0021-972X/06/$15.00/0
*Printed in U.S.A.*

The Journal of Clinical Endocrinology & Metabolism 91(8):2821–2826

doi: 10.1210/jc.2005-2230

# CLINICAL CASE SEMINAR

# Homozygous Mutation of P450 Side-Chain Cleavage Enzyme Gene (CYP11A1) in 46, XY Patient with Adrenal Insufficiency, Complete Sex Reversal, and Agenesis of Corpus Callosum

Hessa al Kandari, Noriyuki Katsumata, Suzan Alexander, and Majedah Abdul Rasoul

*Pediatric Endocrinology Unit (H.a.K.), Farwania Hospital, Kuwait; Department of Endocrinology and Metabolism (N.K.), National Research Institute for Child Health and Development, Tokyo 157-8535, Japan; Pathology Department (S.A.), Sabah Hospital, 13001 Kuwait; and Endocrinology Unit (M.A.R.), Farwania Hospital, 3455 Kuwait*

**Context:** The cholesterol side-chain cleavage enzyme catalyzes the conversion of cholesterol to pregnanalone in the first step of steroidogenic pathways. Defective enzyme activity leads to the deficiency of all steroid hormones, including progesterone, which is essential to sustain term pregnancy.

**Results:** We report a homozygous point mutation in the CYP11A1 gene in a 46, XY phenotypic female born at term to healthy heterozygous parents, presenting relatively late at the age of 1 yr 9 months with life-threatening adrenal insufficiency and complete sex reversal. She was found to have complete agenesis of corpus callosum. The mutation resulted in a single amino acid substitution: valine for alanine at position 359. The functional analysis of the mutant enzyme revealed markedly reduced enzyme activity, but about 11% residual activity was demonstrated. We explained the completion of pregnancy to term and the late presentation by a possible difference in the mutant enzyme activity *in vivo* and *in vitro* or by the residual mutant activity, which would have been enough to maintain pregnancy and viability of the patient. The clinical findings of nearly undetectable levels of steroid hormones at presentation are explained by the total disruption of steroidogenic cells later on, with recurrent ACTH stimulation leading to intramitochondrial cholesterol accumulation and cell death (a two-hit mechanism).

**Conclusion:** This report of a homozygous mutation in CYP11A1 gene in a child with agenesis of corpus callosum shows that homozygous mutations in CYP11A1 gene can be compatible with term pregnancy and delayed presentation. **(*J Clin Endocrinol Metab* 91: 2821–2826, 2006)**

STEROID HORMONES ARE essential for normal sexual development, accommodation of stresses, and fluid electrolyte balance. They are synthesized mainly in adrenal glands, gonads, and fetoplacental unit. Animal studies have shown *de novo* steroid synthesis in the central nervous system; moreover, it was established recently that key steroidogenic enzymes cholesterol side-chain cleavage enzyme (P450scc), aromatase, 5$\alpha$-reductase, 3$\alpha$-hydroxysteroid dehydrogenase, and 17$\beta$-hydroxysteroid dehydrogenase are in human brains, providing evidence that neurosteroids can be produced in human brains. However, pathophysiological significance remains to be elucidated (1).

Although steroid hormones have diverse physiological action, the biosynthesis of all steroids begins with the conversion of cholesterol to pregnanalone, which is the first and rate-limiting step in steroidogenic pathway. The cytochrome P450scc system catalyzes the conversion of cholesterol to pregnanalone. It is located in the matrix side of inner mitochondrial membrane and cooperates with two coenzymes, ferredoxin and ferredoxin reductase, to carry out three successive oxidative reduction reactions of cholesterol through an electron transport chain using reduced nicotinamide adenine dinucleotide phosphate as an electron donor, and it is encoded in humans by a single gene on chromosome 15, the CYP11A1 gene (2, 3), expression of which is under stringent control (4–8). Disruption of this early step in the steroidogenic pathway causes impaired production of gonadal and adrenal steroids *in utero* and postnatally leading to accumulation of cholesterol in the adrenal cortex and resulting in the most severe genetic disorder of steroid synthesis, congenital lipoid adrenal hyperplasia (CLAH). CLAH is due to impaired conversion of cholesterol to pregnanolone resulting in deficiency of all steroids, including mineralocorticoid, glucocorticoid, and sex steroids; affected individuals are phenotypic females with a severe salt-losing syndrome. It is believed to result from a two-hit event, the initial genetic loss of steroidogenesis, and the subsequent loss due to cellular damage from accumulation of cholesterol and cholesterol esters (9). CLAH was formerly named 20,22 desmolase deficiency and was thought to be caused by mutations in P450scc (10–12), but the gene for P450scc was found to be

**First Published Online May 16, 2006**

Abbreviations: CLAH, Congenital lipoid adrenal hyperplasia; hCG, human chorionic gonadotropin; P450scc, cholesterol side-chain cleavage enzyme; SF, steroidogenic factor; StAR, steroidogenic acute regulatory protein.

**JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

normal in these patients (13, 14). Recently, it was reported that CLAH is because of defects in the steroidogenic acute regulatory protein (StAR), which is a protein required for the rapid flux of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane that is needed to induce acute response of steroid synthesis (9, 15–18). Nevertheless, the description of Yang *et al.* (19) that rabbits that are homozygous for P450scc gene deletion have a phenotype that closely corresponds with CLAH together with the recent reports of CLAH resulting from deficient P450scc activity in animals (20, 21) and in humans (22–24) have provided the evidence that some of these patients may harbor P450scc mutations.

P450scc is essential for progesterone synthesis, which is important to sustain pregnancy in mammals. The origin of progesterone during pregnancy can be the ovarian corpus luteum, the placenta, and the fetal adrenal. The mechanisms by which progesterone synthesis is maintained vary between species (25, 26). In rabbits, the role of placental progesterone production is less obvious because luteal progesterone synthesis is indispensable throughout term. Thus, a homozygous P450scc mutation in the fetus will not disrupt progesterone production by the maternal corpus luteum, and the pregnancy will be carried to term (19). In contrast, in humans, the corpus luteum is active during the first trimester, and the placenta assumes the steroidogenic role of the ovary at 8 wk gestation (luteoplacental shift) (27–30). It was thought that deficiency of cytochrome P450scc is incompatible with term pregnancy, and spontaneous abortion is expected at about 6–7 wk, when production of progesterone from maternal corpus luteum wanes, and the mutant placental system is unable to take over (31), but recent reports of three cases (22–24) gave evidence that deficiency of cytochrome P450scc can be compatible with term pregnancy if the *in vivo* enzyme level is sufficient to sustain the pregnancy.

The corpus callosum is the largest interhemispheric commissure. It forms between the 8th and 20th wk of gestation. Agenesis of corpus callosum can occur in isolation or in association with the central nervous system or systemic malformation (32). We report the first patient with a homozygous point mutation in CYP11A1 leading to single amino acid substitution, which was inherited in an autosomal-recessive fashion from healthy parents. The 46, XY patient was born at term and presented with severe adrenal insufficiency at the age of 1 yr 9 months and had complete sex reversal. Furthermore, she was found to have complete agenesis of corpus callosum by computed tomography of the brain. Thus, severe P450scc deficiency can be compatible with term gestation and with survival for a considerable time without replacement.

## Patient and Methods

### Case report

The proband was the third child for a healthy first-degree consanguineous couple from Syria. She has two full siblings and 15 stepbrothers and stepsisters; eight of them are married and have children, and all are healthy. The mother had a history of two early miscarriages. The patient was born by spontaneous vaginal delivery after an uneventful 40-wk gestation. Birth weight was 3 kg. She had normal developmental milestones with no past history of major illness but a minor episode of upper respiratory tract infection associated with vomiting at the age of 10 months, which was treated with iv rehydration for a few hours in a primary health care center; no investigation was done. She presented for the first time to the emergency department at the age of 1 yr 9 months with a history of vomiting, reduced oral intake, and an attack of tonic clonic convulsion. She was drowsy and moderately dehydrated with generalized tanning of skin and markedly increased pigmentation of lips and gums, nape of the neck, and abdomen. Her weight was 8 kg (below the 5th centile for age). She had normal female external genitalia with separate vaginal and urethral openings. Small bilateral inguinal masses were felt. She had metabolic acidosis (pH 7.2 and bicarbonate level of 13 mmol/liter) with hypoglycemia (0.6 mmol/liter), hyponatremia (113 mmol/liter), and hyperkalemia (6.6 mmol/liter). She had extremely elevated ACTH (>1250 pg/liter; normal range, 10–84) and high plasma renin activity (1900 mIU/liter; normal range, 5–75), with a very low or undetectable level of aldosterone (<30 pmol/liter; normal range, 50–850). The diagnosis of glucocorticoid and mineralocorticoid deficiency was made; treatment with hydrocortisone and fludrocortisone was commenced, after which the child improved dramatically. Her karyotype result was 46, XY. The child was then admitted for ACTH stimulation test after 24-h discontinuation of medication under medical supervision. A short synacthen test showed that levels of steroid hormones were undetectable and failed to show any rise after stimulation with 0.25 mg of synacthen [ACTH-(1–24)] (Table 1). The serum levels of FSH and LH were elevated (10.4 IU/liter, normal range, 0.7–3.39; 0.78 IU/liter, normal range, 0.33–0.55, respectively). Human chorionic gonadotropin (hCG) stimulation failed to raise serum testosterone (0.295 and 0.24 nmol/liter pre- and poststimulation, respectively). Ultrasound and magnetic resonance imaging of the abdomen identified bilateral small gonads, 0.4 cm, in inguinal regions with testis-like echo texture, and normal-sized adrenal glands (right, $6.4 \times 5.3 \times 9$ mm; left, $5.8 \times 4.6 \times 8.6$ mm; normal range, $5.6 \pm 0.8 \times 4.6 \pm 0.7 \times 8.3 \pm 0.6$ mm) with complete lack of Müllerian structures. A genitogram showed a short, blunt-end vagina. Computed tomography of the brain revealed complete agenesis of corpus callosum.

Histopathology of the gonads revealed normal testicular tissue for age; normal seminiferous tubule and Sertoli cells. However, Leydig cells and germ cells were not visualized, which could be normal for age. The epididymis was abnormally wrapped around the testis but with normal histology. Seminal vesicles and vas deference were not identified. The direct sequencing of analysis of the candidate genes of the patient including StAR, dosage-sensitive sex adrenal hypoplasia congenita critical region on X, and steroidogenic factor (SF)-1 revealed normal DNA sequences (data not shown).

### *PCR amplification and direct sequencing of the CYP11A1 gene*

DNA was extracted from peripheral white blood cells of the patient and her parents after informed consent for genetic analyses was ob-

**TABLE 1.** Baseline steroid levels and steroid responses to 0.25 mg ACTH

| | Prestimulation | Poststimulation | Normal age range (basal) |
|---|---|---|---|
| Cortisol | 16.53 nmol/liter | 16.99 nmol/liter | (88–331) nmol/liter |
| Pregnanalone | <0.5 nmol/liter | <0.5 nmol/liter | (0.8–2.2) nmol/liter |
| 17OH-Pregnanalone | | <10 ng/100 ml/liter | (15–235) ng/100 ml |
| 17OH-Progesterone | <0.3 nmol/liter | <0.3 nmol/liter | (0.16–2.27) nmol/liter |
| Dehydroepiandrosterone | <0.8 μmol/liter | <0.8 μmol/liter | 0.3–1.6 μmol/liter |
| Aldosterone | <30 pmol/liter | <30 pmol/liter | 166–940 pmol/liter |

Normal age ranges according to Ref. 33. Poststimulation × 5–10 the initial values for all.

tained. Exons of the *CYP11A1* gene were amplified and directly sequenced as reported previously (23).

### *Restriction enzyme analysis*

The A359V mutation found in the patient and her parents, which destroys a recognition site for a restriction enzyme *Hha*I (New England Biolabs, Inc., Beverly, MA), was confirmed by digestion of PCR products with the restriction enzyme followed by electrophoresis on a 2% NuSieve GTG agarose gel (FMC Bioproducts, Rockland, ME).

### *Functional expression of the mutant P450scc*

The wild-type and R353W mutant P450scc cDNAs were prepared as previously described (23). The bovine adrenodoxin and bovine adrenodoxin reductase expression plasmids and the human StAR expression plasmid were obtained as previously described (23). The A359V amino acid substitution was introduced into the wild-type P450scc cDNA by the recombinant PCR method (34). The primer sequences for introduction of the mutation were as follows: a sense primer 359VF, 5′-CTTGGCTGTGCGGCACCAGGCCCAGGGAGA-3′; and an antisense primer 359VR, 5′-GGGCCTGGTGCCGCACAGCCAAGACCTCTG-3′ (the mutated nucleotides are *underlined*). The wild-type or mutant P450scc cDNAs were ligated to a mammalian expression plasmid, pRK 5, and expressed transiently as previously reported (23). In brief, COS-1 cells were transfected with 1 $\mu$g each of bovine adrenodoxin, bovine adrenodoxin reductase, and human StAR expression plasmids, and 1 $\mu$g of either the wild-type or the mutant P450scc expression plasmid by the electroporation method using Gene Pulsor II (Bio-Rad Laboratories, Richmond, CA). The P450scc activity was determined by measuring the amount of pregnenolone using RIA, which was synthesized from cholesterol in the media during 48 h of incubation. In addition, pRK-GH1, a human GH expression plasmid, was included in the transfection mixture and used as an internal control for transfection efficiency. The experiments, each performed in quadruplicate, were done four times, and the results were presented as the means ± SEM (n = 4). Statistical analysis was performed using unpaired Student's *t* test.

Western blot analyses of mitochondrial fractions isolated from transfected cells were carried out with a polyclonal antibody raised against a recombinant human P450scc protein, which was provided by Dr. Bon-chu Chung (Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan) (35).

## Results

### *Sequencing analysis of the CYP11A1*

We directly sequenced the *CYP11A1* gene from the patient and found a homozygous mutation (Fig. 1). The mutation was a C to T transition in the second nucleotide of codon 359 in exon 6, which resulted in a substitution of Val for Ala and was designated A359V. No other mutation was found in the remainder of the coding region or the splice sites of the *CYP11A* gene. The parents were heterozygous for the A359V mutation.

### *Restriction enzyme analysis*

The A359V mutation destroys an *Hha*I recognition site in the wild-type *CYP11A* sequence. Thus, amplification of a 287-bp fragment from exon 6 of the wild-type *CYP11A* followed by digestion with *Hha*I should yield 157- and 130-bp fragments, whereas the PCR products containing the mutation should remain uncut. As shown in Fig. 2, restriction analysis of the patient gave only the undigested 287-bp fragment, indicating that the patient does not carry the wild-type sequence. The analysis of the parents gave a mixed digestion pattern, confirming their heterozygosity.

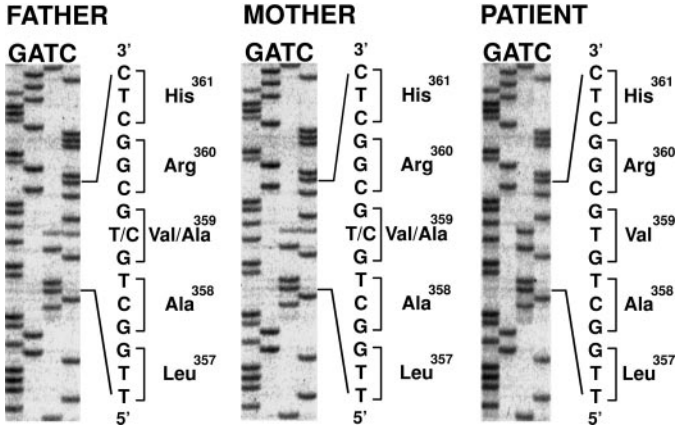


FIG. 1. Partial nucleotide sequences of exon 6 in the *CYP11A1* gene from the patient and her parents. The patient had a homozygous C to T transition in the second nucleotide of codon 359, which resulted in a substitution of Val for Ala, designated A359V. In the parents, both T and C were present in the second nucleotide of codon 359, indicating heterozygosity.

### *P450scc activity of replacement mutants*

To examine the functional consequences of the amino acid substitutions, we transiently expressed the mutants along with adrenodoxin, adrenodoxin reductase, and StAR in COS-1 cells. Western blot analyses detected similar amounts of the wild-type P450scc and the mutants (data not shown). The COS-1 cells expressing the wild-type P450scc successfully converted cholesterol to pregnenolone, whereas those transfected with the empty pRK5 plasmid produced virtually no pregnenolone (Table 2). The A359V replacement resulted in marked reduction in pregnenolone production, so did the R353W, but the A359V mutant had significantly higher activity than the R353W mutant (Table 2).

## Discussion

We report the first homozygous point mutation in the CYP11A1 gene with a single amino acid substitution, valine

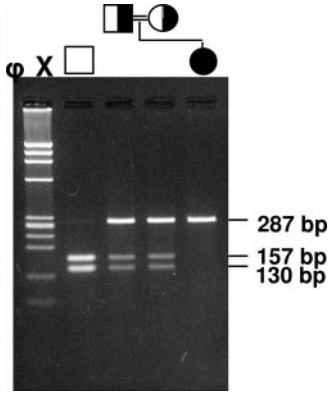


FIG. 2. Electrophoretic pattern of the digested PCR product from the patient (*black circle*), her parents (*black semisquare* and *black semicircle*), and a normal male control (*white square*). The patient had only the undigested 287-bp fragment, indicating that the patient does not carry the wild-type sequence. The parents had the 157- and 130-bp fragments as well as the undigested 287-bp fragment, indicating their heterozygosity. φX, *Hae*III-digested φX174 RF DNA, which was used as a size marker.

**TABLE 2.** Enzyme activities of wild-type and mutant P450scc

| | Pregnanalone (ng/ml) |
|---|---|
| pRK5 | <0.1[a] |
| pRK-SCC | 49.3 ± 7.0 |
| pRK-R353W | 1.4 ± 0.2[b] |
| pRK-A359V | 5.8 ± 1.6[b,c] |

pRK5, Empty plasmid; pRK-SCC, wild-type P450scc; pRK-R353W, R353W replacement P450scc; pRK-A359V, A359V replacement P450scc. The values represent the pregnanalone concentrations in the culture media obtained from four independent experiments, each performed in quadruplicate, and are shown as the mean ± SEM (n = 4).

[a] Lower than detection limit of the assay.

[b] $P < 0.01$ *vs.* pRK-SCC.

[c] $P < 0.01$ *vs.* pRK-R353W.

for alanine, leading to a severe functional defect in cytochrome P450scc enzyme activity *in vitro*, which was consistent with a severe functional defect *in vivo*. All steroidogenic pathways were proven severely defective in our patient, and the steroid hormone levels were below the lowest levels measurable by our laboratory both pre- and post-ACTH stimulation done at the age of presentation. However, this severe loss of function does not explain the continuation of pregnancy to term or the ability of the patient to survive for approximately 1.5 yr without replacement before presenting with life-threatening adrenal insufficiency. We postulate that the residual enzyme activity of the mutant allowed some progesterone synthesis to sustain full-term gestation and some adrenal cortisol synthesis, which has been enough for the patient to survive until the presentation. The severe loss of function that occurred later can be the result of the two-hit mechanism that accounts for the pathophysiology of CLAH caused by StAR mutations (9), in which the disease progresses from mutation-induced impairment of steroidogenesis (the first hit) to the loss of steroidogenesis from cell death due accumulation of cholesterol esters (the second hit). If we had had the chance to investigate the patient when she had her minor illness at the age of 10 months, we could perhaps have demonstrated a compensated state of low levels of steroid hormones in the presence of very high ACTH and plasma rennin activities before the patient progressed to the complete loss of function (the second hit) at presentation. However, there can be a difference in the second-hit mechanism between pathophysiology caused by StAR mutations and that by the CYP11A1 mutations as discussed later.

Two patients reported to have CYP11A1 mutation were born at term and presented with adrenal insufficiency relatively late. One 46, XX patient presenting in late infancy was found to have compound heterozygous for two point mutations (23), and the other was 46, XY, presenting at 4 yr of age, and was found to have a heterozygous point mutation (22). Like our child, these subjects had partial inactivating mutations of CYP11A1, which allowed them to retain some enzymatic ability to produce enough progesterone to complete term pregnancy and to maintain adrenal function to some extent before presentation.

Hiort *et al.* (24) were the first to report homozygous deletion in CYP11A1, presenting with adrenal insufficiency and sex reversal. However, their patient was born preterm and developed symptoms of adrenal insufficiency in the first day of life. The mutation was inherited from heterozygous parents. Similarly, our patient inherited the CYP11A1 point mutation from heterozygous, healthy, fertile parents. Investigation of the father revealed normal levels of serum electrolytes, cortisol, and ACTH; we did not have the chance to investigate the mother. This proves that heterozygous carriers of this disruptive mutation have normal adrenal function, normal sexual differentiation and fertility. Moreover, the mother reported a history with two early miscarriages, in the Hiort *et al.* (24) case report, which supports the suggestion made by the author that disrupted mutation in CYP11A1 might be a cause of recurrent abortion.

The gonads of our child were confirmed to be testes with testicular tissue normal for age by histopathological examination of the gonadal biopsy, but they were atrophied and unable to produce testosterone even after hCG stimulation. The high levels of FSH and LH also suggested this gonadal failure. However, because of the absence of any müllerian structures and the presence of epididymis, it is most likely that the testes differentiated normally in the early fetal life and produced anti-Müllerian hormone to cause regression of Müllerian structures and produced some testosterone to allow epididymis formation. The low P450scc enzyme activity allowed some production of testosterone by the Leydig cells that was enough to support Wolffian duct differentiation into epididymis but did not last long enough to allow any virilization of the external genitalia. We suppose that the Leydig cells failed completely to produce testosterone before 9 wk of fetal life (the time the male external genital formation commences). In the absence of testosterone after this time, the bipotential fetal external genitalia developed into normal female external genitalia. We suggest that this early complete failure of testosterone production by the Leydig cells was secondary to the recurrent trophic stimulation by placental hCG, which led to intramitochondrial cholesterol accumulation and cell death (the two-hit mechanism).

We demonstrated by magnetic resonance imaging that our patient had normal-sized adrenal glands, and this finding is consistent in the three patients reported to date with disruption of CYP11A1 (22–24). This might be a point of differentiation from the classic CLAH due to the StAR mutation, which is usually but not always associated with enlarged adrenal glands, and it was used as a tool for early detection of the disease (36). We propose that there are differences in the second hit between StAR and SCC deficiency. In case of P450scc deficiency, cholesterol would be accumulated not only in the cytoplasm but also in the mitochondria because the StAR activity is normal. This intramitochondrial accumulation of cholesterol would lead to the release of apoptotic signals such as cytochrome C and consequently leads to apoptosis of the steroidogenic cells before the accumulation of cholesterol in the cytoplasm causes adrenal lipoid hyperplasia.

Other causes of male pseudohermaphrodite and adrenal insufficiency were excluded for our patient. CLAH due to StAR mutation was ruled out because no mutation was detected in the StAR gene. SF-1 mutation is another possible differential diagnosis, but these patients usually have normally developed Müllerian structures, which are not present in our patient, and no mutation was detected in the SF-1 gene. The dosage-sensitive sex adrenal hypoplasia congenita crit-

ical region on X-1 mutation was also considered, although usually it causes adrenal insufficiency but does not cause male pseudohermaphroditism; it was also reported to be negative.

We consider our case to be different from the previously reported cases of P450scc enzyme deficiency, in that our child was born with a structural brain defect in the form of complete agenesis of corpus callosum not associated with any other central nervous system abnormality. The child was totally asymptomatic until the date of writing this report and had normal development. Callosal agenesis may be an isolated anomaly or may be syndromic as part of more extensive malformations, metabolic and genetic disorder, and is reported to have a higher incidence with consanguinity, which is present in our patient. It was found that isolated agenesis of corpus callosum has a favorable outcome (37, 38), although it can be a coincidental finding in our patient. As we mentioned earlier, brain steroid synthesis *de novo* has been reported in animals; furthermore, CYP11A1 mRNA is expressed in the cerebral cortex and the subcortical white matter of the human brain, together with other key enzymes of steroidogenesis, which provides evidence that steroids can be produced in human brain (1). It is not known how or even whether the steroidogenic enzymes are involved in the pathophysiology of the central nervous system. The significance of this association may be revealed with future expansion of our knowledge about the role of neurosteroids and neuroactive steroids in the development and function of the human brain and with reporting of more cases of P450scc mutations.

## Acknowledgments

We are grateful to Ms. Shoko Mikami-Kaneko and Ms. Atsuko Nagashima-Miyokawa for excellent technical assistance. We thank Dr. Yoshiyasu Yabusaki (Sumitomo Chemical, Co., Ltd., Takarazuka, Japan) and Dr. Shigeaki Kato (Tokyo University, Tokyo, Japan) for providing the bovine adrenodoxin and adrenodoxin reductase expression plasmids. We thank Dr. Bon-chu Chung (Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan) for providing the antihuman P450scc antibody. We thank Dr. M. Haider from the Pediatric Department, Faculty of Medicine Kuwait University for his assistance in DNA extraction.

Received October 14, 2005. Accepted May 5, 2006.

Address all correspondence and requests for reprints to: Hessa al Kandari, King Fahad Hospital, King Abdul Aziz Medical City, P.O. Box 22490, Pediatric Department 1510, Riyadh 11426, Kingdom of Saudi Arabia. E-mail: hesahabeeb@yahoo.com.

This work was supported in part by a Grant-in Aid on Priority Area from the Ministry of Education, Science, Sports and Culture, Japan (16086215).

Present address for H.a.K.: Pediatric Endocrinology, King Fahad Hospital, King Abdul Aziz Medical City, Riyadh 11426, Saudi Arabia.

H.a.K., N.K., S.A., and M.A.R. have nothing to declare.

## References

1. **Stoffel-Wagner B** 2001 Neurosteroid metabolism in the human brain. Eur J Endocrinol 145:669–679
2. **Chung BC, Matteson KJ, Voutilainen R, Mohandas TK, Miller WL** 1986 Human cholesterol side-chain cleavage enzyme, P450scc: cDNA cloning, assignment of the gene to chromosome 15, and expression in the placenta. Proc Natl Acad Sci USA 83:8962–8966
3. **Morohishi K, Sogawa K, Omura T, Fujii-Kuriyama Y** 1987 Gene structure of human cytochrome P-450(SCC), cholesterol desmolase. J Biochem 101:879–887
4. **Guo I-C, Hu M-C, Chung B-C** 2003 Transcriptional regulation of CYP11A1 J Biomed Sci 10:593–598
5. **Been-Zimra M, Koler M, Orly J** 2002 Transcription of cholesterol side-chain cleavage cytochrome P450 in the placenta: activating protein-2 assumes the role of steroidogenic factor-1 by binding to an overlapping promoter element. Mol Endocrinol 16:1864–1880
6. **Gizard F, Lavallee B, DeWitte F, Hum DW** 2001 A novel zinc finger protein TReP-132 interacts with CBP/p300 to regulate human CYP11A1 gene expression. J Biol Chem 276:33881–33892
7. **Huang N, Miller WL** 2005 LBP proteins modulate SF1-independent expression of P450scc in human placental JEG-3 cells. Mol Endocrinol 10:409–4020
8. **Moore CCD, Brentano ST, Miller WL** 1990 Human P450scc gene transcription is induced by cyclic AMP and repressed by 12-O-tetradecanoylphorbol-13-acetate and A23187 through independent cis elements. Mol Cell Biol 10:6013–6023
9. **Bose HS, Sugawara T, Strauss JF, Miller WL** 1996 The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. N Engl J Med 335:1870–1879
10. **Degenhart HJ, Visser KHA, Boon H, O'Doherty NJD** 1972 Evidence for deficiency of 20α cholesterol hydroxylase activity in adrenal tissue of a patient with lipoid adrenal hyperplasia. Acta Endocrinol (Copenh) 71:512–518
11. **Koizumi S, Kyoya S, Miyawaki T, Kidani H, Funabashi T, Nakashima H, Nakanuma Y, Ohta G, Itagaki E, Katagiri M** 1977 Cholesterol side-chain cleavage enzyme activity and cytochrome P450 content in adrenal mitochondria of a patient with congenital lipoid adrenal hyperplasia (Prader Disease). Clin Chim Acta 77:301–306
12. **Hauffa BP, Miller WL, Grumbach MM, Conte FA, Kaplan SL** 1985 Congenital adrenal hyperplasia due to deficient cholesterol side-chain cleavage activity (20,22 desmolase) in a patient treated for 18 years. Clin Endocrinol (Oxf) 23:481–493
13. **Lin D, Gitelman SE, Saenger P, Miller WL** 1991 Normal genes for cholesterol side chain cleavage enzyme, P450scc, in congenital lipoid adrenal hyperplasia. J Clin Invest 88:1955–1962
14. **Sakai Y, Yanase T, Okabe Y, Hara T, Waterman MR, Takayanagi R, Haji M, Nawata H** 1994 No mutatin in cytochrome P450 side chain cleavage in a patient with congenital lipoid adrenal hyperplasia. J Clin Endocrinol Metab 79:1198–1201
15. **Lin D, Sugawara T, Strauss III JF, Clark BJ, Stocco DM, Saenger P, Rogol A, Miller WL** 1995 Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. Science 276:1828–1831
16. **Nakae J, Tajima T, Sugawara T, Arakane F, Hanaki K, Hotsubo T, Igarashi N, Igarashi Y, Ishii T, Koda N, Kando T, Kohno H, Nakagawa Y, Tachibana K, Takeshima Y, Tsubouchi K, Strauss III JF, Fujieda K** 1997 Analysis of the steroidogenic acute regulatory protein (StAR) gene in Japanese patients with congenital lipoid adrenal hyperplasia. Hum Mol Genet 6:571–576
17. **Okuyama E, Nishi N, Onishi S, Itoh S, Ishii Y, Miyanaka H, Fujita K, Ichikawa Y** 1997 A novel splicing junction mutation in the gene for the steroidogenic acute regulatory protein causes congenital lipoid adrenal hyperplasia. J Clin Endocrinol Metab 82:2337–2342
18. **Stocco DM** 2001 Tracking the role of a StAR in the sky of the new millennium. Mol Endocrinol 15:1245–1254
19. **Yang X, Iwamoto K, Wang M, Artwohl J, Manson JI, Pang S** 1993 Inherited congenital adrenal hyperplasia in the rabbit is caused by a deletion in the gene encoding cytochrome P450 cholesterol side-chain cleavage enzyme. Endocrinology 132:1977–1982
20. **Pang S, Yang X, Wang M, Tissot R, M Nino, Manaligod J, Bullock LP, Mason JI** 1992 Inherited congenital adrenal hyperplasia in the rabbit: absent cholesterol side-chain cleavage cytochrome P450 gene expression. Endocrinology 131:181–186
21. **Hu MCH, Hsu NCH, El Hadj NB, Pai CI, Chu HP, Wang CHK, Chung BCH** 2002 Steroid deficiency syndromes in mice with targeted disruption of CYP11a1. Mol Endocrinol 16:1943–1950
22. **Tajima T, Fujieda K, Kouda N, Nakae J, Miller WL** 2001 Heterozygous mutation in the cholesterol side chain cleavage enzyme (P450scc) gene in a patient with 46, XY sex reversal and adrenal insufficiency. J Clin Endocrinol Metab 86:3820–3825
23. **Katusumata N, Ohtake M, Hojo T, Ogawa E, Hara T, Sato N, Tanaka T** 2002 Compound heterozygous mutations in the cholesterol side-chain cleavage enzyme gene (CYP11A) cause congenital adrenal insufficiency in humans. J Clin Endocrinol Metab 87:3808–3813
24. **Hiort O, Holterhus P-M, Werner R, Marschke C, Hoppe U, Partsch C-J, Riepe FG, Achermann JC, Struve D** 2005 Homozygous disruption of P450 side-chain cleavage (CYP11A1) is associated with prematurity, complete 46,XY sex reversal, and severe adrenal failure. J Clin Endocrinol Metab 90:538–541
25. **Niswender GD, Juengel JL, Silva PJ, Rollyson MK, McIntush EW** 200 Mechanisms controlling the function and life span of the corpus luteum. Physiol Rev 80:1–29
26. **Hilliard J** 1973 Corpus luteum function in Guinea pig, hamster, rats, mice and rabbits. Biol Reprod 8:203–221
27. **Csapo AI, Pulkkinen M** 1978 Indispensability of the human corpus luteum in the maintenance of early pregnancy. Luteectomy evidence. Obstet Gynecol Surv 33:69–81

28. **Scott R, Navot D, Liu HC, Rosenwaks Z** 1991 A human in vivo model for luteoplacental shift. Fertil Steril 56:481–484
29. **Christenson LK, Devoto L** 2003 Cholesterol transport and steroidogenesis by the corpus luteum. Reprod Biol Endocrinol 1:90–98
30. **Strauss III JF, Martinez F, Kiriakidou M** 1996 Placenta steroid hormone synthesis: unique features and unanswered questions. Biol Reprod 54:303–311
31. **Miller WL** 1998 Why no body has P450scc (20,22 desmolase) deficiency. J Clin Endocrinol Metab 83:1399–1400
32. **Sztriha L** 2005 Spectrum of corpus callosum agenesis. Pediatr Neurol 32:94–101
33. **Lashansky G, Saenger P, Fishman K, Gautier T, Mayes D, Berg G, Dimartino-Nardi J, Reiter E** 1991 Normative data for adrenal steroidogenesis in a healthy pediatric population: age- and sex-related changes after adrenocorticotropin stimulation. J Clin Endocrinol Metab 73:674–686
34. **Higuchi R, Krummel B, Saiki RD** 1998 A general method of in vitro preparation and specific mutagenesis of DNA fragments: study of protein and DNA interaction. Nucleic Acids Res 16:7351–7367
35. **Hu M-C, Guo I-C, Lin J-H, Chung B-C** 1991 Regulated expression of cytochrome P-450scc (cholesterol-side-chain cleavage enzyme) in cultured cell lines detected by antibody against bacterially expressed human protein. Biochem J 274:813–817
36. **Ogata T, Ishikawa K, Kohda E, Matsuo N** 1988 Computed tomography in the early detection of congenital lipoid adrenal hyperplasias. Pediatr Radiol 18:360–361
37. **Moutard ML, Lewein F, Baron JM, Kieffer V, Descamps P** 1998 Prognosis of isolated agenesis of the corpus callosum. Neurochirurgie 44:96–98
38. **Belhocine O, Andre C, Kalifa G, Adamsbaum C** 2005 Does asymptomatic septal agenesis exist? A review of 34 cases. Pediatr Radiol 35:410–418

**JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**