Genetics of congenital adrenal hyperplasia and genotype-phenotype correlation

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Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is caused by mutations in the CYP21A2 gene, located on the short arm of chromosome 6. The two main underlying mechanisms of CYP21A2 defects are large gene deletion and conversion. Anticipation of the phenotypes associated with different combinations of CYP21A2 mutations remains the most important determinant in prenatal diagnosis and counseling of the expectant couple who are determined to be at risk for congenital adrenal hyperplasia. (Fertil Steril® 2019;111:24–9. ©2018 by American Society for Reproductive Medicine.)

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Synthesis of adrenal steroid hormones and cortisol is mediated by five major enzymes whose impaired activity leads to the group of disorders called congenital adrenal hyperplasia (CAH). The five steroidogeneic enzymes are cytochrome P450 side chain cleavage enzyme encoded by the *CYP11A1* (cytochrome P450, family 11, subfamily A, member 1), 21 α hydroxylase (encoded by the *CYP21A2* gene: cytochrome P450 family 21 subfamily A member 2), 11 β hydroxylase (encoded by the *CYP11B1* gene: cytochrome P450, family 11, subfamily B, member 1), 3β-hydroxysteroid dehydrogenase 2 (encoded by the *HSD3B2* gene), and 17-hydroxylase/17, 20-lyase deficiency (encoded by the *CYP17A1* gene: cytochrome P450, family 17, subfamily A, member 1) (1–5).

This Views and Reviews centers on 21-hydroxylase deficiency CAH due to genetic defects in the CYP21A2 gene, with a focus on genotype-phenotype correlations. Pathophysiology and biochemical findings in CAH are discussed in this Views and Reviews section by Gomes et al. Depending on the degree of impairment of the 21-hydroxylase, three distinct phenotypes have been described: classical salt wasting, classical simple virilizing, and nonclassical CAH (6, 7).

Virilization of external genitalia in the female newborn, salt wasting (potentially fatal), rapid somatic growth, and skeletal maturation with a subsequent compromised adult height, precocious adrenarche and/or puberty, acne, hirsutism, irregular menses, and fertility concerns are all features of classical salt-wasting CAH. Patients with classical simple virilizing CAH present with similar features but with an intact mineralocorticoid pathway. The main distinctive clinical feature between classical and nonclassical CAH is the normal external genitalia in the newborn female with nonclassical CAH and the lack of genital ambiguity; patients with nonclassical CAH may present with mild features of hyperandrogenemia or may be asymptomatic (6, 7).

CYP21A2 GENE AND PSEUDOGENE

The *CYP21A2* gene is a 10-exon, 3.1 kb gene that is mapped on the short arm of chromosome number 6 within the major histocompatibility complex region (locus 6p21.31). The *CYP21A2* gene is specifically located within proximity of three other genes along a 730 kb region called the *RCCX* module. The three neighboring genes, *RP1*, *C4*, and *TNXB*, respectively, encode a nuclear serine/threonine protein kinase, which is the fourth complement system component, and an extracellular protein matrix called *Tenascin X* that is expressed in the skin, tendons, and blood vessels.

Tandem duplication, a well-established phenomenon of molecular evolution, may result in a mono-, bi-, or tri-modular RCCX module. The most common outcome of this tandem

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duplication is the bimodular RCCX module, *RP1-C4A-CYP21A1-TNXA/RP2-C4B-CYP21A2-TNXB*.

The active gene encoding the 21-hydroxylase enzyme *CYP21A2* and the inactive gene *CYP21A1* "pseudogene" are highly similar, with 98% homology; 15 mutations render the *CYP21A1* inactive. Four promoter region mutations—an intronic mutation, two frameshift mutations on exons 3 and 7, and eight single base pair missense mutations (on exons 1, 4, 7, three on exon 6, and two on exon 8)—comprise the 15 *CYP21A1*/pseudogene mutations. Knowledge of these 15 *CYP21A1*/pseudogene mutations is vital to understanding the molecular pathophysiology of 21-hydroxylase deficiency CAH. Figure 1 demonstrates the CYP21A2 gene and its location within the RCCX module on chromosome number 6. The location of the 15 *CYP21A1*/pseudogene mutations is also shown (7, 8).

MECHANISM OF MUTATIONS

Hundreds of *CYP21A2* disease-causing defects have been described to date, with gene conversions and deletions as the underlying mechanisms in the vast majority of cases. Other mechanisms, such as de novo mutations and uniparental disomy, have also been described (7).

Gene Conversions

Misalignment of sister chromatids during mitosis leads to exchange of genetic material between the CYP21A2 and CYP21A1. This results in transfer of CYP21A1/pseudogene mutations to the CYP21A2 gene. Pseudogene-derived CYP21A2 mutations are sometimes referred to as "common mutations" on commercial testing panels (7).

CYP21A1/Pseudogene-Derived Mutations

Four promoter region mutations (g.-103A>G, g.-110T>C, g.-113G>A, g.-126C>T). Present in the noncoding region of the CYP21 and associated with reduction of transcriptional activity to 20%.

Exon 1 mutation (g.89C>T [p.P30L]). A mild missense single base pair mutation associated with 40%–70% of 21-hydroxylase activity (9).

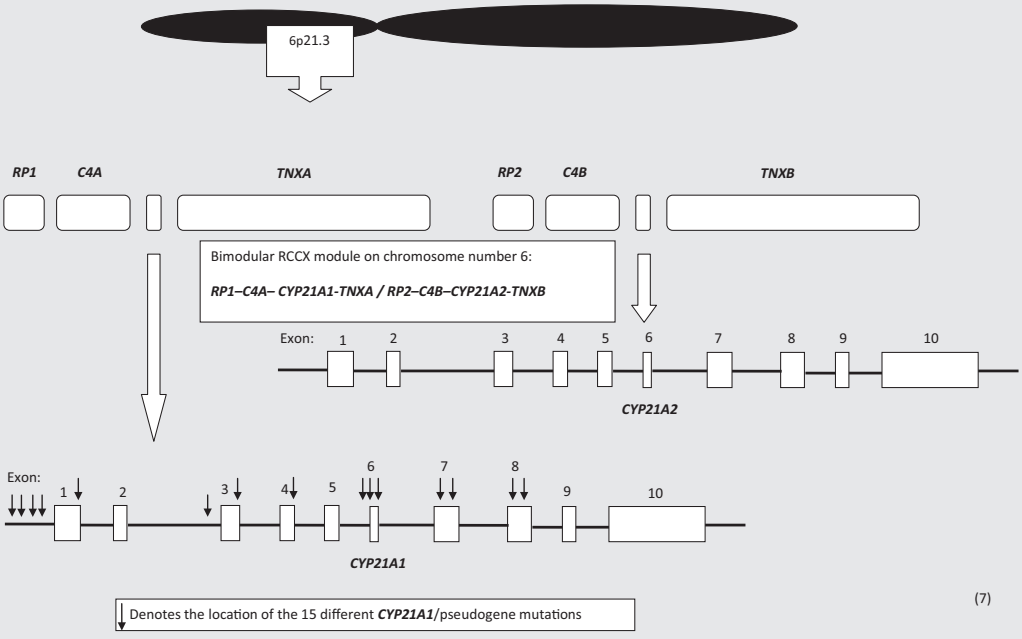
Intron 2 mutation (g.655C/A>G). Associated with aberrant slicing due to upstream activation of a splice acceptor site and <5% of 21-hydroxylase enzyme activity (6, 9).

Exon 3 mutation (g.707_714delGAGACTAC [p.G110fs]). A frameshift mutation associated with an eight base pair deletion, a premature termination codon, and complete loss of 21-hydroxylase enzyme activity (9).

Exon 4 mutation [g.999T>A (p.I172N)]. Associated with loss of the hydrophobic pocket and reduction of 21-hydroxylase enzyme activity to 2% (1, 6, 10).

Exon 6 mutations [g.1380T>A (p.I236N), g.1383T>A (p.V237E) and g.1389T>A (p.M239K)]. This triple mutation cluster always occurs together and is associated with a substrate-binding defect depleting the

FIGURE 1



The RCCM module: CYP21A2 and CYP21A1 gene with mutation sites.

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21-hydroxylase enzyme activity to 0%. Of note, p.I236N and p.M239K do not appear to cause significant structural disruption if present alone; nevertheless, both always occur together in conjunction with the highly disruptive p.V237E (1, 9).

Exon 7 mutations [g.1683G>T (p.V281L)]. Another mild missense single base pair mutation that is associated with 20%–50% of 21-hydroxylase activity.

[g.1762_1763insT(p.L307fs)]. Another frameshift mutation but here associated with a single base insertion and premature termination codon associated with complete loss of 21-hydroxylase enzyme activity (9).

Exon 8 mutations [g.1994C>T (p.Q318X) and g.2108C>T (p.R356W)] Associated with disruption of H-bonding and loss of 21-hydroxylase enzyme (1).

Large Gene Deletion via Unequal Crossover

Misalignment of sister chromatids during meiosis and subsequent unequal crossover of genetic material of the RCCX module result in a chromatid with one RCCX module (monomodular) and another chromatid with three RCCX modules (trimodular). The effect of this misalignment and unequal crossover is determined by the site of crossover. Crossover can happen at the C4, CYP21, or TNX genes, with three different resultant chimeras (7).

A simplified demonstration of the three possible scenarios and the resultant chimeric hybrids for the possible crossover sites is shown in Figure 2.

The first possible scenario (crossover site is C4) results in a trimodular RCCX chromatid with one CYP21A2 copy and two CYP21A1 copies (inheritance of this allele does not carry a risk for CAH due to the presence of an intact CYP21A2 copy) (11) and a monomodular RCCX chromatid with one CYP21A2 copy and a functional C4A/C4B chimera (inheritance of this allele does not carry a risk for CAH due to the presence of an intact CYP21A2 copy) (7). Thus, crossover at C4 does not result in 21-hydroxylase deficiency CAH because each chromosome will ultimately harbor an intact CYP21A2 (7).

The second possible scenario (crossover site is TNX) results in a monomodular RCCX chromatid with a deleted CYP21A2 and one CYP21A1 copy (clearly, inheritance of this allele is associated with risk for CAH) (11) and a trimodular RCCX chromatid with two CYP21A2 copies and one CYP21A1 copy (inheritance of this allele does not carry a risk for CAH due to the presence of an intact CYP21A2) (7). Thus, crossover at TNX may result in 21-hydroxylase deficiency CAH if the offspring inherits the chromosome with the deleted CYP21 (7).

The third possible scenario (crossover site is CYP21) results in a trimodular RCCX chromatid with one CYP21A2 copy, one CYP21A1 copy, and a CYP21A1CYP21A2 chimera (inheritance of this allele does not carry a risk for CAH due to the presence of an intact CYP21A2) (11) and a monomodular RCCX chromatid with a CYP21A1CYP21A2 chimera (because CYP21A1CYP21A2 harbors mutations, the inheritance of this allele is associated with risk for CAH). Nine different junction sites result in nine different CYP21A1CYP21A2 chimeras, each of which will harbor CYP21A1 mutations (7).

Figure 3 shows the nine possible CYP21A1CYP21A2 chimeras with the respective mutations. CYP21A1(pseudogene) and CYP21A2 are denoted by gray and white arrows, respectively.

GENOTYPE-PHENOTYPE CORRELATION IN CAH AND THE CLINICAL RELEVANCE

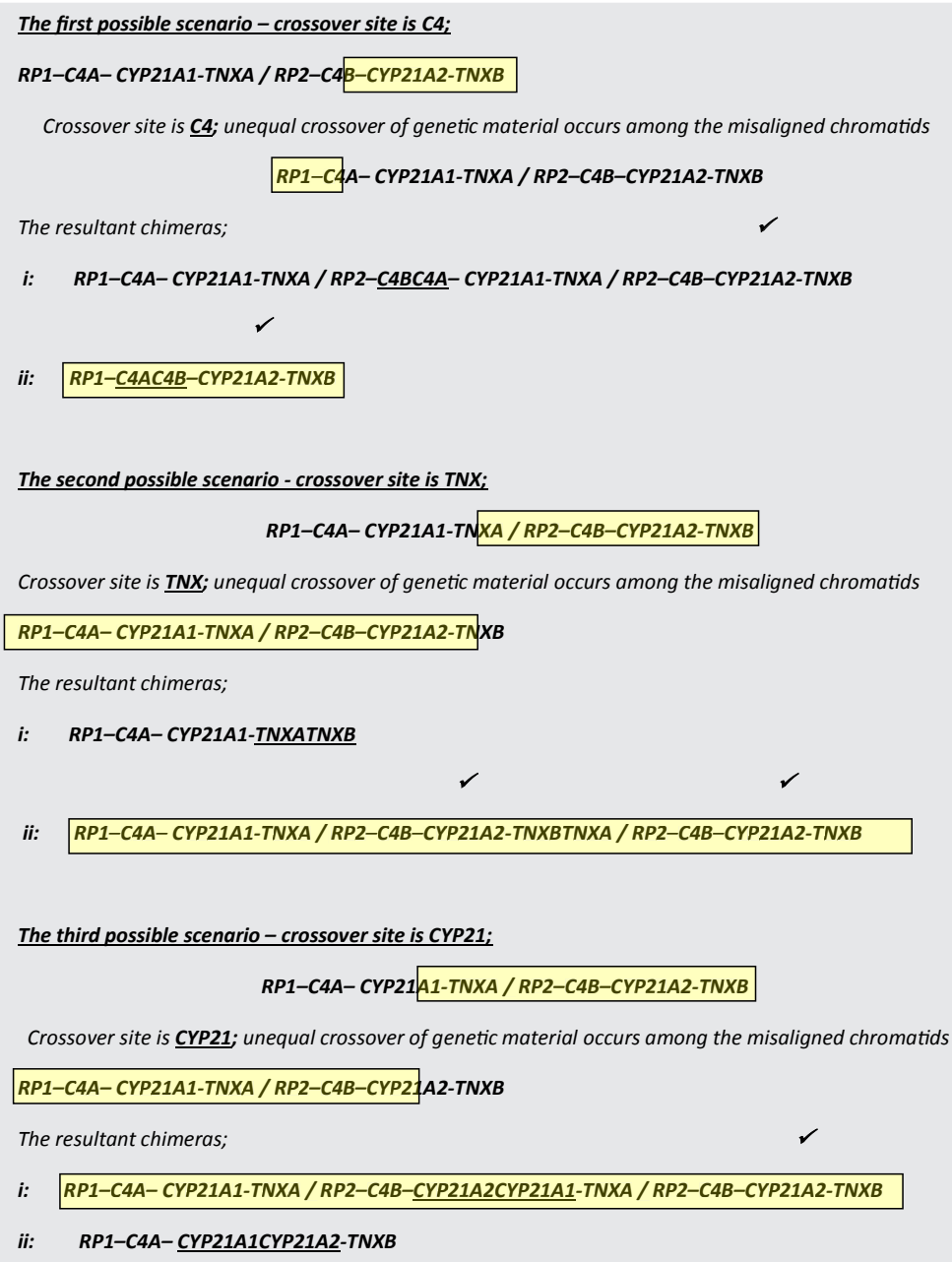
In addition to pseudogene-derived mutations, which are believed to account for more than 90% of mutations in the CYP21A2 gene, hundreds of other mutations associated with different disruptive effects have been reported. Of the pseudogene-derived mutations, promoter region mutations exon 1 (p.P30L) and exon 7 (p.V281L) are usually associated with nonclassical CAH. Exon 3 (p.G110fs), exon 4 (p.I172N), exon 6 cluster mutation (p.I236N) (p.V237E) and (p.M239K), exon 7 (p.L307fs), exon 8 (p.Q318X) and (p.R356W), and intron 2 G (g.655C/A>G) are associated with an expected clinical phenotype of classical CAH and female genital ambiguity (1, 6).

Variability in phenotype has been described with p.P30L, intron 2 G, and p.I172N mutations. A clinical phenotype of classical CAH has been associated with p.P30L in about 30% of cases; simple virilizing CAH has been associated with intron 2 G in 20% of cases; and salt-wasting CAH has been associated with p.I172N mutations in 25% of cases (7).

The autosomal-recessive nature of inheritance is well established in CAH, with the allele harboring the less severely affected mutation determining the phenotype. For patients to manifest classical CAH, they have to possess two classical CAH mutations on each of the maternally and paternally inherited CAH allele. Patients who inherit a mutation known to be associated with nonclassical CAH and another mutation associated with classical CAH will be expected to manifest nonclassical CAH and so on (1, 6, 7, 12).

The postnatal diagnosis of CAH is established on clinical grounds via biochemical testing and genetic confirmation. The risk of CAH in the fetus is 1 in 4 when the expectant couple are both carriers. Although amniotic fluid testing for biochemical markers has been used to diagnose a fetus with CAH, current diagnostic practices involve amniotic fluid/chorionic villous tissue sampling for CYP21A2 genotyping. Further management of an affected pregnancy, which is discussed in detail by Simpson et al. in this Views and Reviews section, is usually based on the genotype of the fetus. When couples who are carriers for CYP21A2 mutations already have an affected child (a proband), the clinical phenotype associated with their combination of mutations will be known. In the absence of a proband in the family, the counseling team relies solely on genetic data and established phenotypic correlations for prenatal diagnosis of CAH. This calls for an accurate correlation between the genotype and phenotype in CAH (7, 12).

FIGURE 2



Three possible scenarios and resultant chimeric hybrids for the possible crossover sites in large gene deletion. Check marks indicate a functional gene.

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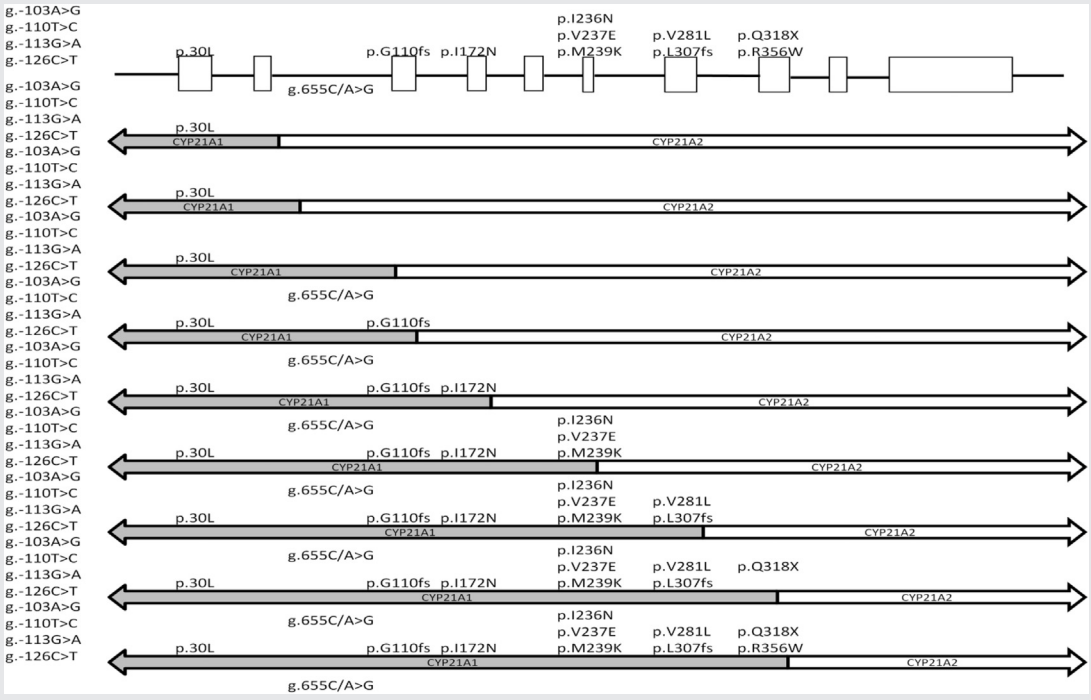
An extensive genotype-phenotype correlation in 1,507 patients with CAH has been published recently by New et al. (6). Unfortunately, genotype-phenotype discordances exist, some of which are explained. An example of an unexplained genotype-phenotype discordance is from a report of a patient who was homozygous for the exon 7 (p.V281L) mutations and had a clearly expected phenotype of nonclassical CAH manifesting with clinical features consistent with salt-wasting CAH (1, 6).

Duplication Silencing a Mutation

The exon 8 mutation (p.Q318X) is known to be associated with a phenotype of salt-wasting CAH but is frequently associated with duplication in the CYP21A2 gene. The duplication results in an allele that carries an intact CYP21A2 gene and a mutated one (the disruptive effects of the p.Q318X mutation are hence muted). In 2013, Lekarev et al. reported a case of erroneous prenatal diagnosis of CAH that was finally

FIGURE 3



Nine different CYP21A1CYP21A2 chimeras with the respective mutations that result when the crossover site is CYP21.

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explained after the duplication was discovered; this pregnancy was unnecessarily treated with dexamethasone to prevent falsely anticipated genital ambiguity in the fetus (13).

The Extent of the Deletion

The most commonly encountered CYP21A2, a 30 kb deletion, is known to be associated with classical CAH. An "attenuated form" of the deletion, which spares mutations downstream of the intron 2 splice site, is thought to be associated with less severe disease because the hybrid CYP21A1CYP21A2 chimera will only harbor the promotor region mutations (with secondary impairment of transcriptional activity) and the exon 1 mutation (associated with nonclassical CAH) (14, 15).

Of note, deletions of CYP21A2 that extend into TNXB result in a "contiguous gene syndrome" consisting of classical CAH and Ehler-Danlos syndrome (8). Siblings with identical CYP21A2 mutations have shown phenotypic variability, indicating the involvement of other genes in the clinical spectrum of CAH (16).

In summary, the established genotype-phenotype correlation in CAH continues to display discordances. The diagnosis and treatment of patients with CAH must account for hormonal and clinical evidence in addition to genetic confirmation. Physicians providing anticipatory guidance during management of at-risk pregnancies and preconceptional counseling must always account for genotype-phenotype discordances in CAH.

REFERENCES

1. Haider S, Islam B, D'Atri V, Sgobba M, Poojari C, Sun L, et al. Structure-phenotype correlations of human CYP21A2 mutations in congenital adrenal hyperplasia. Proc Nat Acad Sci U S A 2013;110:2605–10.
2. Khattab A, Haider S, Kumar A, Dhawan S, Alam D, Romero R, et al. Clinical, genetic, and structural basis of congenital adrenal hyperplasia due to 11beta-hydroxylase deficiency. Proc Nat Acad Sci U S A 2017;11:E1933–40.
3. Simard J, Rheaume E, Sanchez R, Laflamme N, de Launoit Y, Luu-The V, et al. Molecular basis of congenital adrenal hyperplasia due to 3 beta-hydroxysteroid dehydrogenase deficiency. Mol Endocrinol 1993;7:716–28.
4. Picado-Leonard J, Miller WL. Cloning and sequence of the human gene for P450c17 (steroid 17 alpha-hydroxylase/17,20 lyase): similarity with the gene for P450c21. DNA 1987;6:439–48.
5. Meimaridou E, Hughes CR, Kowalczyk J, Chan LF, Clark AJ, Metherell LA. ACTH resistance: genes and mechanisms. Endocr Dev 2013;24:57–66.
6. New MI, Abraham M, Gonzalez B, Dumic M, Razzaghy-Azar M, Chitayat D, et al. Genotype-phenotype correlation in 1,507 families with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. Proc Nat Acad Sci U S A 2013;110:2611–6.
7. New MI, Lekarev O, Mancenido D, Parsa A, Yuen T. Chapter 3A—Congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. In: New MI, Lekarev O, Parsa A, Yuen TT, O'Malley BW, Hammer GD, editors. Genetic steroid disorders. San Diego: Academic Press; 2014:29–51.
8. Miller WL, Merke DP. Tenascin-X, congenital adrenal hyperplasia, and the CAH-X syndrome. Horm Res Paediatrics 2018;89:352–61.
9. White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 2000;21:245–91.
10. Tusie-Luna MT, Traktman P, White PC. Determination of functional effects of mutations in the steroid 21-hydroxylase gene (CYP21) using recombinant vaccinia virus. J Biol Chem 1990;265:20916–22.
11. Rafii S, Butler JM, Ding B-S. Angiocrine functions of organ-specific endothelial cells. Nature 2016;529:316.

12. New MI, Tong YK, Yuen T, Jiang P, Pina C, Chan KC, et al. Noninvasive prenatal diagnosis of congenital adrenal hyperplasia using cell-free fetal DNA in maternal plasma. J Clin Endocrinol Metab 2014;99:E1022–30.
13. Lekarev O, Tafuri K, Lane AH, Zhu G, Nakamoto JM, Buller-Burckle AM, et al. Erroneous prenatal diagnosis of congenital adrenal hyperplasia owing to a duplication of the CYP21A2 gene. J Perinatol 2013;33:76–8.
14. L'Allemand D, Tardy V, Gruters A, Schnabel D, Krude H, Morel Y. How a patient homozygous for a 30-kb deletion of the C4-CYP 21 genomic region can have a nonclassic form of 21-hydroxylase deficiency. J Clin Endocrinol Metab 2000;85:4562–7.
15. Concolino P, Costella A. Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency: a comprehensive focus on 233 pathogenic variants of CYP21A2 gene. Mol Diag Ther 2018;22:261–80.
16. Khattab A, Yuen T, Al-Malki S, Yau M, Kazmi D, Sun L, et al. A rare CYP21A2 mutation in a congenital adrenal hyperplasia kindred displaying genotype-phenotype nonconcordance. Ann N Y Acad Sci 2016;1364:5–10.