ISSN 2472-1972

Pubertal and Adult Testicular Functions in Nonclassic Lipoid Congenital Adrenal Hyperplasia: A Case Series and Review

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Lipoid congenital adrenal hyperplasia (LCAH) is caused by mutations in *STAR* and characterized by a defect in steroidogenesis and lipid droplet accumulation in steroidogenic cells. Patients with 46,XY and classic LCAH will typically present with female-type external genitalia. However, those with nonclassic LCAH will have masculinized external genitalia. The rarity of the nonclassic form has precluded the clarification of the long-term outcomes of testicular function in nonclassic LCAH. We report the cases of three adult males with nonclassic LCAH in whom primary adrenal insufficiency had been diagnosed at 5 days, 4 years, and 5 years of age. All exhibited complete male external genitalia and had completed pubertal development without androgen replacement. The endocrinological data showed preserved gonadal function in patients 1 and 2 and hypergonadotropic hypogonadism in patient 3. Semen analyses showed normozoospermia in patient 1 and mild oligozoospermia in patient 2. Electron microscopic analysis of a testicular biopsy specimen from patient 2 at 13 years of age revealed prominent lipid accumulation in the cytosol of Leydig cells. Patients 1 and 2 shared the same compound heterozygous mutations in *STAR* (p.Glu258* and p.Arg272Cys). Patient 3 possessed a heterozygous dominant-negative mutation in *STAR* (p.Gly22_Leu59del). A functional assay of a variant STAR-Arg272Cys determined the residual activity as 35% of the wild-type STAR. The results from the present case series and a review of four previously reported adult cases indicate that testosterone synthesis can be preserved in most males with nonclassic LCAH to complete pubertal development and induce germ cell maturation despite lipid accumulation in the Leydig cells.

Freeform/Key Words: lipoid congenital adrenal hyperplasia, nonclassic form, steroidogenic acute regulatory protein, testicular function, spermatogenesis

Lipoid congenital adrenal hyperplasia (LCAH; OMIM 201710) is caused by loss-of-function mutations in the *STAR* encoding steroidogenic acute regulatory protein (StAR) [1]. Because StAR functions as a cholesterol transporter from the outer to the inner mitochondrial membrane in the first step of steroidogenesis [2], LCAH is characterized clinically by the

LCAH, lipoid congenital adrenal hyperplasia; PAI, primary adrenal insufficiency; StAR, *STAR* encoding steroidogenic acute regulatory protein.

Received 5 March 2019
Accepted 10 May 2019
First Published Online 16 May 2019

Case Report
July 2019 | Vol. 3, Iss. 7
doi: 10.1210/js.2019-00086 | Journal of the Endocrine Society | 1367–1374

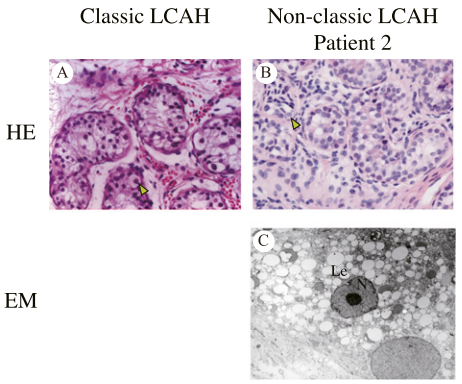

Figure 1. Histopathological features of a testicular biopsy specimen. (A) Classic LCAH at 1 y of age (not from the patients in the present report). (B) Patient 2 at 5 y of age. (C) Patient 2 at 13 y of age. (A,B) The findings were obtained using light microscopy with hematoxylin-eosin (HE) staining. (C) The findings were obtained using electron microscopy (EM). Yellow arrowheads indicate spermatocytes. Le, Leydig cell; N, nucleus.

impairment of steroidogenesis in the adrenal glands and gonads and pathologically by the accumulation of cholesterol ester in the cytosol of adrenal and gonadal steroidogenic cells [1]. Patients with LCAH will typically exhibit neonatal-onset or early infantile-onset primary adrenal insufficiency (PAI) and female-type or minimally masculinized external genitalia, regardless of their chromosomal sex.

Baker *et al.* [3] reported patients with atypical 46,XY who had had late infantile-onset or childhood-onset PAI and completely masculinized external genitalia. They defined these cases as a nonclassic form of LCAH [3]. Thirteen families with nonclassic LCAH, including those reported by Baker et al. [3] have been reported, including 13 males who showed complete male external genitalia or hypospadias [3–8]. Testicular biopsy and semen analysis were described in only one case each [7]. To the best of our knowledge, the testicular function through puberty into adulthood of males with nonclassic LCAH has not been comprehensively elucidated. We present the pubertal and adult testicular functions of three Japanese adult males with nonclassic LCAH and assessed whether their testosterone synthesis was sufficient for pubertal development or germ cell maturation.

1. Case Report

A. Patient 1

A boy was born as the only child of nonconsanguineous Japanese parents through a vaginal delivery at 40 weeks of gestation. His birth weight and length was 3690 g and 54.5 cm, respectively. He had complete male external genitalia with descended testes. At 1 year of age, hyperpigmentation of the lips was noticed. The hyperpigmentation gradually spread to the skin and buccal mucosa. At 5 years of age, PAI was diagnosed based on high plasma ACTH (>6000 pg/mL; >1320 pmol/L) and low serum cortisol (1.7 μg/dL; 46.9 nmol/L) levels. He was subsequently treated with hydrocortisone and fludrocortisone. Detailed information on pubertal development for this patient was not available.

At 35 years of age, his height and weight were 159.1 cm and 52.7 kg, respectively. The hydrocortisone dosage was 25 mg/d. His plasma ACTH and serum cortisol levels during replacement therapy were 109.0 pg/mL (24.0 pmol/L) and 16.9 μg/dL (466.2 nmol/L), respectively. Fludrocortisone replacement was discontinued after he developed hypertension. The plasma active renin concentrations (range, 9.2 to 37.3 pg/mL; 0.22 to 0.88 pmol/L) were not elevated during hydrocortisone replacement. The stretched penile length, testicular volume, and serum gonadotropin and testosterone levels were within the normal range (Table 1). Semen analysis revealed a sperm count of 60×10^6/mL (lower reference limit, 15×10^6/mL) with a motility of 60% (lower reference limit, 40%) and normal morphology of 23.5% (lower reference limit, 4%).

Table 1. Adult Testicular Function of Males With Nonclassic LCAH

Case	External Genitalia	Age at Pubertal Entry, y	Pubertal Development	Age at Evaluation, y	Testicular Volume, mL	Pubic Hair (Tanner Stage)	LH, IU/L	FSH, IU/L	T, ng/mL; nmol/L	Semen Volume, mL	Sperm Count, $\times 10^6$/mL)	*STAR* Genotype
Our cases												
1	Normal male	NA	Spontaneously completed	35	20	IV	5.2	3.7	4.46; 15.5	3.8	60	p.Gln258*; p.Arg272Cys
2	Normal male	11.6	Spontaneously completed	30	R20, L12	IV	8.4 → 90.6[a]	6.4 → 20.4[a]	5.55 → 10.70[b]	6.0	14	p.Gln258*; p.Arg272Cys
3	Normal male	11.3	Spontaneously completed	20	5	IV	18.8	34.2	5.02; 1.7	NA	NA	p.Gly22_Leu59del
Previously reported cases[c]												
4	NA	NA	NA	36	NA	NA	12	24	2.80; 1.0	NA	NA	p.Arg192Cys; p.Arg192Cys
5	Glanular hypospadias	NA	Spontaneously completed	28	Normal	NA	15.7	NA	4.09; 1.4	NA	Normal	p.Arg188Cys; p.Arg188Cys
6	Normal male	11.5	NA	29	25	NA	7.3	7.2	6.69; 2.3	NA	NA	p.Gly221Ser; p.Thr44Hisfs
7	Severe hypospadias	NA	Required androgen replacement	27	NA	NA	15.2	16.7	0.78; 0.3	NA	NA	p.Phe267Ser; p.Leu260Pro

Abbreviations: L, left; NA, not available; R, right; T, testosterone.
[a]GnRH stimulation test.
[b]Human chorionic gonadotropin stimulation test (19.3 → 37.1 nmol/L).
[c]The cases of patients 4 and 5 were reported by Metherell *et al.* [7], patient 6 by Flück *et al.* [6], and patient 7 by Sahakitrungruang *et al.* [5].

B. Patient 2

A boy was born as the first child of nonconsanguineous Japanese parents through an elective cesarian section because of uterine inertia at 44 weeks of gestation. His birth weight and length was 2985 g and 52.0 cm, respectively. He had complete male external genitalia. He showed hyperpigmentation of the lips at 3 years of age. At 4 years of age, hyperpigmentation of the skin was noticed when he had an episode of recurrent vomiting. PAI was diagnosed based on a high plasma ACTH level (4600 pg/mL; 1012 pmol/L), a low serum cortisol level (2.4 μg/dL; 66.2 nmol/L), high plasma renin activity (24.0 ng/mL/h), and relatively low serum aldosterone level (59.0 pg/mL; 163.7 pmol/L) and was subsequently treated with hydrocortisone only. He underwent orchiopexy of his left testis at 5 years of age. Testicular biopsy revealed the presence of germ cells in the seminiferous tubules with hyaline-like hypertrophy of the basement membrane and the broad interstitial regions without Leydig cell hyperplasia (Fig. 1B). The second testicular biopsy at 13 years of age detected prominent accumulation of lipid droplets in the cytosol of Leydig cells using light and electron microscopy (Fig. 1C). These biopsy specimens were histologically analyzed using the same method as in our previous study [9]. His testicular volume had increased to 3 mL at 11.6 years of age and had gradually increased to 20 mL in the right and 12 mL in the left [10]. His pubic hair had started to develop at 14.1 years of age, and his voice had deepened at 14.5 years.

Although he had been continuously instructed to take hydrocortisone (30 mg/d), he had stopped taking hydrocortisone and attending regular follow-up examinations at 17 years of age. He had experienced three episodes of severe fatigue and nausea at 24, 25, and 30 years of age. At 30 years of age, he returned to the outpatient clinic for reevaluation of these episodes. His height was 170.6 cm and weight, 76.5 kg. He had severe hyperpigmentation of the skin, lips, and buccal mucosa. At 7 AM, the plasma ACTH level was 5960 pg/mL (1311 pmol/L) and serum cortisol level was 7.6 μg/dL (209.7 nmol/L). A standard ACTH stimulation test showed a blunted response of the serum cortisol levels from 6.5 to 7.4 μg/dL (179.3 to 204.2 nmol/L). An abdominal computed tomography scan revealed multiple irregularly shaped nodules in the adrenal glands. He agreed to resume hydrocortisone replacement at 15 mg/d. During replacement therapy, his plasma ACTH and serum cortisol levels were 739 pg/mL (162.8 pmol/L) and 6.5 μg/dL (179.3 nmol/L), respectively. Although the response of serum gonadotropin to GnRH appeared to be brisk, the serum gonadotropin and testosterone levels at baseline were within the normal range (Table 1). A testicular ultrasound scan at 31 years of age detected two microcalcifications in the left testis. Semen analysis revealed a sperm count of 14×10^6 /mL. The patient refused to undergo further analysis of fertility.

C. Patient 3

A boy was born as the second child of nonconsanguineous Japanese parents through an uneventful vaginal delivery at 41 weeks of gestation. His birth weight and length was 3090 g and 49.0 cm, respectively. He had complete male external genitalia with descended testes. Skin hyperpigmentation was already observed at birth. At 5 hours of age, he showed intermittent movements, indicating seizure. His plasma glucose, serum sodium, potassium, and chloride level was 26 mg/dL (1.4 mmol/L), 139 mEq/L, 5.6 mEq/L, and 111 mEq/L, respectively. PAI was diagnosed based on a high plasma ACTH level (4858 pg/mL; 1069 pmol/L), low serum cortisol level (0.3 μg/dL; 8.3 nmol/L), and high plasma renin activity (>80.0 ng/mL/h). He was subsequently treated with hydrocortisone and fludrocortisone.

His testicular volume had increased to 3 mL at 11.3 years of age and to 5 mL at 12.5 years of age but did not increase further [10]. His pubic hair had started to develop at 15.3 years of age. At 20 years of age, the hydrocortisone and fludrocortisone dosage was 20 mg/d and 0.05 mg/d, respectively. His height and weight was 152.2 cm and 40.2 kg, respectively. His plasma ACTH and serum cortisol levels during replacement therapy were 360.0 pg/mL (79.3 pmol/L) and 15.1 μg/dL (416.6 nmol/L), respectively. The high serum gonadotropin levels indicated the

presence of hypergonadotropic hypogonadism (Table 1). A testicular ultrasound scan did not show any abnormalities, other than small testes. He decided not to undergo a semen analysis.

D. Genetic and Functional Studies

The patients or their parents provided written informed consent for the molecular studies, which were approved by the ethics committee of Keio University School of Medicine. We extracted genomic DNA from their peripheral lymphocytes and analyzed *STAR* using PCR-based Sanger sequencing. The primer sequences and PCR conditions are provided in an online repository [11]. We identified compound heterozygous mutations (p.Glu258* and p.Arg272Cys) in patients 1 and 2 and a heterozygous mutation (p.Gly22_Leu59del) in patient 3. The results of the genetic analysis of patients 1 and 2 have been previously reported [12]. p.Glu258* is a functionally null mutation that has been reported to be the most common mutation of *STAR* in East Asia [4]. p.Arg272Cys has been previously reported but has not been functionally analyzed [13]. We assessed the activity of STAR-Arg272Cys to enhance pregnenolone production using *in vitro* analysis in COS-1 cells, which were cotransfected by a plasmid F2 expressing the NH2-CYP11A1-FDXR-FDX1 fusion protein (a gift from Dr. Walter Miller, University of San Francisco, San Francisco, CA) [14]. We found that STAR-Arg272Cys retained 35% of the wild-type STAR activity (Table 2). STAR-Gly22_Leu59del was previously reported as having a dominant-negative effect on wild-type STAR [8].

2. Review of Reported Data

Thirteen males with nonclassic LCAH have been previously reported [3, 5–7]. All 13 males had required glucocorticoid replacement, and 4 had also required mineralocorticoid replacement. Of these 13 patients, 3 had "normal" male external genitalia, and 4 showed abnormal virilization, including glanular hypospadias in 2, severe hypospadias in 1, and micropenis in 1. Testicular function during adulthood was reported for only 4 of these 13 patients (Table 1). Of these four patients, three showed hypergonadotropic hypogonadism. Of the three patients, patient 5 exhibited a normal sperm count without androgen replacement [7], patient 4 had impaired fertility [7], and patient 7 had required androgen replacement therapy for pubertal development [5].

3. Discussion

We reported the cases of three adult males with nonclassic LCAH, two of whom had the same compound heterozygous mutations and one of whom had the heterozygous mutation in *STAR*. We had previously identified p.Arg272Cys in Japanese female patients with nonclassic LCAH who had presented with preserved estradiol and progesterone syntheses sufficient enough to enable normal pubertal development and even successful pregnancy and delivery without progesterone replacement [13]. To the best of our knowledge, the present

Table 2. Functional Analysis of STAR-Arg272Cys

Variable	Pregnenolone, ng/mL/48 h
pRK5	5.3 ± 0.29
pRK–wild-type STAR	58.1 ± 3.1[a]
pRK–STAR-Arg272Cys	20.3 ± 0.95[a,b]

Data presented as the pregnenolone concentrations in the culture media obtained from four independent experiments, each performed in quadruplicate, and shown as the mean ± SEM (n = 4).
Abbreviation: pRK5, empty plasmid.
[a] $P < 0.01$ vs pRK5.
[b] $P < 0.01$ vs pRK–wild-type STAR.

study is the first to have determined that the residual activity of STAR-Arg272Cys is 35% of the wild type. This activity is consistent with those of other mutant proteins causing nonclassic LCAH, with a range of 6% to 40% [3–7]. p.Gly22_Leu59del has been already reported as a dominant-negative mutation in a Brazilian female patient with 46,XY and LCAH with neonatal-onset PAI and severe undermasculinization [8]. Considering that the reported residual activity of STAR-Gly22_Leu59del was ~60% [8], it is not surprising that the p.Gly22_Leu59del was identified in our male-assigned case of nonclassic LCAH. Patient 3 showed the earliest onset of PAI in association with nonclassic LCAH. The onset age of PAI overlaps between classic and nonclassic LCAH and likely depends on the timing and degree of physical stress. Thus, it is quite difficult to differentiate between these two forms of LCAH, especially in 46,XX females who exhibit female external genitalia, regardless of the form of LCAH. These results have expand our knowledge of the genotypic and phenotypic variability of nonclassic LCAH.

All our patients developed male external genitalia and had completed pubertal development without androgen replacement therapy. However, their testicular function during adulthood varied. Patients 1 and 2 did not show hypergonadotropic hypogonadism. However, patient 3 had developed compensated hypergonadotropic hypogonadism. Patient 1 showed a normal sperm count and quality, patient 2 exhibited oligospermia, and patient 3 likely had impaired fertility due to hypoplastic testes. The compromised spermatogenesis could reflect a decreased intratesticular testosterone level [15]. Metherell *et al.* [7] described four males with nonclassic LCAH, including one (patient 5 in Table 1) with moderately compensated hypergonadotropic hypogonadism and a normal sperm count at 28 years of age. They reported another case (patient 4 in Table 1) that showed impaired fertility and no lipid accumulation in the cytosol of possibly hyperplastic Leydig cells at 36 years of age. Inconsistent with patient 4, we observed prominent lipid droplets in the Leydig cells in patient 2. To the best of our knowledge, this the first identification in nonclassic LCAH. The lipid accumulation could vary with age or otherwise depend on the residual activity of mutant STAR. The estimated residual activity of STAR in patient 2, who was compound heterozygous for p.Glu258* and p.Arg272Cys (~18%), was less than that in patient 4, who was homozygous for p.Arg192Cys (40%). These data indicate that the testosterone-producing capacity can be preserved in most males with nonclassic LCAH to enable the development of male external genitalia, achieve complete pubertal development, and, even, induce germ cell maturation despite lipid accumulation in the Leydig cells.

All our patients with nonclassic LCAH had severe PAI, irrespective of the variable onset of PAI and variable degrees of hypogonadism. The accumulating evidence for the phenotype of human patients with nonclassic LCAH and the previously reported phenotypes of various mouse models could provide valuable insights into the role of StAR in the steroidogenesis of different tissues. A BAC transgene of Star-Met1_Trp47del had rescued PAI in 75% and undermasculinization in 100% of Star knockout mice [16]. Star-Met1_Trp47del in those mice and STAR-Gly22_Leu59del in patient 3 could lose the mitochondrial targeting signal in the amino terminus. Both the Star-Met1_Trp47del–rescued mice and patient 3 exhibited male external genitalia. Thus, the mitochondrial targeting signal of StAR is not essential for the steroidogenesis of fetal Leydig cells. Another BAC transgene of Star-Ser195Ala did not rescue PAI but partially rescued undermasculinization in 33% of Star knockout mice [17]. The phosphorylation at Ser195 is essential for the steroidogenesis of adrenocortical cells. These phenotypes of BAC-rescued mice and patients with nonclassic LCAH have demonstrated that the steroidogenesis of fetal Leydig cells might be more retrievable than that of adrenocortical cells in cases in which the StAR protein has been partially rescued. The difference in terms of StAR dependency remains unknown. It could be due to the compensation of StAR-independent steroidogenesis or the vulnerability of cell damage from cytosolic cholesterol ester accumulation. Collectively, the human and murine phenotypes in partial StAR deficiency suggest that StAR-dependent steroidogenesis is more important in adrenocortical cells than in fetal or adult Leydig cells.

The present study addressed two important issues in the phenotype of nonclassic LCAH. We found no clear distinction between the classic and nonclassic forms with respect to the onset of PAI. The nonclassic form clearly applies only to 46,XY patients who develop sufficient male external genitalia to be considered male. Another issue is the phenotypic variability in testicular function between male patients with nonclassic LCAH with the same *STAR* mutations. Steroidogenesis and spermatogenesis can be affected by other factors, including cryptorchidism or variations in disease susceptibility genes. These follow-up data could contribute to the improved treatment of males with nonclassic LCAH.

Acknowledgments

We are grateful to Dr. Walter L. Miller (University of California, San Francisco, California) for providing the F2 plasmid.

Financial Support: The present study was supported by the Japanese Society for the Promotion of Science Grants-in-Aid for Scientific Research (grant JP16K09996/JP19K08987 to T.I. and grant JP17K10091 to N.K.) and the Ministry of Health, Labour and Welfare of Japan (grant Nanbyo-Ippan-046 to T.H., N.K., and T.I. for research on intractable diseases).

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Disclosure Summary: The authors have nothing to disclose.

References and Notes

1. Bose HS, Sugawara T, Strauss JF III, Miller WL, Consortium ICLAH; International Congenital Lipoid Adrenal Hyperplasia Consortium. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. *N Engl J Med*. 1996;**335**(25):1870–1878.
2. Clark BJ, Wells J, King SR, Stocco DM. The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells: characterization of the steroidogenic acute regulatory protein (StAR). *J Biol Chem*. 1994;**269**(45):28314–28322.
3. Baker BY, Lin L, Kim CJ, Raza J, Smith CP, Miller WL, Achermann JC. Nonclassic congenital lipoid adrenal hyperplasia: a new disorder of the steroidogenic acute regulatory protein with very late presentation and normal male genitalia. *J Clin Endocrinol Metab*. 2006;**91**(12):4781–4785.
4. Nakae J, Tajima T, Sugawara T, Arakane F, Hanaki K, Hotsubo T, Igarashi N, Igarashi Y, Ishii T, Koda N, Kondo T, Kohno H, Nakagawa Y, Tachibana K, Takeshima Y, Tsubouchi K, Strauss JF III, Fujieda K. Analysis of the steroidogenic acute regulatory protein (StAR) gene in Japanese patients with congenital lipoid adrenal hyperplasia. *Hum Mol Genet*. 1997;**6**(4):571–576.
5. Sahakitrungruang T, Soccio RE, Lang-Muritano M, Walker JM, Achermann JC, Miller WL. Clinical, genetic, and functional characterization of four patients carrying partial loss-of-function mutations in the steroidogenic acute regulatory protein (StAR). *J Clin Endocrinol Metab*. 2010;**95**(7):3352–3359.
6. Flück CE, Pandey AV, Dick B, Camats N, Fernández-Cancio M, Clemente M, Gussinyé M, Carrascosa A, Mullis PE, Audi L. Characterization of novel StAR (steroidogenic acute regulatory protein) mutations causing non-classic lipoid adrenal hyperplasia. *PLoS One*. 2011;**6**(5):e20178.
7. Metherell LA, Naville D, Halaby G, Begeot M, Huebner A, Nürnberg G, Nürnberg P, Green J, Tomlinson JW, Krone NP, Lin L, Racine M, Berney DM, Achermann JC, Arlt W, Clark AJL. Nonclassic lipoid congenital adrenal hyperplasia masquerading as familial glucocorticoid deficiency. *J Clin Endocrinol Metab*. 2009;**94**(10):3865–3871.
8. Baquedano MS, Guercio G, Marino R, Berensztein E, Costanzo M, Bailez M, Vaiani E, Maceiras M, Ramirez P, Chaler E, Rivarola MA, Belgorosky A. Unique dominant negative mutation in the N-terminal mitochondrial targeting sequence of StAR, causing a variant form of congenital lipoid adrenal hyperplasia. *J Clin Endocrinol Metab*. 2013;**98**(1):E153–E161.
9. Aya M, Ogata T, Sakaguchi A, Sato S, Matsuo N. Testicular histopathology in congenital lipoid adrenal hyperplasia: a light and electron microscopic study. *Horm Res*. 1997;**47**(3):121–125.
10. Figshare. Deposited. 2019;25. 10.6084/m9.figshare.7764038.
11. Figshare. Deposited. 2019;22. 10.6084/m9.figshare.8020106.
12. Amano N, Narumi S, Hayashi M, Takagi M, Imai K, Nakamura T, Hachiya R, Sasaki G, Homma K, Ishii T, Hasegawa T. Genetic defects in pediatric-onset adrenal insufficiency in Japan. *Eur J Endocrinol*. 2017;**177**(2):187–194.

13. Hatabu N, Amano N, Mori J, Hasegawa Y, Matsuura H, Sumitomo N, Nishizawa K, Suzuki M, Katakura S, Kanamoto N, Kamimaki T, Ishii T, Hasegawa T. Pubertal development and pregnancy outcomes in 46,XX patients with nonclassic lipoid congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2018;**104**(5):1866–1870.
14. Harikrishna JA, Black SM, Szklarz GD, Miller WL. Construction and function of fusion enzymes of the human cytochrome P450scc system. *DNA Cell Biol*. 1993;**12**(5):371–379.
15. Anderson RA, Baird DT. Male contraception. *Endocr Rev*. 2002;**23**(6):735–762.
16. Sasaki G, Ishii T, Jeyasuria P, Jo Y, Bahat A, Orly J, Hasegawa T, Parker KL. Complex role of the mitochondrial targeting signal in the function of steroidogenic acute regulatory protein revealed by bacterial artificial chromosome transgenesis *in vivo*. *Mol Endocrinol*. 2008;**22**(4):951–964.
17. Sasaki G, Zubair M, Ishii T, Mitsui T, Hasegawa T, Auchus RJ. The contribution of serine 194 phosphorylation to steroidogenic acute regulatory protein function. *Mol Endocrinol*. 2014;**28**(7): 1088–1096.