

## Review Article

# Cholesterol Side-Chain Cleavage Enzyme (SCC) Deficiency

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**Abstract.** Cholesterol side-chain cleavage enzyme (SCC) catalyzes the conversion of cholesterol to pregnenolone, the first step in the biosynthesis of all steroid hormones. It was once postulated that SCC deficiency would be lethal, but recent studies have established that SCC deficiency is an autosomal recessive disorder caused by inactivating mutations in the *CYP11A1* gene. Clinical manifestations include adrenal insufficiency and 46,XY sex reversal due to disrupted steroidogenesis, which are similar to StAR deficiency. Further accumulation of patients with SCC deficiency should clarify the similarities and differences between SCC deficiency and StAR deficiency.

**Key words:** cholesterol side-chain cleavage enzyme (SCC), *CYP11A1*, steroidogenic acute regulatory protein (StAR), *STAR*, congenital lipoid adrenal hyperplasia

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### Introduction

Cholesterol side-chain cleavage enzyme (SCC) catalyzes the conversion of cholesterol to pregnenolone in steroidogenic cells, which is the first step in the biosynthesis of all steroid hormones (1). SCC is a mitochondrial cytochrome P450 enzyme, which resides in the inner mitochondrial membrane and is regulated by substrate availability (1, 2).

The *CYP11A1* gene, encoding SCC of 521 amino acid residues, is located on chromosome 15q23-24 and contains 9 exons (3). It was once postulated that no one could have SCC deficiency caused by homozygous mutations in *CYP11A* (4),

but it has been demonstrated that this postulation is not the case, because three patients with the autosomal recessive form of SCC have been described.

In this review article, we summarize these patients with SCC deficiency.

### History and Genetics of SCC Deficiency

Congenital lipoid adrenal hyperplasia (CLAH), originally described by Prader and Siebenmann (5), is the most severe form of congenital adrenal hyperplasia, leading to impaired production of all steroids including glucocorticoids, mineralocorticoids and sex steroids. This disorder is inherited as an autosomal recessive trait, and the affected individuals are all phenotypically female with a severe salt-losing syndrome that is fatal unless treated with steroid replacement therapy (6). Because mitochondria from affected adrenal glands and gonads fail to convert cholesterol to

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pregnenolone, it was postulated that this disorder might be caused by a defect in SCC (7, 8). However, no mutations have been revealed in the *CYP11A1* gene encoding SCC in affected individuals (9–11). Therefore, it was concluded that CLAH could not be caused by mutations in the *CYP11A1* gene, and that the defect had to lie in a factor that is not expressed in placenta, since progesterone produced by fetal trophoblasts is essential for pregnancy (9). In rabbits, a naturally occurring deletion in the gene encoding SCC has been reported. The homozygotes of the deletion are affected by CLAH, and the heterozygotes are phenotypically normal (12). Recently, *Cyp11a1* null mice created by targeted disruption of the gene have been described, demonstrating a similar phenotype (13).

Steroidogenic acute regulatory protein (StAR) is a 30-kDa phosphorylated protein that rapidly appears in mitochondria of steroidogenic cells following tropic stimulation, and is required in the acute regulation of steroidogenesis (14). Exogenous expression of this protein in MA-10 mouse Leydig tumor cells resulted in increased steroid production in the absence of hormone stimulation. The most striking evidence for the importance of StAR in the acute regulation of steroidogenesis has been provided by studies on CLAH patients that have demonstrated inactivating mutations in the *STAR* gene cause CLAH (15). As predicted, StAR has been shown to be primarily expressed in all steroidogenic tissues except for placenta (16), and mutations in the *STAR* gene have been identified in most, but not all, CLAH patients (17).

Recently, inactivating mutations in the *CYP11A1* gene were described in a 46,XX patient with adrenal deficiency and in 46,XY patients with sex reversal and adrenal insufficiency, who have no mutations in the *STAR* gene (18–21). The first inactivating mutation in the *CYP11A1* gene was reported as a heterozygous state in a patient with 46,XY sex reversal and adrenal

insufficiency (16). The patient had a heterozygous 6-nucleotide insertion between codons 271 and 272, resulting in the insertion of Gly and Asp (271\_272insGlyAsp). Neither of the patient's parents carried the mutation, indicating the mutation arose *de novo* in the patient. An *in vitro* expression study demonstrated that the mutant SCC had no activity to convert cholesterol to pregnenolone, and failed to have a dominant negative effect over the wild-type SCC, suggesting that haploinsufficiency in the *CYP11A1* gene might cause SCC deficiency, and thus, that SCC deficiency might be inherited as an autosomal dominant trait in humans.

Soon after this report, we reported a patient with hereditary SCC deficiency caused by compound heterozygous mutations in the *CYP11A1* gene (17). This report was crucial, because the family of our patient indicated at least three important clinical aspects of SCC deficiency. The first aspect is that the mother, who was a heterozygote for the inactivating mutation, had normal phenotype; thus heterozygous mutations in the *CYP11A1* gene do not cause SCC deficiency. The second aspect is that compound heterozygous mutations in the *CYP11A1* gene were the cause of the SCC deficiency; thus SCC deficiency is an autosomal recessive disorder. The third and most important aspect is that our patient was born uneventfully after a normal pregnancy; thus SCC deficiency due to homozygous and compound heterozygous mutations is not necessarily lethal, contrary to a previous prediction (4). These three aspects were confirmed to be the case by the finding of two additional families with hereditary SCC deficiency due to homozygous inactivating mutations in the *CYP11A1* gene (20, 21). Therefore, it is now obvious that haploinsufficiency in the *CYP11A1* gene by itself cannot account for SCC deficiency. Since it is confusing to include the patient with the apparent autosomal dominant form of SCC deficiency (16), this review will focus only on

**Table 1** Molecular and clinical findings in three patients with SCC deficiency

	Patient 1	Patient 2	Patient 3
Mutation			
Type	A189V(splicing)*/R353W	279delA/279delA	A359V/A359V
Location	Exon 3/Exon 6	Exon 5/Exon 5	Exon 6/Exon 6
Activity	some*/3%	0%/0%	11%/11%
Clinical features			
Karyotype	46,XX	46,XY	46,XY
Social sex	Female	Female	Female
Gestation	40 wk	31 wk	40 wk
Birth weight	3150 g	Not described	3100 g
External genitalia	Female	Female	Female
Müllerian derivatives	Present	Absent	Absent
Gonadal development	Ovary	Undetectable	Testes
Onset of adrenal failure	7–9 mo	9 d	1 yr 9 mo
Image of adrenals	Undetectable	Undetectable	Normal
Reference	19	20	21

\*The A189V(splicing) mutation was shown to have residual activity, but the activity remains to be quantified.

the patients with the autosomal recessive form of SCC deficiency in further discussion as summarized in Table 1 (19–21).

### **CYP11A1 Mutations and Activities of Mutants**

Patient 1, the first patient with hereditary SCC deficiency had compound heterozygous mutations in the *CYP11A1* gene (19). One mutation A189V(splicing) in exon 3, changing codon 189 (GCG) encoding Ala to GTG encoding Val, arose *de novo* in the paternal allele. When the A189V SCC was expressed *in vitro* by introducing the A189V replacement in the SCC cDNA, the mutant did not show any reduction in activity of converting cholesterol to pregnenolone. When the *CYP11A1* minigene having the A189V(splicing) mutation was expressed *in vitro*, the majority of mRNA was aberrantly spliced and lacked the last 61 nucleotides of exon 3. The 61-nucleotide deletion seen in the mRNA is expected to cause a shift in

the open reading frame after codon 189 with a premature termination at codon 205 and total loss of SCC activity. It is noteworthy that a small amount of mRNA was spliced normally to give rise to the active A189V SCC. Thus the A189V(splicing) mutation partially inactivates the *CYP11A1* gene by introducing a novel alternative splice-donor site. The other mutation in this patient was a maternally inherited missense R353W mutation in exon 6, changing codon 353 (CGG) encoding Arg to TGG encoding Trp. Since the Arg residue at codon 353 is highly conserved, this residue is expected to be important for SCC function. An *in vitro* expression study demonstrated that the R353W mutation reduced the enzymatic activity to approximately 3% of the wild type (19, 21). It should be noted that both *CYP11A1* alleles retained some activity in this patient, although the activity of the A189V(splicing) mutant allele remains to be quantified.

Patient 2, the second patient with SCC deficiency had a homozygous deletion of the first

nucleotide of codon 279 (ATA) in exon 5 (279delA), and both parents were heterozygous for the deletion (20). The 279delA mutation is expected to cause a premature termination at codon 288, and a deletion of the carboxy-terminal 242 amino acids of the SCC enzyme, which is an extremely critical region for SCC function. Thus the 279delA mutation is very likely to result in total loss of SCC activity, although the 279delA mutant has not been expressed *in vitro* to determine the activity.

Patient 3, the third patient with SCC deficiency had a homozygous missense mutation A359V in exon 6, changing codon 359 (GCG) encoding Ala to GTG encoding Val, and both parents, who were first cousins, were heterozygous for the mutation (21). The Ala residue at codon 359 is also highly conserved, suggesting the importance of this amino acid residue for SCC function. The *in vitro* expression study demonstrated that the A359V mutation reduced the activity to approximately 11% of the wild type (21).

## Clinical Features

### Genetic and social sex

Patient 1 had a 46,XX karyotype, and Patients 2 and 3 had a 46,XY karyotype, and all three patients were reared as females (19–21).

### Gestation and birth weight

Patients 1 and 3, having residual activity of SCC, were born at 40 wk of gestation, and their birth weights were 3150 g and 3100 g, respectively (19, 21). Thus restricted SCC activity in fetal trophoblast does not necessarily disrupt pregnancy, or disturb fetal growth. Patient 2, who is expected to have no SCC activity, was born at 31 wk of gestation, and her birth weight was not described (20). The preterm delivery of this patient may be related to total absence of SCC activity. Noteworthy, this fetus with no predicted SCC activity was

able to survive beyond the period of the luteoplacental shift of progesterone production, which usually occurs between 6 to 9 wk of gestation (4). Thus progesterone produced by fetal trophoblasts might not be essential for the maintenance of pregnancy.

### External genitalia

All of the three patients had normal female external genitalia irrespective of karyotype and mutant enzyme activity (19–21). No ambiguities in external genitalia were described in these patients. Thus, the 46XY patients were not able to synthesize enough testosterone to virilize external genitalia due to compromised SCC activity during fetal development.

### Internal genitalia and gonads

Patient 1 is expected to have normal Müllerian derivatives, but her ovarian functions still need to be estimated (19).

Patients 2 and 3 had blunt-end vaginae and no uteri (20, 21). The gonads were not identified in Patient 2 (20). Bilateral small gonads were identified in inguinal regions in Patient 3, and they were histologically confirmed to be testes (21). The epididymis was abnormally wrapped around the testis but with normal histology. Seminal vesicles and vasa deferentia were not identified. Thus confined or even absent SCC activity does not disrupt testicular differentiation, and allows anti-Müllerian hormone secretion to introduce regression of the Müllerian ducts in 46,XY patients.

### Adrenal glands

Patient 1, having residual SCC activity, developed adrenal insufficiency over 7 to 9 mo (19). <sup>131</sup>I-aldosterol adrenal scanning at 9 mo age failed to reveal adrenal glands in the patient (unpublished data). Patient 3, also having residual SCC activity, developed adrenal insufficiency at 1 yr and 9 mo of age, when the patient had normal-sized adrenal glands (21).

Patient 2, who was expected to have no SCC activity, exhibited signs of adrenal insufficiency at 9 d of age, and ultrasound and magnetic resonance imaging failed to demonstrate adrenal tissue at the age of 2 yr (20). Thus, there seems to exist correlation between mutant SCC activity and onset of adrenal failure. Unlike patients with *STAR* mutations, no patients with *CYP11A1* mutations have been demonstrated to have adrenal hyperplasia, and lipid-filled adrenal so far. In cases of SCC deficiency, cholesterol would accumulate not only in the cytoplasm but also in the mitochondria in the presence of normal StAR activity. This intramitochondrial accumulation would damage the mitochondrial function and lead to the release of apoptotic signals such as cytochrome C, and consequently lead to apoptosis of steroidogenic cells before the accumulation of cholesterol in the cytoplasm causes lipid adrenal hyperplasia.

### Heterozygotes

The mother of Patient 1 and both parents of Patients 2 and 3 carried heterozygous deteriorating mutations in the *CYP11A1* gene (19–21). They had no symptoms or signs of adrenal insufficiency just like heterozygotes for mutations in the genes encoding all other adrenal steroidogenic enzyme genes (*HSD3B2*, *CYP17A1*, *CYP21A2*, *CYP11B1*, and *CYP11B2*), and it is needless to say that all of them were fertile. The mother of Patient 1 had no history of miscarriage (19). The mother of Patient 2 had a history of two miscarriages, as did the mother of Patient 3 (20, 21). These miscarriages might have been caused by the homozygous mutations in the *CYP11A1* gene, but the exact cause of the miscarriages was not determined in these families.

### Conclusion

SCC deficiency is now clearly recognized as a clinical entity. SCC deficiency is an autosomal

recessive disorder. The phenotype of SCC deficiency is similar to that of StAR deficiency. Further accumulation of patients with SCC deficiency should clarify genotype-phenotype correlation in SCC deficiency, and the similarities and differences between SCC deficiency and StAR deficiency.

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