Both genes and hormones regulate human sexual development. Although ovarian hormones are not essential for female external genitalia development, male sexual development requires the action of testicular testosterone and dihydrotestosterone (DHT). DHT is the most active endogenous androgen formed by the conversion of testosterone in genital skin. This synthesis route from cholesterol to DHT is called the conventional classic pathway. Recent investigations have reported an alternative ("backdoor") route for DHT formation that bypasses fetal testicular testosterone. This alternative route plays a crucial role in human hyperandrogenic disorders like congenital adrenal hyperplasia caused by P450c21 deficiency, polycystic ovary syndrome, and P450 oxidoreductase deficiency. In addition, mutations in AKR1C2 and AKR1C4, genes encoding 3α-reductases, have been implicated in disorders of sexual development, indicating that both the classic and backdoor routes are required for normal human male sexual development. More recently, androsterone was found to be the primary androgen of the human backdoor route. Androsterone and steroidal substrates specific to the backdoor route are predominantly found in the placenta, liver, and adrenal glands rather than in the testes. These findings are essential to understanding human sexual development.

Keywords: Androgen, Testosterone, Sex development, Backdoor pathway

Highlights

- Male sexual development requires the action of testicular testosterone and dihydrotestosterone (DHT). DHT is the most active endogenous androgen, and it is converted from testosterone in genital skin. This route from cholesterol to DHT is called the conventional classic pathway. Recent reports have revealed a backdoor route to DHT that circumvents testosterone production. Androsterone is the primary circulating backdoor androgen. Furthermore, androsterone and steroidal substrates specific to the backdoor route are predominantly found in the placenta, liver, and adrenal glands rather than in the testes. These findings are crucial to our understanding of human sexual development.

Introduction

Sex development mainly occurs during the first half of gestation, with the end form of sexual structures dependent on sex chromosomes. 46,XX chromosomes in addition to genetic factors like DAX1 (dosage-sensitive/sex-reversal adrenal hypoplasia on the X chromosome), WNT-4, and RSPO1 are required for the development of ovaries. However, the development of the male sex phenotype is more complex. 46,XY chromosomes with an intact SRY (sex-determining region on the Y chromosome) gene, as well as transcription factors like SOX9, SF-1 (steroidogenic factor-1), and WT1 (Wilms tumor 1), are needed for testicular development. Fetal testicular Sertoli cells produce anti-Müllerian hormone (AMH), which is responsible for
regression of Müllerian duct derivatives, including the uterus, fallopian tubes, cervix, and upper vagina. AMH activation is most likely mediated by the SF-1 gene. By approximately 8 weeks of gestation, Leydig cells produce testosterone from cholesterol, which is sequentially mediated by several enzymes. Subsequently, testosterone facilitates the development of the Wolffian duct into the epididymis, vas deferens, and seminal vesicles. Complete development of external genitalia requires the action of dihydrotestosterone (DHT), the most potent testosterone metabolite. DHT is essential for fusing genital folds to form the penis and scrotum. In genital skin, testosterone undergoes conversion into DHT via the action of 5α-reductase type 2, which is encoded by the SRD5A2 gene. This process is called the conventional classic pathway. Recent studies revealed that DHT is also generated via an alternative ('backdoor') route from androstanediol, and this route must be intact for normal and complete prenatal masculinization. In this review, we highlight new evidence clarifying this backdoor pathway.

### Classic pathway of androgen synthesis

The classic route of androgen biosynthesis, both in the testis and adrenal zona reticularis, is well established. Cholesterol is the precursor for androgen biosynthesis, and cholesterol transformation to pregnenolone is the initial rate-limiting step. The steroidogenic acute regulatory protein (StAR) initiates the transport of cholesterol into the mitochondria. Removal of the cholesterol side-chain occurs in the inner mitochondrial membrane, producing pregnenolone; this step is catalyzed by a cholesterol side-chain cleavage enzyme (P450sc; previously termed 20,22 desmolase), which is a cytochrome P450 (CYP) enzyme. P450sc engages cholesterol 20α-hydroxylation, 22-hydroxylation, and side-chain cleavage to produce pregnenolone. Three distinct enzymes were originally thought to mediate these reactions, but P450sc, encoded by the CYP11A1 gene on chromosome 15, was found to catalyze all the steps that mediate pregnenolone formation from cholesterol. P450sc receives electrons from a reduced nicotinamide adenine dinucleotide phosphate-dependent mitochondrial electron transport system consisting of 2 accessory proteins, ferredoxin (also termed adrenodoxin) and ferredoxin reductase (adrenodoxin reductase). P450sc can only work within mitochondria, hence, cholesterol transfer to the inner mitochondrial membrane by StAR is a critical step for androgen synthesis. The zona reticularis and, to some extent, the zona fasciculata, express both 17α-hydroxylase and 17, 20 lyase activities of P450c17, thereby yielding dehydroepiandrosterone (DHEA). The 17, 20 lyase activity of P450c17 requires allosteric action of cytochrome b5 in the adrenal zona reticularis and testicular Leydig cells. Human P450c17 catalyzes 17OH-progesterone to androstenedione with only 2%–3% of 17OH-pregnenolone transformed into DHEA, so testosterone production occurs through DHEA and not through 17OH-progesterone; however, rodent and ungulate P450c17 can effectively mediate this reaction (Fig.

![Fig. 1. Classic pathway of steroidogenesis. P450sc cleaves the cholesterol side-chain to produce pregnenolone. 3βHSD2 mediates the conversion of Δ5 steroids present in the left-hand column to Δ4 steroids. The 17, 20 lyase activity of P450c17 transforms 17OH-pregnenolone into DHEA in the zona reticularis and testicular Leydig cells; only tiny amounts of 17OH-progesterone are transformed to androstenedione in humans, but this reaction occurs more commonly in other mammals. Testicular 17β-hydroxysteroid dehydrogenase type 3 (17βHSD3) transforms DHEA to androstenediol and androstenedione to testosterone; low concentrations of adrenal 17βHSD5 (AKR1C3) in the zona reticularis yield small amounts of testosterone. In ovarian granulosa cells, P450aro (aromatase) transforms androstenedione to estrone and testosterone to estradiol; in estrogenic tissues (breast, ovary, and fat), 17βHSD1 transforms estrone into estradiol. In genital skin, 5α-reductase type 2 further activates testosterone to dihydrotestosterone (DHT). StAR, steroidogenic acute regulatory protein; DOC, deoxycorticosterone; 17OH-Preg, 17OH-pregnenolone; DHEA, dehydroepiandrosterone.](image-url)
Backdoor pathway of androgen synthesis

An alternative route of androgen synthesis has been studied in the gonads of the tammar wallaby and immature mouse testes. The tammar wallaby, a small marsupial mammal, is an advantageous model for investigating male phenotypic development, as male genital development occurs over a prolonged period postnatally in the mother’s pouch, allowing easy access for tissue sampling without placental intervention. Research using young tammar wallabies revealed an alternative route for DHT production. This route circumvents conventional intermediate steroids such as DHEA, androstenedione, and testosterone. Alternatively, 17OH-progesterone can be produced from 17OH-pregnenolone, with the subsequent reduction of 5α- and 3α-, subject to the 17, 20 lyase activity of P450c17, followed by 17βHSD3-mediated catalysis to form androstanediol as the circulating product. As observed in eutherian mammals, target tissues carry out the final enzymatic conversion to generate DHT. In addition, similar reports in immature mouse testes have revealed 2 different routes for DHT formation: the classic route mediated by testosterone and the backdoor route mediated by androstanediol, generated from progesterone through dihydroprogesterone (5α-pregnan-3,20-dione), allopregnanolone (5α-pregnan-3α-ol-20-one), 17OH-allopregnanolone, and androsterone. These studies revealed the existence of a pathway sequentially converting cholesterol into DHT without the requirement for testosterone.

The backdoor route is controlled by several enzymes. First, 5α-reductase is important for this pathway, which is expressed in human fetal testes. Second, P450c17, is involved in the classic pathway, is also essential to the backdoor route. Dihydroprogesterone and allopregnanolone are precursors for the 17α-hydroxylase of P450c17, and 17OH-allopregnanolone is the most effective precursor for 17, 20 lyase. In addition, other enzymes like AKR1C2/4, 17βHSD5 (AKR1C3), and 17βHSD6 (also called retinol dehydrogenase, RoDH) are critical mediators of this pathway. Various factors determine the predominance of the classic or backdoor routes. The most significant factor is P450c17 when compared with 5α-reductase type 1, given that this activity determines whether 17OH-progesterone and progesterone are utilized in the classic or backdoor pathway. The functional level of 17, 20 lyase is also an important factor. The 17, 20 lyase activity of P450c17 was proportionally low in tammar wallabies, resulting in elevated 17OH-progesterone levels in the classic route, thereby inducing entry into the backdoor route.

![Fig. 2. Backdoor pathway of androgen biosynthesis. The backdoor route is similar to the classic route presented in Fig. 1, except for the sequential 5α- and 3α-reduction of 17OH-progesterone, first by SfRed1 to 17OH-dihydroprogesterone, and then 3α-reduction by AKR1C2 or AKR1C4 to produce 17OH-allopregnanolone. P450c17 can catalyze 17, 20 lyase activity using 17OH-allopregnanolone as the substrate, this reaction produces androsterone, which can be catalyzed by testicular 17β-hydroxysteroid dehydrogenase type 3 (17βHSD3) or adrenal 17βHSD5 (AKR1C3) to produce androstanediol. Androstanediol may then undergo 3α-oxidization, probably mediated by 17βHSD6 (also called retinol dehydrogenase, RoDH), to produce the most active androgen, DHT. StAR, steroidogenic acute regulatory protein; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; AKR, aldo-ketoreductase.](www.e-apem.org)
Backdoor pathway in male sexual development

The human backdoor route has been implicated as an essential route of androgen synthesis for male genital development. This alternative pathway plays a crucial role in human hyperandrogenic disorders like congenital adrenal hyperplasia caused by P450c21 deficiency, polycystic ovary syndrome, and POR deficiency, and in physiological male minipuberty during infancy. In addition, mutations in AKR1C2 and AKR1C4, the genes encoding 3α-reductases (3αHSD), reportedly cause disorders of sexual development (DSD), suggesting that both the classic and backdoor routes are required for normal human male sexual development.

1. Backdoor pathway in P450c21 deficiency

Congenital adrenal hyperplasia is an autosomal recessive disorder. P450c21 (21-hydroxylase) deficiency reportedly underlies >90% of cases of congenital adrenal hyperplasia, owing to mutations in the CYP21A2 gene. This enzyme transforms progesterone into deoxy corticosterone and 17OH-progesterone into 11-deoxycortisol. Female infants with classic P450c21 deficiency may present with abnormal development of external genitalia along with varying degrees of masculinization. With P450c21 deficiency, androgens are produced via all three routes. First, the conventional route from cholesterol to DHEA remains intact; although a considerable amount of DHEA is inactivated to DHEA sulfate, the increased DHEA yield will induce some DHEA to undergo transformation into testosterone and DHT. Second, while trivial amounts of 17OH-progesterone are transformed to androstenedione in the normal adrenal gland, the considerable 17OH-progesterone levels generated in P450c21 deficiency induce 17OH-progesterone transformation into androstenedione and subsequently into testosterone. As 17βHSD5 (AKR1C3) enzyme is present in the fetal adrenal gland, it can induce the transformation of some androstenedione into testosterone, predominantly throughout the fetal period via the interrelationship between DHEA transformation into androstenedione and androstenedione transformation into testosterone. Third, the backdoor route is reportedly involved in the masculinization of female patients with P450c21 deficiency, which is associated with elevated 17OH-progesterone entering the backdoor route in the adrenal gland. This alternate route relies on the 5α and 3α reduction of 17-OH progesterone to 17OH-allopregnanolone; this steroid is readily transformed to androstenediol, which can then be oxidized to DHT by an oxidative 17βHSD6 (RdDH) enzyme (Fig. 2). This backdoor route is evidenced by elevated levels of unique steroidal intermediate metabolites in the urine of infants, children, and adults with P450c21 deficiency.

Kamrath et al. studied urine steroid profiles in 142 individuals with P450c21 deficiency and revealed notably elevated concentrations of 17OH-allopregnanolone, the main steroidal intermediate of the backdoor route. The authors also presented a higher androsterone to etiocholanolone (a metabolite of DHEA) ratio in individuals with P450c21 deficiency than in controls. Androsterone can not only originate from the conventional route but also the backdoor route. However, etiocholanolone is produced most only via the conventional route. Accordingly, the ratio of androsterone to etiocholanolone acts as a barometer for the amount of androsterone originating from the alternative backdoor route.

2. Backdoor pathway in POR deficiency

The alternative backdoor route was suggested following a urinary steroid metabolite study in POR deficiency. POR deficiency is an autosomal recessive disease occurring as a result of mutations in the POR gene, which encodes electron transfer to microsomal enzymes involving P450c17, P450c21, and P450aro. POR deficiency presents diverse clinical phenotypes, including skeletal defects and adrenal steroidogenic impairment with elevated 17OH-progesterone, undervirilization in 46,XY patients, and masculinization of 46,XX patients. While it remains perplexing that affected females could be masculinized despite incomplete P450c17 function (which is needed for androgen synthesis), an alternative backdoor route reportedly exists in which 17OH-progesterone undergoes transformation to 17OH-allopregnanolone, a more useful substrate for the 17, 20 lyase activity of P450c17 than the conventional substrate, 17OH-pregnenolone. Subsequently, 17OH-allopregnanolone is transformed via several enzymatic steps to DHT, the most active androgen. Furthermore, the function of P450aro in the placenta is reduced, resulting in the unimpeded action of androgens generated by the fetal adrenals. This, in turn, aggravates female fetus masculinization and might also induce the masculinization of women pregnant with an affected fetus. This has been confirmed by decreased estril levels found in women pregnant with a fetus exhibiting POR deficiency. Patients with POR deficiency excrete urinary steroid substances, suggesting the involvement of the backdoor pathway. In patients with POR deficiency, pregnanediol (a metabolite of progesterone), pregnanetriol (a metabolite of 21-deoxycortisol), and pregnanetriol (a metabolite of 17OH-progesterone) were notably increased, and urine steroid ratios showing P450c17 and P450c21 activity were decreased. Moreover, etiocholanolone (a metabolite of DHEA) and 11-hydroxyandrosterone, associated with the classic pathway, exhibited normal or slightly decreased levels; however, androsterone, which is associated with both pathways, was elevated. This indicates the existence of the backdoor route in POR deficiency.

3. Backdoor pathway in DSD

Mutations causing DSD have been shown in AKR1C2 and AKR1C4 genes encoding 3α-reductases (3αHSD), which function exclusively in the backdoor pathway (Fig. 2). AKR1C2 is an enzyme involved in the backdoor pathway but not the...
conventional classic pathway. Fluck et al.\textsuperscript{5,6} reported AKR1C2 gene mutations with X-linked recessive inheritance in patients with 46,XY DSD. These patients presented with moderate to severe undermasculinization at birth. Patients with 46,XY DSD also exhibit a mutation that causes abnormal splicing in the AKR1C4 gene, encoding an enzyme with similar activity. An individual with 46,XY DSD was found to exhibit AKR1C2 gene mutations on both alleles, supporting the crucial role of AKR1C2.\textsuperscript{5,6} This report revealed that both the classic and backdoor pathways are required for normal human male sexual development.\textsuperscript{5,6}

4. Major human backdoor androgen

Previous works on the backdoor pathway have only provided indirect evidence, as circulating and tissue androgen levels were not determined in examined fetuses. O’Shaughnessy et al.\textsuperscript{7} measured plasma and tissue levels of steroids implicated in the classic and backdoor routes in second-trimester human fetuses using multidimensional and high-resolution mass spectrometry. The study revealed that androsterone, rather than testosterone, is the main backdoor androgen in male fetal circulation; however, significantly lower androsterone and testosterone concentrations were detected in female fetuses. In males, steroidal substrates specific to the backdoor route were predominant in the placenta and fetal liver, with significant androsterone concentrations detected in the fetal adrenal gland. Backdoor substrates, including androsterone, were only present at markedly low concentrations in the fetal testes. A PCR study revealed similar distributions of P450c17, 5α-reductase type 2, and AKR1C2/4 mRNA expression levels in the same tissues, indicating the generation of these steroids within these tissues.\textsuperscript{7} These findings indicated that androsterone is the principal backdoor androgen in the human fetus and revealed that circulating concentrations are sex-dependent; however, small amounts of de novo synthesis occur in the testis. Also, data suggest that placental progesterone acts as a substrate to produce backdoor androgens, which reportedly occurs across several tissues. Accordingly, virilization of the human male fetus is mediated via testosterone and androsterone production by both the fetal testes and nongonadal tissues, resulting in DHT synthesis in genital skin.\textsuperscript{8,9}

Conclusions

DHT is the most active endogenous androgen, and is generated from testosterone in genital skin. This DHT synthesis route, cholesterol to DHT generation, is called the conventional classic pathway. The human fetal testis is essential but insufficient to generate androgenic steroids responsible for male genital development. Recent investigations have suggested that the backdoor route generating DHT can circumvent testosterone production. The backdoor route appears to participate in normal physiological virilization, as well as in abnormal masculinization during pathological states. It has been suggested that androsterone is the primary androgen of the human backdoor route, which reportedly originates from placental progesterone metabolized to androsterone in nontesticular tissues. However, some characteristics of the human backdoor pathway need to be further clarified. Future investigations require to focus on elucidating steroidal pathways in various tissues mediated via several steroidogenic steps.

Notes

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References

1. Witchel SF, Lee PA. Ambiguous genitalia. In: Sperling MA, editor. Pediatric endocrinology. 5th ed. Philadelphia (PA): Elsevier; 2020:123-74.
2. Witchel SF. Disorders of sex development. Best Pract Res Clin Obstet Gynaecol 2018;48:90-102.
3. Siiteri PK, Wilson JD. Testosterone formation and metabolism during male sexual differentiation in the human embryo. J Clin Endocrinol Metab 1974;38:113-25.
4. Wilson JD, Griffin JE, Russell DW. Steroid 5α-reductase 2 deficiency. Endocr Rev 1993;14:577-93.
5. Miller WL, Auchus RJ. The “backdoor pathway” of androgen synthesis in human male sexual development. PLoS Biol 2019;17:e3000198.
6. Fluck CE, Meyer-Boni M, Pandey AV, Kempna P, Miller WL, Schoenle EJ, et al. Why boys will be boys: two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation. Am J Hum Genet 2011;89:201-18.
7. O’Shaughnessy PJ, Antignac JP, Le Bizec B, Morvan M-L, Svechnikov K, Soder O, et al. Alternative (backdoor) androgen production and masculinization in the human fetus. PLoS Biol 2019;17:e3000002.
8. Kamrath C, Hochberg Z, Hartmann MF, Remer T, Wudy SA. Increased activation of the alternative “backdoor” pathway in patients with 21-hydroxylase deficiency: evidence from urinary steroid hormone analysis. J Clin Endocrinol Metab 2012;97:E367-75.
9. Fukami M, Homma K, Hasegawa T, Ogata T. Backdoor pathway for dihydrotestosterone biosynthesis: implications for normal and abnormal human sex development. Dev Dyn 2013;242:320-9.
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10. Miller WL, Auchus RJ. The molecular biology, biochemistry and physiology of human steroidogenesis and its disorders. Endocr Rev 2011;32:81-151.

11. Miller WL. Congenital lipoid adrenal hyperplasia: the human gene knockout for the steroidogenic acute regulatory protein. J Mol Endocrinol 1997;19:227-40.

12. Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. Endocr Rev 1996;17:221-44.

13. Black SM, Harikrishna JA, Szklarz GD, Miller WL. The mitochondrial environment is required for activity of the cholesterol side-chain cleavage enzyme, cytochrome P450scC. Proc Natl Acad Sci USA 1994;91:7247-51.

14. Miller WL. Minireview: regulation of steroidogenesis by electron transfer. Endocrinology 2005;146:2544-50.

15. Miller WL. Disorders of androgen synthesis-from cholesterol to dehydroepiandrosterone. Med Princ Pract 2005;14 Suppl 1:58-68.

16. Renfree MB, Wilson D, Short RV, Shaw G, George FW. Steroid hormone content of the gonads of the tammar wallaby during sexual differentiation. Biol Reprod 1999;61:644-7.

17. Wilson JD, Auchus RJ, Leihy MW, Guryev OL, Estabrook RW, Osborn SM, et al. 5α-androstan-3α,17β-diol is formed in tammar wallaby pouch young testes by a pathway involving 5α-pregnane-3α,17α-diol-20-one as a key intermediate. Endocrinology 2003;144:575-80.

18. Mahendroo M, Wilson JD, Richardson JA, Auchus RJ. Steroid 5α-reductase 1 promotes 5α-androstan-3α,17β-diol synthesis in immature mouse testes by two pathways. Mol Cell Endocrinol 2004;222:113-20.

19. Butler CM, Shaw G, Renfree MB. Development of the penis and clitoris in the tammar wallaby, Macropus eugenii. Anat Embryol 1999;199:451-7.

20. Auchus RJ. The backdoor pathway to dihydrotestosterone. Trends Endocrinol Metab 2004;15:432-8.

21. Ghayee HK, Auchus RJ. Clinical implications of androgen backdoor pathway for dihydrotestosterone synthesis in male sexual differentiation. Mol Cell Endocrinol 2013;371:124-32.

22. White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 2000;21:245-91.

23. Gidlof S, Falhammar H, Thilen A, von Dobeln U, Ritzen M, Wedell A, et al. One hundred years of congenital adrenal hyperplasia in Sweden: a retrospective, population-based cohort study. Lancet Diabetes Endocrinol 2013;1:35-42.

24. White PC, New MI, Dupont B. HLA-linked congenital adrenal hyperplasia results from a defective gene encoding a cytochrome P450 specific for steroid 21-hydroxylation. Proc Natl Acad Sci USA 1984;81:7505-9.

25. Krone N, Dhir V, Ivison HE, Arlt W. Congenital adrenal hyperplasia and P450 oxidoreductase deficiency. Clin Endocrinol (Oxf) 2007;66:162-72.

26. Storbeck KH, Schiffer L, Baranowski ES, Chortis V, Prete A, Barnard L, et al. Steroid metabolome analysis in disorders of adrenal steroid biosynthesis and metabolism. Endocr Rev 2019;40:1605-25.

27. Wood PC, Speiser PW. Congenital adrenal hyperplasia and P450 oxidoreductase deficiency. J Clin Endocrinol Metab 2006;91:2643-9.

28. Dhayat NA, Dick B, Frey BM, d’Uscio CH, Vogt B, Fluck CE. Androgen biosynthesis during sexual differentiation. J Clin Endocrinol Metab 2012;97:E257-67.

29. Biason-Lauber A, Miller WL, Pandey AV, Fluck CE. Of marsupials and men: “Backdoor” dihydrotestosterone synthesis in male sexual differentiation. Mol Cell Endocrinol 2013;371:124-32.

30. Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. Endocr Rev 1996;17:221-44.

31. Marti N, Galvan JA, Pandey AV, Trippel M, Tapia C, Muller M, et al. Genes and proteins of the alternative steroid backdoor pathway for dihydrotestosterone synthesis are expressed in the human ovary and seem enhanced in the polycystic ovary syndrome. Mol Cell Endocrinol 2017;441:116-23.

32. Shackleton C, Marcos J, Malunowicz EM, Szarras-Czapnik M, Jira P, Taylor NE, et al. Biochemical diagnosis of Antley-Bixler syndrome by steroid analysis. Am J Med Genet A 2004;128A:223-31.

33. Homma K, Hasegawa T, Nagai T, Adachi M, Horikawa R, Fujiwara I, et al. Urine steroid hormone profile analysis in cytochrome P450 oxidoreductase deficiency: implication for the backdoor pathway to dihydrotestosterone. J Clin Endocrinol Metab 2006;91:2643-9.
41. Arlt W, Walker EA, Draper N, Ivison HE, Ride JP, Hammer F, et al. Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. Lancet 2004;363:2128-35.

42. Fukami M, Nishimura G, Homma K, Nagai T, Hanaki K, Uematsu A, et al. Cytochrome P450 oxidoreductase deficiency: identification and characterization of biallelic mutations and genotype-phenotype correlations in 35 Japanese patients. J Clin Endocrinol Metab 2009;94:1723-31.

43. Shackleton C, Marcos J, Arlt W, Hauffa BP. Prenatal diagnosis of P450 oxidoreductase deficiency (ORD): a disorder causing low pregnancy estriol, maternal and fetal virilization, and the Antley-Bixler syndrome phenotype. Am J Med Genet A 2004;129A:105-12.

44. Fukami M, Horikawa R, Nagai T, Tanaka T, Naiki Y, Sato N, et al. Cytochrome P450 oxidoreductase gene mutations and Antley-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients. J Clin Endocrinol Metab 2005;90:414-26.