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EXPERT OPINION

Prenatal diagnosis of congenital adrenal hyperplasia owing to 21-hydroxylase deficiency

Mabel Yau†, Christian Pina, Ahmed Khattab, Ariella Barhen & Maria I New
Icahn School of Medicine at Mount Sinai, Department of Pediatrics, Adrenal Steroid Disorder Group, New York, NY, USA

A non-invasive prenatal diagnostic method has been developed for congenital adrenal hyperplasia (CAH) owing to 21-hydroxylase deficiency. Excess fetal androgen production causes genital virilization in female fetuses affected with classical forms of CAH. In order to prevent genital ambiguity, prenatal dexamethasone treatment must be administered before the 9th week of gestation when genital organogenesis occurs. Invasive prenatal diagnostic methods do not yield a genetic diagnosis until after genital organogenesis begins. This new methodology could allow for the targeted treatment of affected female fetuses and avoid unnecessary prenatal treatment of males or unaffected females.

Keywords: 21-hydroxylase congenital adrenal hyperplasia, congenital adrenal hyperplasia, dexamethasone treatment, prenatal diagnosis

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Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder affecting 1:15,000 live births worldwide [1,2]. This disease caused by enzymatic defects in adrenal steroidogenesis is characterized by varying levels of impairments in mineralocorticoid and glucocorticoid synthesis and overstimulation of the androgen pathway [2,3]. The most common defect arises from mutations of the *CYP21A2* gene causing a deficiency of 21-hydroxylase. The majority of mutations causing 21-hydroxylase deficiency are the result of recombination events between the *CYP21A2* gene and its non-functional pseudogene, *CYP21P* [3].

In severe cases of CAH, excess fetal androgen production causes genital virilization in female fetuses. Clitoral and perineal surgical reconstruction for severe virilization (Prader score of 3 or greater) performed by an experienced surgeon remains the standard of care for females affected with classical CAH [4]. Forest *et al*. and Morel and coworkers first showed that fetal hyperandrogenemia and genital ambiguity are preventable by low-dose dexamethasone given orally to pregnant at-risk mothers [5,6]. In our experience with prenatal diagnosis and treatment of CAH from 1978 to 2011, prenatal dexamethasone treatment diminished the degree of virilization in 100% of severely affected female fetuses of mothers compliant with prenatal treatment, thus eliminating the need for genitoplasty [7]. As genital organogenesis begins early in the first trimester, prenatal dexamethasone must be administered to the pregnant mother before 9 weeks of gestation to be effective [7].

CAH is currently diagnosed prenatally by chorionic villus sampling at ~ 10 weeks of gestation, or later, by amniocentesis at 14 weeks. A number of strategies have been developed that utilize polymerase chain reaction (PCR)-based techniques to specifically amplify the CYP21A2, not the pseudogene [8-10]. Techniques for molecular diagnosis of point mutations include allele-specific oligonucleotide hybridization [11], amplification-created restriction sites [12], single-stranded conformation polymorphisms [13], allele-specific PCR [10], ligation detection reactions [14] and

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multiplex minisequencing [15]. Varying degrees of 21-hydroxylase inactivation are associated with different phenotypic expression. In a large cohort of 1507 patients, the genotype correlates with the severity of disease in 92% of cases [2]. Cautious anticipatory counseling to expectant families may be given when the genotype correlates well with the phenotype.

These invasive prenatal diagnostic methods do not yield genetic results until after the deadline to initiate prenatal dexamethasone treatment of 9 gestational weeks when genital organogenesis begins. With current protocols for prenatal dexamethasone treatment utilizing invasive prenatal diagnostic methods, all fetuses at risk for CAH are treated for several weeks before sex and affection status are obtained. This means that mothers bearing male and unaffected female fetuses receive dexamethasone unnecessarily. In our clinic's experience from 1978 to 2011, 392 of 719 subjects referred for prenatal diagnosis of CAH chose to be treated with prenatal dexamethasone [7]. Of these 392 treated cases, prenatal dexamethasone has not been associated with long-term sequelae in mothers or offspring [7,16]. However, other studies have reported concerns regarding cognitive functioning in offspring [17]. There is, thus, a need for diagnosing CAH before genital organogenesis begins at ~ 9 weeks, so that therapy can be offered only to mothers with an affected female fetus and omit unnecessary prenatal treatment if the fetus is male or unaffected female.

In 1997, Lo *et al.* reported the detection of cell-free fetal DNA (cffDNA) in maternal plasma – ushering in new possibilities for the non-invasive prenatal diagnosis of monogenic disorders, including CAH [18]. Prior to the discovery of cffDNA, non-invasive prenatal diagnosis was possible only by isolating nucleated fetal cells in the maternal circulation for years which remained in maternal circulation, contaminating the prenatal genetic analysis of subsequent pregnancies by former pregnancies [19,20]. In contrast, cffDNA disappears from the maternal circulation within 24 h after birth, effectively eliminating the possibility of contamination from a previous pregnancy [20]. Massively parallel sequencing (MPS) of cffDNA in maternal plasma has also opened new possibilities for the diagnosis of monogenic disorders *in utero* [20]. This new non-invasive technique of prenatal diagnosis allows for earlier analysis of fetal genotype, thus avoiding invasive antenatal procedures that are currently the standard of care. Earlier diagnosis with non-invasive prenatal diagnosis presents the opportunity for early prenatal treatment of CAH using a low dose of dexamethasone to the mother and prevent the unnecessary treatment of males and unaffected females. There is also potential that this new non-invasive method may prove successful in the diagnosis and treatment of other monogenic disorders.

In May 2014, we first reported the development and validation of targeted MPS for the non-invasive prenatal diagnosis of CAH using maternal plasma [21]. In our study, 14 expectant families, each with a child affected with classical CAH (proband), and parents with at least one mutant CYP21A2 gene were recruited and fetal inheritance of parental haplotypes was determined using this new method. The fetal CAH affection status and sex were correctly deduced in 100% of fetuses using this method [21]. Seven pregnancies had CAH, five were carriers and two were normal. Invasive prenatal diagnostic methods of chorionic villus sampling and amniocentesis were used to confirm the diagnosis in seven cases. The CAH status of the remaining cases was confirmed postnatally. In one family, CAH affection status was deduced from maternal plasma drawn as early as 5 weeks and 6 days [21].

In principle, blood drawn from expectant mothers as early as 6 weeks of gestation can be used for the *in utero* genetic diagnosis not only of CAH, but for other monogenic disorders. This method has been studied in β-thalassemia [22], hemophilia [23] and cystic fibrosis [24]. However, unlike the other inherited diseases, our technology is particularly relevant to classical CAH owing to 21-hydroxylase deficiency and 11-β-hydroxylase deficiency, as these disorders can be effectively treated *in utero* by administering dexamethasone to the mother to prevent genital ambiguity in affected female fetuses. Non-invasive prenatal sex determination by SRY can be determined as early as 4 weeks and 5 days as mentioned by Tardy-Guidollet *et al.* [25]. In this study, prenatal dexamethasone was initiated prior to fetal sex determination in the maternal serum. The percentage of male fetuses treated with prenatal dexamethasone significantly decreased over time (from 60% in 2002 to 5 – 10% by 2010 – 2011) [25]. These data demonstrate that sex determination has significantly reduced the unnecessary prenatal dexamethasone treatment of male fetuses. The methodology of prenatal sex determination by SRY when performed early and accurately has the potential to eliminate the need for unnecessary treatment in males.

This new non-invasive technique for prenatal diagnosis offers advantages over invasive antenatal examinations that are currently the standard of care. The risk associated with invasive prenatal procedures is low but still clinically significant risk. Invasive procedures early in gestation are associated with a higher risk of pregnancy loss and spontaneous miscarriage as well as a low risk of malformations such as talipes and hemangiomas [26,27]. In our clinic's experience from 1978 to 2011, the average Prader score of 63 affected female newborns prenatally treated with dexamethasone was 1.7 compared with the average Prader score of 3.73 in the 11 untreated affected females [7]. Long-term studies of women who underwent cosmetic genitoplasty in infancy or early childhood disclose impaired genital sensitivity, sexual difficulties, decreased intercourse frequency and stress urinary leakage [28,29]. There are reports of complications resulting from genitoplasty such as strictures, fistulas, urinary infection, fibrosis and scarring, which have caused stress and depression in patients, especially with regard to their sexual relationships [30] The complications that may be associated with genitoplasty along with impaired

fertility may result in non-disclosure, and distress in patients who require repeated surgery and genital examinations that reinforce their disorder [30]. Thus, the successful prenatal dexamethasone treatment of affected female fetuses may allow them to have more successful sexual lives with improved body image.

In conclusion, we report a strategy to avoid unnecessary prenatal dexamethasone treatment of male fetuses and unaffected female fetuses by utilizing non-invasive prenatal testing for CAH using targeted MPS on fetal cell-free DNA in maternal plasma. This focused treatment cannot be accomplished through genetic diagnosis of CAH by amniocentesis and chorionic villus sampling. It has been shown that detection of fetal chromosomal aneuploidy from cffDNA in the maternal plasma using MPS has a high sensitivity and specificity leading to its commercial use [31-33]. While our data are promising, the number of studied cases is still relatively small, requiring further validation in large-scale prospective studies. Currently, the American College of Obstetricians and Gynecologists guidelines state "Cell free fetal DNA does not replace the accuracy and diagnostic precision of prenatal diagnosis with CVS or amniocentesis, which remain an option for women". In the long term, however, families with CAH may be willing to pursue prenatal genetic diagnosis to identify an affected female fetus at risk of genital virilization if a safe non-invasive option for accurate diagnosis is readily available. The benefit of prenatal dexamethasone treatment can then be weighed directly against the potential risk to these affected female fetuses as the exposure of male fetuses and unaffected female fetuses to dexamethasone can be eliminated.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Pang SY, Wallace MA, Hofman L, et al. Worldwide experience in newborn screening for classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Pediatrics 1988;81:866-74
2. New MI, Abraham M, Gonzalez B, et al. Genotype-phenotype correlation in 1,507 families with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. Proc Natl Acad Sci USA 2013;110:2611-16
3. New MI, Lekarev O, Mancenido D, et al. Genetic steroid disorders. In: New MI, Lekarev O, Parsa A, Yuen T, O'Malley BW, Hammer GD, editors. Congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. Elsevier; London, UK: 2014. p. 29-51
4. Speiser PW, Azziz R, Baskin LS, et al. A Summary of the endocrine society clinical practice guidelines on congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency. Int J Pediatr Endocrinol 2010;2010:494173
5. Forest MG, Betuel H, David M. Prenatal treatment in congenital adrenal hyperplasia due to 21-hydroxylase deficiency: up-date 88 of the French multicentric study. Endocr Res 1989;15:277-301
6. Forest MG, David M, Morel Y. Prenatal diagnosis and treatment of 21-hydroxylase deficiency. J Steroid Biochem Mol Biol 1993;45:75-82
7. New MI, Abraham M, Yuen T, et al. An update on prenatal diagnosis and treatment of congenital adrenal hyperplasia. Semin Reprod Med 2012;30:396-9
8. White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 2000;21:245-91
9. Keen-Kim D, Redman JB, Alanes RU, et al. Validation and clinical application of a locus-specific polymerase chain reaction- and minisequencing-based assay for congenital adrenal hyperplasia (21-hydroxylase deficiency). J Mol Diagn 2005;7:236-46
10. Wilson RC, Wei JQ, Cheng KC, et al. Rapid deoxyribonucleic acid analysis by allele-specific polymerase chain reaction for detection of mutations in the steroid 21-hydroxylase gene. J Clin Endocrinol Metab 1995;80:1635-40
11. Speiser PW, White PC, Dupont J, et al. Prenatal diagnosis of congenital adrenal hyperplasia due to 21-hydroxylase deficiency by allele-specific hybridization and Southern blot. Hum Genet 1994;93:424-8
12. Tajima T, Fujieda K, Mikami A, et al. Prenatal diagnosis of steroid 21-hydroxylase deficiency by the modified polymerase chain reaction to detect splice site mutation in the CYP21 gene. Endocr J 1998;45:291-5
13. Tajima T, Fujieda K, Nakayama K, et al. Molecular analysis of patient and carrier genes with congenital steroid 21-hydroxylase deficiency by using polymerase chain reaction and single strand conformation polymorphism. J Clin Invest 1993;92:2182-90
14. Day DJ, Speiser PW, White PC, et al. Detection of steroid 21-hydroxylase alleles using gene-specific PCR and a multiplexed ligation detection reaction. Genomics 1995;29:152-62
15. Ohlsson G, Schwartz M. Mutations in the gene encoding 21-hydroxylase detected by solid-phase minisequencing. Hum Genet 1997;99:98-102
16. Meyer-Bahlburg HF, Dolezal C, Haggerty R, et al. Cognitive outcome of offspring from dexamethasone-treated pregnancies at risk for congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Eur J Endocrinol 2012;167:103-10
17. Hirvikoski T, Nordenstrom A, Lindholm T, et al. Cognitive functions in children at risk for congenital adrenal hyperplasia treated prenatally with

dexamethasone. J Clin Endocrinol Metab 2007;92:542-8

18. Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485-7
19. Bianchi DW, Zickwolf GK, Weil GJ, et al. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. Proc Natl Acad Sci USA 1996;93:705-8
20. Lo YM. Fetal DNA in maternal plasma: biology and diagnostic applications. Clin Chem 2000;46:1903-6
21. New MI, Tong YK, Yuen T, 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

•• **This reference reports new methodologies for early non-invasive genetic diagnosis of CAH and sex determination.**

22. Lam KW, Jiang P, Liao GJ, et al. Noninvasive prenatal diagnosis of monogenic diseases by targeted massively parallel sequencing of maternal plasma: application to beta-thalassemia. Clin Chem 2012;58:1467-75
23. Tsui NB, Kadir RA, Chan KC, et al. Noninvasive prenatal diagnosis of hemophilia by microfluidics digital PCR analysis of maternal plasma DNA. Blood 2011;117:3684-91
24. Nasis O, Thompson S, Hong T, et al. Improvement in sensitivity of allele-specific PCR facilitates reliable noninvasive prenatal detection of cystic fibrosis. Clin Chem 2004;50:694-701
25. Tardy-Guidollet V, Menassa R, Costa JM, et al. New management strategy of pregnancies at risk of congenital adrenal hyperplasia using fetal sex determination in maternal serum: French cohort of 258 cases (2002-2011). J Clin Endocrinol Metab 2014;99:1180-8

•• **This reference reports new methodologies for early non-invasive genetic diagnosis of CAH and sex determination.**

26. Alfirevic Z, Sundberg K, Brigham S. Amniocentesis and chorionic villus sampling for prenatal diagnosis. Cochrane Database Syst Rev 2003(3):CD003252
27. Bauland CG, Smit JM, Scheffers SM, et al. Similar risk for hemangiomas after amniocentesis and transabdominal chorionic villus sampling. J Obstet Gynaecol Res 2012;38:371-5
28. Crouch NS, Liao LM, Woodhouse CR, et al. Sexual function and genital sensitivity following feminizing genitoplasty for congenital adrenal hyperplasia. J Urol 2008;179:634-8
29. Crouch NS, Creighton SM. Long-term functional outcomes of female genital reconstruction in childhood. BJU Int 2007;100:403-7
30. Zainuddin AA, Grover SR, Shamsuddin K, et al. Research on quality of life in female patients with congenital adrenal hyperplasia and issues in developing nations. J Pediatr Adolesc Gynecol 2013;26:296-304
31. Jensen TJ, Zwiefelhofer T, Tim RC, et al. High-throughput massively parallel sequencing for fetal aneuploidy detection from maternal plasma. PLoS One 2013;8:e57381
32. Ehrich M, Deciu C, Zwiefelhofer T, et al. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. Am J Obstet Gynecol 2011;204:205 e1-11
33. Bianchi DW, Platt LD, Goldberg JD, et al. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstet Gynecol 2012;119:890-901

Affiliation

Mabel Yau[†] MD, Christian Pina, Ahmed Khattab MD, Ariella Barhen MD & Maria I New MD
[†]Author for correspondence
Icahn School of Medicine at Mount Sinai, Department of Pediatrics, Adrenal Steroid Disorder Group, One Gustave Levy Place, Box 1198, New York, NY, USA
Tel: +1 212 241 7847;
Fax: +1 212 241 5405;
E-mail: mabel.yau@mssm.edu