes in a patient with cerebrotendinous xanthomatosis. J Lipid Res 46:287–296
17. Braverman N, Steel G, Obie C, Moser A, Moser H, Gould SJ, Valle D (1997) Human *PEX7* encodes the peroxisomal PTS2 receptor and is responsible for rhizomelic chondrodysplasia punctata. Nat Genet 15:369–376
18. Motley AM, Hettema EH, Hogenhout EM, Brites P, ten Asbroek AL, Wijburg FA, Baas F, Heijmans HS, Tabak HF, Wanders RJ, Distel B (1997) Rhizomelic chondrodysplasia punctata is a peroxisomal protein targeting disease caused by a non-functional PTS2 receptor. Nat Genet 15:377–380
19. Heymans HS, Oorthuys JW, Nelck G, Wanders RJ, Schutgens RB (1985) Rhizomelic chondrodysplasia punctata: another peroxisomal disorder. N Engl J Med 313:187–188
20. Hoefler G, Hoefler S, Watkins PA, Chen WW, Moser A, Baldwin V, McGillivary B, Charrow J, Friedman JM, Rutledge L, Hashimoto T, Moser HW (1988) Biochemical abnormalities in rhizomelic chondrodysplasia punctata. J Pediatr 112:726–733