

# Homozygous Disruption of P450 Side-Chain Cleavage (*CYP11A1*) Is Associated with Prematurity, Complete 46,XY Sex Reversal, and Severe Adrenal Failure

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**Disruption of the P450 side-chain cleavage cytochrome (P450<sub>scc</sub>) enzyme due to deleterious mutations of the *CYP11A1* gene is thought to be incompatible with fetal survival because of impaired progesterone production by the fetoplacental unit. We present a 46,XY patient with a homozygous disruption of *CYP11A1*.**

The child was born prematurely with complete sex reversal and severe adrenal insufficiency. Laboratory data showed diminished or absent steroidogenesis in all pathways. Molecular genetic analysis of the *CYP11A1* gene revealed a homozygous single nucleotide deletion leading to a premature termination at codon position 288. This mutation will delete

highly conserved regions of the P450<sub>scc</sub> enzyme and thus is predicted to lead to a nonfunctional protein. Both healthy parents were heterozygous for this mutation.

Our report demonstrates that severe disruption of P450<sub>scc</sub> can be compatible with survival in rare instances. Furthermore, defects in this enzyme are inherited in an autosomal-recessive fashion, and heterozygote carriers can be healthy and fertile. The possibility of P450<sub>scc</sub>-independent pathways of steroid synthesis in addition to the current concept of luteoplacental shift of progesterone synthesis in humans has to be questioned. (*J Clin Endocrinol Metab* 90: 538–541, 2005)

**C**HOLESTEROL SIDE-CHAIN CLEAVAGE cytochrome P450 (P450<sub>scc</sub>) is a key regulator of steroidogenesis. P450<sub>scc</sub> is a single mitochondrial enzyme that involves adrenodoxin and nicotinamide adenine dinucleotide phosphate reduced adrenodoxin reductase. P450<sub>scc</sub> catalyzes the conversion of cholesterol to pregnenolone as the first and rate-limiting step in the biosynthesis of all steroid hormones. P450<sub>scc</sub> is necessary, therefore, for fetal and postnatal glucocorticoid, mineralocorticoid, and sex steroid biosynthesis. Disruption of these early steroidogenic pathways leads to accumulation of cholesterol in the adrenal cortex, causing congenital lipoid adrenal hyperplasia (CLAH). CLAH is due to severe impairment of conversion of cholesterol to pregnenolone both in the adrenals and gonads *in utero* and postnatally, resulting in salt wasting and lack of virilization. CLAH is believed to occur as a two-stage event, during which the initial genetic loss is aggravated by subsequent cellular damage due to accumulation of cholesterol esters with gross enlargement of the adrenal glands (1). Recently it was reported that CLAH is not due to defects in P450<sub>scc</sub> but rather to changes in steroidogenic acute regulatory protein (StAR), which facilitates cholesterol entry into the mitochondria to induce an acute response of steroid synthesis (1, 2).

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Abbreviations: CLAH, Congenital lipoid adrenal hyperplasia; P450<sub>scc</sub>, P450 side-chain cleavage cytochrome; SF-1, steroidogenic factor-1; StAR, steroidogenic acute regulatory protein.

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P450<sub>scc</sub> is essential for progesterone synthesis in all steroidogenic tissues in primates. However, several tissues including the placenta maintain steroidogenesis independent of StAR (3). In normal primate pregnancies, the maternal corpus luteum produces progesterone in the first trimester but the fetally derived placenta takes over progesterone production from the second trimester to term (luteoplacental shift) (4). Thus, the current paradigm is that pregnancies with fetal StAR deficiency are not compromised, whereas severe disruption of P450<sub>scc</sub> would be incompatible with term pregnancy in humans, and affected fetuses would not be viable (5). In rabbits, in which the pregnancy is supported by progesterone synthesis by the maternal corpus luteum, a naturally occurring deletion of P450<sub>scc</sub> has been described, demonstrating the phenotype of this deficiency and also proving an autosomal-recessive inheritance mode for the disorder (6). To date, only two patients have been reported with P450<sub>scc</sub> deficiency due to molecular abnormalities of the *CYP11A1* gene. In one patient P450<sub>scc</sub> haploinsufficiency due to a heterozygous mutation was reported (7). In the second patient, compound heterozygous mutations of P450<sub>scc</sub> partially inactivated the enzyme in *in vitro* experiments. However, clinical manifestation of CLAH did not occur until the seventh month of life. Thus, *in vivo* steroidogenesis had been sufficient throughout the neonatal period (8).

P450<sub>scc</sub> is encoded by the *CYP11A1* gene, which is 29 kb long and consists of nine exons and eight introns. The resulting protein is 521 amino acids in length and contains a highly conserved heme binding site as well as a mitochon-

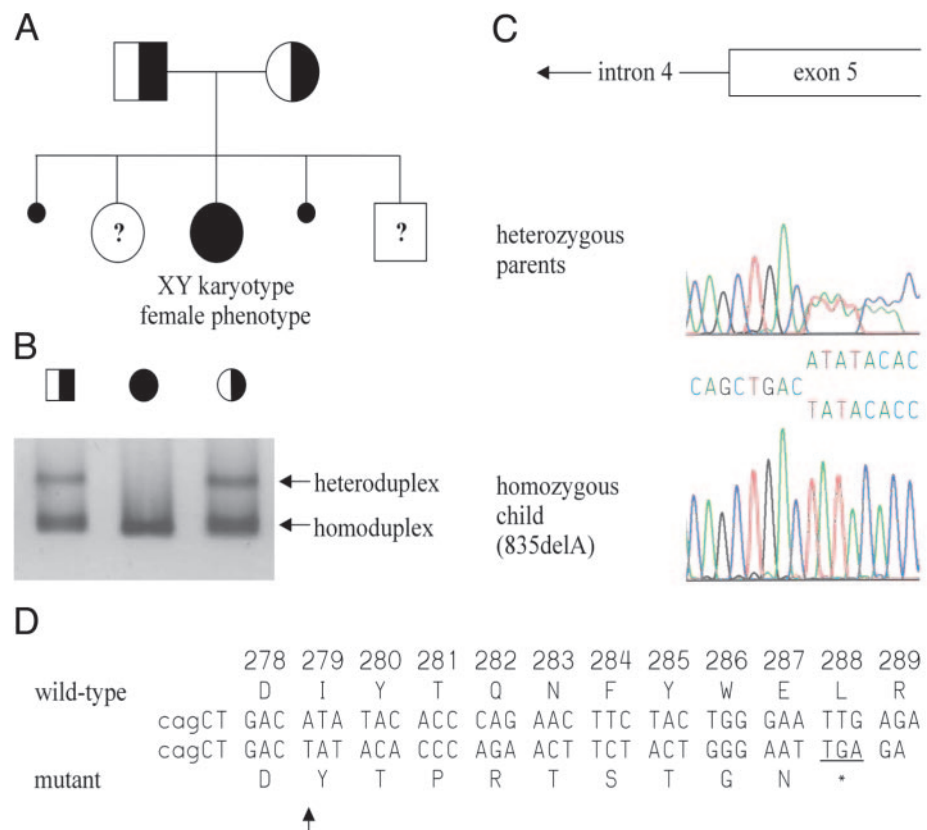
drial transport signal (9). We report the first patient with a homozygous small deletion mutation in *CYP11A1* leading to a premature termination of P450scc. This mutation was inherited in an autosomal-recessive fashion from healthy parents. The resulting enzyme will lack a major part of the protein structure including the heme binding motif and thus be nonfunctional. The child was born prematurely with severe adrenal insufficiency present in the first days of life. Furthermore, the 46,XY patient presented with complete sex reversal. This is the first description of an inherited disruptive mutation in the *CYP11A1* gene. Thus, in rare cases, children with complete P450scc deficiency may survive.

**Case Report**

The child was born at 31 wk gestation to nonconsanguineous Caucasian parents. The 31-yr-old mother had a history of two early miscarriages and had two healthy children (Fig. 1A). During this pregnancy, low maternal plasma estriol [0.2 ng/ml (0.69 nmol/liter); reference 1.55–1.95 ng/ml] was detected in the 18th gestational wk, prompting amniocentesis and determination of 46,XY fetal karyotype. The mother received betamethasone to induce fetal lung maturation because of impending preterm delivery in the 30th wk of gestation. Progesterone was not given and 4 d later, the child was born by cesarean section due to unavoidable labor. Surprisingly, the baby’s phenotype was unequivocally female with an unusual bronze skin color. After an uneventful postpartal period, the child developed signs of adrenal insufficiency with serum so-

dium of 123 mmol/liter, potassium of 5.2 mmol/liter, and blood glucose of 31 mg/dl (1.7 mmol/liter) on d 9 post partum. Hydrocortisone in a dose of 40 mg/m<sup>2</sup> was given because congenital adrenal hyperplasia was suspected but was discontinued on d 21 because a transient adrenal insufficiency was suspected and the prenatal determined karyotype was not known at this time. Within 24 h, the child again developed signs of severe salt wasting with serum sodium 123 mmol/liter and potassium 6.6 mmol/liter. Blood glucose was 59 mg/dl (3.2 mmol/liter), blood pressure was 31/18 mm Hg. The child developed respiratory failure and was put on a respiratory ventilator. At this time, plasma ACTH was excessively elevated at 2789 ng/liter (normal < 60 ng/liter) and plasma renin activity was more than 3200 ng/liter (normal < 308 ng/liter). Plasma cortisol and 17-hydroxyprogesterone were undetectable, consistent with severe glucocorticoid and mineralocorticoid deficiency. Treatment with hydrocortisone and fludrocortisone was commenced, and the child improved rapidly. Urinary steroid analysis at the age of 2 months did not demonstrate any excretion of 3β-hydroxy-5-ene steroids, suggesting complete loss of steroidogenesis. The 46,XY karyotype was confirmed. Therefore, a presumptive diagnosis of congenital lipoid adrenal hyperplasia was made. At the age of 2 yr, medication was discontinued under medical supervision. Within 24 h, the child went into adrenal crisis. Although ACTH was normal on treatment, severe adrenal insufficiency was evident from plasma hormone levels determined in adrenal crisis (Table 1). Ultrasound and magnetic resonance imaging failed

FIG. 1. Localization and pattern of inheritance of the *CYP11A1* gene mutation. A, Pedigree of the family, indicating the homozygous propositus, the heterozygous parents, two healthy siblings with unknown genetic status, and two previous miscarriages. B, Single-strand conformation polymorphism/heteroduplex analysis demonstrates the heterozygous status of the parents and homozygous status of their child. C, Electropherograms of DNA sequences confirm the heterozygous deletion of an adenine within exon 5 of *CYP11A1* in the parents and the homozygous deletion of this nucleotide in their child. D, The deletion of adenine in codon 279 (arrow) leads to a frame shift and premature stop codon at position 288 (underlined). The intron 4/exon 5 boundary is indicated by lower-/uppercase letters.



**TABLE 1.** Hormone analysis at the age of 2 yr after discontinuation of steroid medication for 24 h demonstrated highly elevated ACTH and low or undetectable levels of steroid hormones

	ACTH (ng/liter)	Progesterone (ng/ml)	17-OH-pregnenolone (ng/ml)	17-OHP (ng/ml)	Aldosterone (ng/ml)	Cortisol (ng/ml)	DHEA (ng/ml)
Patient	479	0.12	0.38	0.01	0.02	<2.0	<0.05
Normal age range (1–3 yr)	<60	0.6–0.74	0.4–2.4	0.03–0.95	0.1–0.79	17.1–137.0	1.0–785.0

Normal age ranges according to Refs. 18 and 19. Conversion factors (SI) are: for progesterone  $\times 3.18$ ; for 17-OH-pregnenolone  $\times 3.006$ ; for 17-OHP  $\times 3.026$ ; for aldosterone  $\times 2.774$ ; for cortisol  $\times 2.759$ ; and for DHEA  $\times 3.47$ . 17-OHP, 17-Hydroxyprogesterone; DHEA, dehydroepiandrosterone.

to demonstrate gonads or adrenal tissue, and no uterus was seen.

### Materials and Methods

Molecular genetic analysis of *CYP11A1* was performed using published primer sequences (7, 9). All exons were amplified by PCR and subsequently investigated with single-strand conformation polymorphism analysis. An aberrant migration pattern of the patient's DNA amplification product was detected in exon 5 (Fig. 1B). This exon was subjected to direct sequencing on an automated sequencer after reamplification from DNA of the patient and her parents. Molecular analysis of the genes encoding StAR and steroidogenic factor-1 (SF-1, NR5A1) was also performed and did not reveal any abnormalities. DNA from a normal male was used as a negative control. Informed consent was obtained for studies of the propositus and the parents. The parents did not consent to studying their healthy children.

### Results

We detected a homozygous deletion of adenine at nucleotide position 835 in exon 5 of *CYP11A1* in the proband, leading to a frame shift within codon 278 and a premature termination codon at position 288 (Fig. 1 C and D). Both parents were heterozygous for this single nucleotide change (Fig. 1), thus confirming autosomal-recessive inheritance of this mutation. The detected mutation is predicted to cause deletion of the carboxy-terminal 242 amino acids of the P450scc enzyme, a region containing the highly conserved heme binding site, a critical meander domain involved in the interaction of P450scc with its electron transfer partners, and several conserved adrenodoxin binding sites (10–12). The importance of this region for P450scc function has previously been demonstrated by point mutations that abolish cholesterol side chain cleavage (R426Q) or heme binding (R425Q) (10). Therefore, this homozygous 835delA mutation is unlikely to be compatible with expression of a functional P450scc enzyme.

### Discussion

Our report demonstrates that, in rare cases, fetuses with inherited homozygous disruptive mutations of the *CYP11A1* gene may be viable. The single nucleotide deletion detected in the patient as well as the parents leading to an early premature termination codon implies that the resulting protein will be completely nonfunctional. Due to this obvious molecular impact, functional studies were not performed. It has to be postulated that maternal steroids have contributed to survival of the child. However, it is also unclear why progesterone and 17-hydroxypregnenolone were detectable after discontinuation of treatment at the age of 2 yr. This may imply that subtle P450scc-independent mechanisms of progesterone synthesis may exist, but this hypothesis should not

be drawn from a single case presentation. Also, whether the earlier miscarriages reported by the mother were due to other affected pregnancies with homozygous *CYP11A1* mutation remains unknown because investigation of abortive material was not performed at that time because the molecular aberration in the affected child was unknown.

This *CYP11A1* mutation was inherited from heterozygous but healthy parents, proving that heterozygosity for a disruptive mutation allows sufficient steroid production for normal adrenal function, sexual differentiation, and fertility. Investigation of the mother revealed normal levels of LH, FSH, ACTH, and plasma renin as well as estradiol, progesterone, and cortisol. Electrolytes were normal.

The gonads of this child have differentiated normally because they produced sufficient antimüllerian hormone to induce regression of Müllerian structures during early fetal development. However, because gonadal structures were not detectable by ultrasound or magnetic resonance imaging, we assume that very early secondary atrophy may have occurred due to cholesterol accumulation comparable with mechanisms observed in patients with StAR defects in later childhood (1). Because surgical procedures have not taken place yet, the exact structure of these organs or the histology of remnants is still unknown.

Recently two reports of patients with partial inactivating mutations of P450scc have been published. One 46,XX patient who presented with adrenal insufficiency in late infancy was found to be compound heterozygous for two point mutations inducing amino acid substitutions. One of these exchanges partially reduced P450scc activity, whereas the other change was predicted to create a novel splice-donor site (8). The other patient, a 46,XY child with clitoromegaly and adrenal failure at 4 yr of age, carried only one heterozygous mutation, which completely abolished enzyme activity *in vitro* (7). The authors postulated that haploinsufficiency of P450scc resulted in subnormal responses to ACTH. This in turn induced cellular damage by cholesterol accumulation due to recurrent ACTH stimulation, finally resulting in adrenal insufficiency. Of note, both previously reported subjects were born after normal pregnancies and did not present with severe illness during the neonatal period, implying sufficient placental progesterone synthesis during pregnancy.

Several examples of the combination of sex reversal and adrenal insufficiency in 46,XY infants have been reported so far. Mutations of the gene encoding SF-1 cause a similar phenotype as the homozygous *CYP11A1* mutation described here (13). Müllerian structures may be present due to early gonadal failure. However, in a recent report, a heterozygous

8-bp deletion within the *NR5A1* locus encoding for SF-1 was associated with 46,XY sex reversal without adrenal insufficiency or the presence of Mullerian structures, demonstrating that during embryogenesis gonads and throughout life, adrenals were functional (14). Mutations in the StAR gene lead to CLAH with secondary adrenal and gonadal atrophy in childhood (1, 2, 15–17). Hence, P450scc deficiency due to homozygous disruptive mutations of the *CYP11A1* gene may be the ultimate cause of CLAH, with early secondary atrophy of steroid producing organs during fetal life. This finding is consistent with earlier observations in rabbits, in which homozygous disruption of P450scc leads to a similar phenotype and heterozygote carriers are healthy and fertile (6). In contrast to humans, affected rabbits are thought to survive pregnancy because progesterone production is maintained by the corpus luteum rather than the placenta. The case presented here may dispute the existing paradigm of the luteoplacental shift of steroidogenesis in humans with alternative pathways of progesterone synthesis. It may also have implications for *CYP11A1* mutations as an inherited cause of recurrent early fetal loss.

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