Mini Review

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Prenatal Diagnosis and Treatment of Congenital Adrenal Hyperplasia

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Key Words
21-hydroxylase deficiency · Congenital adrenal hyperplasia · Prenatal diagnosis · Prenatal treatment · Steroid hydroxylases

Abstract
Congenital adrenal hyperplasia is a group of inherited disorders caused by an enzyme deficiency in steroid biosynthesis. The most common form of congenital adrenal hyperplasia is 21-hydroxylase deficiency, which in its severe form can cause genital ambiguity in females. Steroid 21-hydroxylase deficiency can be diagnosed in utero through molecular genetic analysis of fetal DNA. Prenatal treatment successfully reduces genital ambiguity, and the subsequent problems of sex misassignment and gender confusion. Data from current studies show that prenatal diagnosis and treatment are safe for the mother and the fetus. The evidence also suggests that it is safe over the long term, but all subjects exposed to dexamethasone treatment during embryonic and fetal life should have their physical, cognitive and emotional developments recorded.

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Introduction

Congenital adrenal hyperplasia (CAH) is a group of disorders in steroid biosynthesis, caused by an enzyme deficiency in the conversion of cholesterol to cortisol. CAH is a monogenic, autosomal recessive disorder [1]. More than 90% of CAH cases arise from 21-hydroxylase deficiency (21OHD) [2]. Steroid 21OHD CAH occurs in two forms: a severe, or 'classical' form, and a mild, or 'nonclassical' form. Classical 21OHD CAH is further identified into salt-wasting and simple-virilizing forms. Data from close to 6.5 million newborn screenings worldwide indicate that the incidence of classical CAH ranges between 1:13,000 and 1:15,000 live births [3].

Females with the classical form of 21OHD are born with ambiguous genitalia owing to the overproduction of androgens from the fetal adrenal. The precursors to the 21-hydroxylase enzyme defect are shunted into the androgen pathway. The androgens then cause the virilization of external genitalia. Males do not have sexual ambiguity because the major source of androgens in males is the testes. Unlike the adrenal, the testis does not have a 21-hydroxylation pathway. The nonclassical form of 21OHD does not result in ambiguous genitalia in the 46,XX female, as the prenatal level of androgens is not high enough to virilize the external genitalia of the female.

ACTH stimulation can differentiate forms of CAH. A logarithmic nomogram was developed to provide hor-

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monal standards for diagnosis and further assignment of the 21OHD type by relating baseline to ACTH-stimulated serum concentrations of 17-OHP [4, 5].

Prenatal Development

The adrenal cortex is formed in the 4th week of gestation from coelomic epithelial mesoderm. By the 6th or 7th week of gestation, the provisional zones, the functional adrenal cortex of the fetus [6], secrete steroids. In affected females, elevated levels of circulating adrenal androgens interfere with vaginal and urethral canal separation, leading to a common urogenital sinus. Female genital anatomy is also affected as the androgens interact with the receptors on genital skin. This interaction induces clitoral enlargement, promotes fusion of the labial folds, and causes rostral migration of the urethral/vaginal perineal orifice. Therefore, affected females are born with virilized external genitalia including clitoromegaly and labial fusion [7]. However, internal female genitalia (uterus, fallopian tubes and ovaries) are normal as females cannot produce müllerian-inhibiting hormone since they do not have testicular Sertoli cells. Therefore the müllerian ducts do not regress, and the internal female internal genitalia develop normally.

Molecular Genetics

The gene encoding 21-hydroxylase enzyme (MIM number 201910) is mapped to the short arm of chromosome 6 (6p21.3). There is a corresponding inactive pseudogene, *CYP21A1P,* which is 98% homologous to the active gene *CYP21A2* [8]. Unequal crossing over during meiosis can result in deletion of the gene. Gene conversion transfers deleterious point mutations from the pseudogene gene to the active gene, causing either complete or partial deficiency of 21-hydroxylase activity [9]. More than 100 mutations have been described, including point mutations, small deletions, small insertions and complex rearrangements of the gene [10]. About 20% of the mutant alleles are meiotic recombinations deleting a 30-kb gene segment [11] that encompasses the 3′ end of the *CYP21A1P* pseudogene, all of the adjacent C4B complement gene, and the 5′ end of *CYP21A2*, producing a nonfunctional chimeric pseudogene. Another common mutation is the Intron 2 splice mutation (656 A/C to G), occurring with a frequency of 20–30%.

Genotype-Phenotype Correlation

In 21OHD CAH, genotypes can be used to predict the disease severity. In autosomal recessive disorders, the expressed phenotype reflects the less severe mutation of the patients' alleles. The severity of each mutation is characterized by the percentage of the remaining enzyme activity found by in vitro expression studies. In table 1, the common mutations and the phenotypes are described. Salt-wasters usually have the most severe mutations (homozygous deletions), while nonclassical patients usually have the milder V281L mutation [12]. However, a considerable degree of divergence is observed within mutation groups with intermediate severity [13]. Genotype-phenotype nonconcordance occurs, although infrequently, with less severe allelic mutations such as V281L, P30L, and I172N. In addition, the splice mutation in Intron 2 is commonly associated with phenotypic variation of salt-wasting severity, which can be explained by variable splicing. In the context of prenatal diagnosis, it is important to distinguish classical and nonclassical genotypes in order to determine the necessity of prenatal treatment. In our series of publications on prenatal diagnosis [1, 14], we divided the genotypes into mutation identical groups. V281L is known to cause a nonclassical phenotype, and therefore should not indicate prenatal dexamethasone treatment. However, in approximately 5% of our prenatal diagnoses, the homozygous V281L in combination with a severe allelic mutation (compound heterozygote) resulted in a newborn with the classical disease. We have also found the P30L mutation to cause nonconcordance, but less frequently than the V281L mutation [13, 15, 16]. In our recent review of 723 patients from mixed ethnic backgrounds (unpublished data), the classical versus nonclassical phenotype could be predicted from genotypes in most cases. However, rare exceptions existed when patients carried the V281L and P30L mutations. These mutations conferred the classical phenotype in less than 3% of the patients when a nonclassical phenotype was expected. Stikkelbroeck et al. [17] demonstrated that a very small percentage of patients who carried I172N and another severe mutation presented with a nonclassical phenotype when a classical phenotype was expected.

In families where the proband is a virilized female, predicting the risk of genital virilization in subsequent female fetuses is feasible. If the proband is a male, prediction of phenotype based on genotype is not possible and the subsequent affected female fetus must be treated until term to avoid genital ambiguity.

Table 1. Common gene mutations of the 21-hydroxylase gene *CYP21A2* [49]

Exon/ Intron	Mutation type	Mutation	Phenotype	Severity of enzyme defect (% enzyme activity)	References
1. Nonclassical mutations					
Exon 1	Missense mutation	P30L	NC	Mild (30–60%)	Tusie-Luna, 1991
Exon 7	Missense mutation	V281L	NC	Mild (20–50%)	Speiser, 1988
Exon 8	Missense mutation	R339H	NC	Mild (20–50%)	Helmberg, 1992
Exon 10	Missense mutation	P453S	NC	Mild (20–50%)	Helmberg, 1992; Owerbach, 1992
2. Classical mutations					
Deletion	30-kb deletion	–	SW	Severe (0%)	White, 1984
Intron 2	Aberrant splicing of Intron 2	656 A/C-G	SW, SV	Severe (ND)	Higashi, 1988
Exon 3	Eight-base deletion	G110 Δ8nt	SW	Severe (0%)	White, 1994
Exon 4	Missense mutation	I172N	SV	Severe (1%)	Amor, 1988; Tusie-Luna, 1990
Exon 6	Cluster mutations	I236N, V237E, M239K	SW	Severe (0%)	Amor, 1988; Tusie-Luna, 1990
Exon 8	Nonsense mutation	Q318X	SW	Severe (0%)	Globerman, 1988
Exon 8	Missense mutation	R356W	SW, SV	Severe (0%)	Chiou, 1990
Exon 10	Missense mutation	R483P*	SW	Severe (1–2%)	Wedell, 1993

NC = Nonclassical; ND = not determined; SV = simple virilizing; SW = salt-wasting.

Prenatal Diagnosis of 21OHD

A number of approaches to prenatal identification of affected fetuses have been used. In 1965, Jeffcoate et al. [18] first reported a successful prenatal diagnosis of 21OHD based on elevated levels of 17-ketosteroids and pregnanetriol in amniotic fluid. The hormonal diagnostic test for 21OHD is amniotic fluid 17-OHP. Androstenedione Δ^4 may also be employed as an adjunctive diagnostic assay [19]. Hormonal diagnosis is only currently used when molecular diagnosis is unavailable.

Recent advances in genotyping of the *CYP21A2* gene have made molecular genetic studies of extracted fetal DNA, the ideal method to diagnose 21OHD CAH in the fetus [9]. Approximately 95–98% of the mutations causing 21OHD have been identified through a combination of molecular genetic techniques studying large gene rearrangement, and arrays of point mutations [20–22]. Chorionic villus sampling (CVS), rather than amniocentesis, with molecular genotyping is the preferred diagnostic method in use. CVS is performed at the 8th–9th week of gestation, while amniocentesis is usually performed at the 12th–13th week of gestation. As we only wish to treat affected females till term and only ¼ of the fetuses will be affected and ½ will be males, 7 out of 8 fetuses do not require treatment. Thus, amniocentesis, which is performed later in gestation, results in treatment of unaffected fetuses for a longer period of time than CVS. However, amniocentesis can be used as an alternative, reliable method of prenatal diagnosis when CVS is unavailable. In such instances, the supernatant is used for hormonal measurement and the cells are cultured to obtain a genotype through DNA analysis. The supernatant hormone measurements are only positive when the fetus is a salt-waster [1].

Nonetheless, pitfalls do occur in a small percentage of the patients undergoing prenatal diagnosis, such as undetectable mutations [23], allele drop out [24], or maternal DNA contamination. Determination of satellite markers may increase the accuracy of molecular genetic analysis [25].

Studying fetal DNA from maternal plasma may reduce the need for invasive procedures to obtain fetal samples in at-risk pregnancies if analysis of the fetal DNA becomes possible at the optimal time of gestation. Reports of sex determination from fetal DNA in maternal plasma show promise [26, 27], but require more confirmation of the successful results.

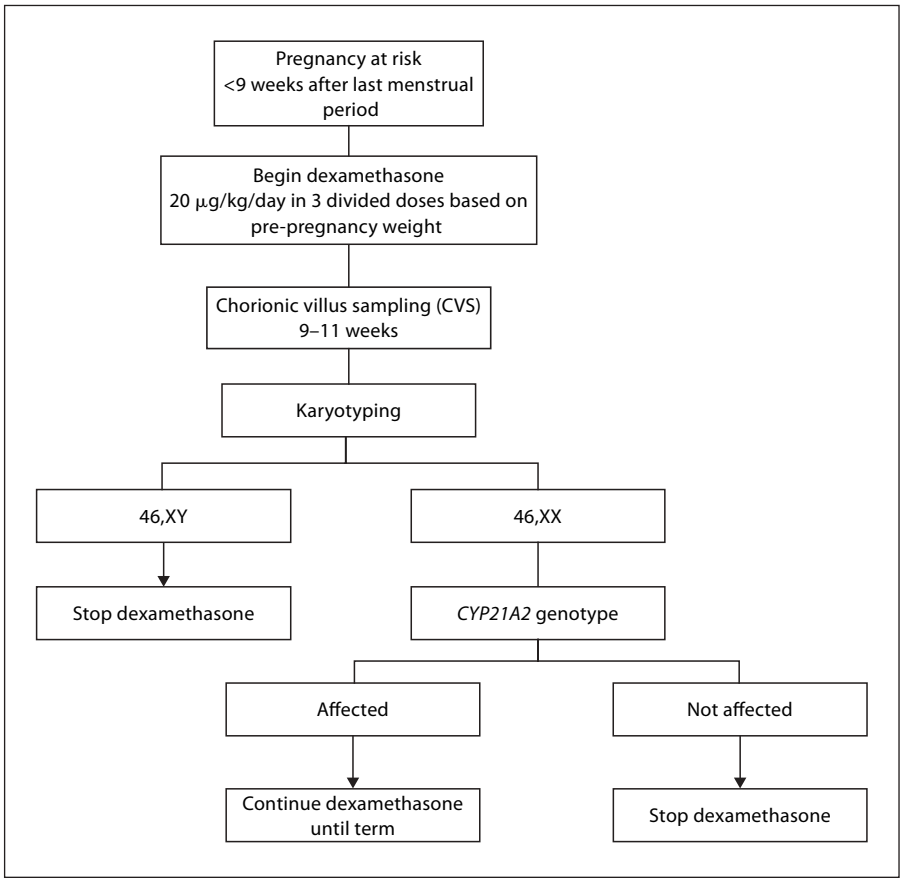

Fig. 1. Simplified algorithm for prenatal diagnosis and treatment of CAH.

Preimplantation Diagnosis

Preimplantation genetic diagnosis identifies genetic abnormalities in preimplantation embryos prior to embryo transfer, so only unaffected embryos established from IVF are transferred. The procedure has been utilized in many monogenic recessive disorders such as cystic fibrosis, hemoglobinopathies, spinal muscular atrophy and Tay-Sachs disease. Preimplantation genetic diagnosis is being used for a growing number of genetic diseases [28]. Preimplantation diagnosis has not been utilized in CAH except for one report which did not result in a pregnancy [28]. It would be desirable to have further studies of preimplantation diagnosis in CAH families.

Prenatal Treatment

In 21OHD, prenatal treatment with dexamethasone has been used since 1984 [29]. Institution of therapy before the 8th week of gestation, prior to the onset of adrenal androgen secretion, effectively suppresses excessive adrenal androgen production and prevents virilization of external female genitalia. Dexamethasone is used because it binds minimally to cortisol-binding globulin in the maternal blood, and unlike hydrocortisone, escapes inactivation by placental 11β-hydroxysteroid dehydrogenase enzyme. Thus, dexamethasone crosses the placenta from the mother to the fetus and suppresses ACTH secretion with a longer half life than other synthetic steroids [14].

When dexamethasone administration begins as early as the 8th week of gestation, the treatment is blind to the disease status and sex of the fetus. If the fetus is later determined upon karyotype to be a male, or an unaffected female upon DNA analysis, treatment is discontinued. Otherwise, treatment is continued to term [19]. A simplified algorithm of management of potentially affected pregnancies is shown in figure 1. The optimal dosage and timing is 20 μg/kg/day of dexamethasone per maternal pre-pregnancy body weight, in three divided doses, starting as soon as pregnancy is confirmed, and no later than

9 weeks after the last menstrual period [30, 31]. The mother's blood pressure, weight, glycosuria, HbA_{1C}, symptoms of edema, striae and other possible adverse effects of dexamethasone treatment should be carefully observed throughout pregnancy. Urinary estriol may be monitored in the mother after 15–20 weeks of gestation to indicate fetal adrenal suppression, and to assure compliance [32].

Outcome of Prenatal Treatment

Although some uncertainties and concerns remain about the long-term safety of prenatal diagnosis and treatment [33], compelling data from large cohorts of pregnancies with prenatal diagnosis and treatment [14, 34] prove its efficacy and safety.

Effects of Prenatal Treatment

Dexamethasone administered at or before the 9th week of gestation is effective in reducing genital virilization as demonstrated by the difference in Prader score of treated versus untreated affected females [32] (fig. 2). In families with both treated and untreated affected females, genital virilization in the treated siblings was decreased (fig. 3). Treated newborns whose genitalia were rated Prader III–IV had delayed treatment initiation, were undertreated by the referring physician, or were incorrectly dosed due to maternal noncompliance.

Maternal and Fetal Tolerance of Dexamethasone Treatment

The Mother

In our experience, mothers receiving dexamethasone treatment did not have enduring side effects [14, 35]. The weight gain, edema and striae disappeared upon cessation of therapy. No differences were demonstrated in terms of hypertension or gestational diabetes. Swedish researchers [36] have also found that treated and untreated pregnant mothers did not differ in blood pressure, glycosuria and proteinuria. In one report from France, 18% of the treated mothers reported some side effects, but only 2% of them were considered to have more severe complications [34]. In our study [14], all the mothers who received prenatal treatment (partial and full-term) stated that they would take dexamethasone again for a future pregnancy. A retrospective survey [37] of 38 mothers' attitudes and experiences towards prenatal diagnosis and treatment indicated that diagnostic procedures of both amniocentesis and CVS were well-tolerated. The anxiety or discomforts from the procedures were outweighed by the knowledge of the disease status of the fetus. All women chose to undergo dexamethasone treatment again in future pregnancies.

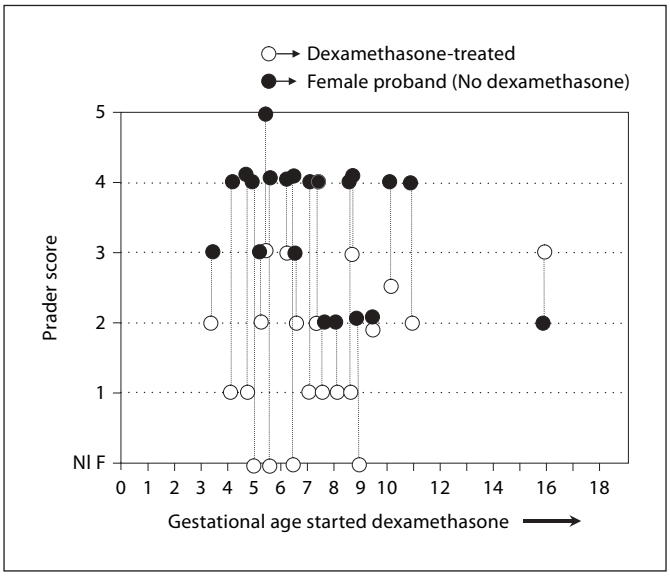

Fig. 2. Genitalia of untreated (Prader IV) versus prenatally dexamethasone-treated (Prader I) siblings with congenital adrenal hyperplasia. Treatment was from 5 weeks of gestation until term.

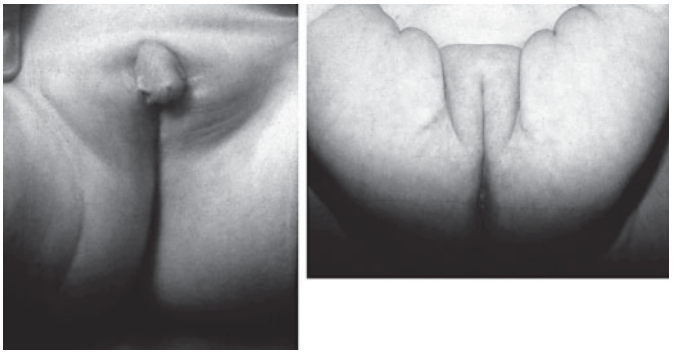

Fig. 3. Prader stages of affected female infants in monitored, prenatally treated pregnancies.

The Fetus

In our comprehensive studies of almost 600 pregnancies [14, 35], prenatally treated newborns did not differ in weight, length or head circumference from untreated, unaffected siblings. The gestational length and rate of fe-

tal wasting did not differ. Postnatal growth has been normal in followed cases: a long-term follow-up study in Scandinavia demonstrated that 44 children treated prenatally had normal pre- and postnatal growth compared to matched controls [36].

Rare and isolated adverse events of prenatal dexamethasone treatment have been reported in treated children, but no harmful effects that can be clearly attributed to the treatment have been documented [38]. The incidence of fetal deaths in treated pregnancies does not exceed that predicted for the general population [39]. Importantly, no cases have been reported of cleft palate, placental degeneration or fetal death, which have been observed in a rodent model of in utero exposure to high-dose glucocorticoids [40].

Long-Term Effects of Dexamethasone Treatment

Long-term follow-up studies of treated fetuses are lacking, but are underway in our group [32]. Long-term follow-up studies providing extensive data on the somatic and neuropsychological outcomes of glucocorticoid treatment are being investigated.

The authors' group has engaged in studying developmental outcomes in children prenatally expose to dexamethasone. The preliminary results are summarized below:

First Study Phase. The first pilot study examined the cognitive and behavioral development of 26 prenatally treated children aged 6 months to 5 1/2 years compared to 14 untreated children. There were no significant differences between the two groups studied, except that the treatment group had more internalizing behaviors than the untreated children, such as shyness, less sociability, greater avoidance, and a marginal increase in emotionality [41]. Another analysis of standard questionnaires regarding gender related behavior was completed in 17 prenatally treated girls among 78 CAH affected girls, ages 2–12. The analysis showed that gender related behavior correlated with the degree of virilization at birth determined by Prader score [42].

Second Study Phase. In the second phase of the study, more thorough questionnaires (also administered to mothers) were used. Development outcomes of 174 prenatally children were compared to 313 unexposed children, ages 1 month to 12 years of age [43]. None of the developmental areas, including cognitive, motor, language, social, and self-help skills, were different between the two groups.

Third Study Phase. In the third phase of the study, physical observation and interviews were completed for 140 children, ages 5–12. Approximately 1/3 of the participants were affected with CAH, and 1/2 of the affected children were treated with dexamethasone. The study confirmed what was found in second study phase: prenatally treated children did not suffer adverse cognitive effects [44]. The study did find that dexamethasone-exposed CAH girls showed less masculine gender role behavior overall than the CAH girls unexposed to dexamethasone. When gender-related behaviors were blindly assessed during interview and observation using several combined tools, there was no difference between the treated and untreated groups in regards to gender identity [45].

Our group continues our commitment to long-term follow-up studies of developmental outcomes of prenatal treatment, focusing on cognition, gender, temperament and handedness (as indicator of prenatal androgen effects). Large-scale, international studies of cognitive function and psychological outcomes have been initiated to confirm the safety of treatment, and to enhance our understanding of prenatal steroid exposure in humans [35, 38].

Treatment Failures

Although we have seen great success with prenatal treatment, treatment failures occur. These failures have been attributed to the cessation of therapy in midgestation, noncompliance or suboptimal dosing, though some reports provide no ready explanation for the failure [46, 47]. Studies before 1993 must be viewed with caution, however, as it was then common practice then to discontinue dexamethasone treatment to obtain hormonal values in amniotic fluid [48]. Discontinuing dexamethasone treatment for even a short period during sexual differentiation increases the likelihood of genital virilization in affected female newborns. Protocols also varied between institutions.

Conclusion

There is accurate, extensive, and compelling data from the human studies pointing to the benefit and safety of prenatal treatment. When properly introduced, prenatal diagnosis and treatment of 21OHD spares the affected female of the consequences of genital ambiguity, which is a crucial and complicated morbidity in 21OHD CAH. Treatment with dexamethasone is effective in reducing genital virilization, the risk of sex misassignment, and unnecessary genitoplasty. In prenatal diagnosis of

21OHD CAH, prompt and accurate genetic diagnosis is crucial to minimize the duration of dexamethasone exposure in the 7 out of 8 fetuses who must be treated, but are not classically affected females. Based on current studies, proper prenatal diagnosis and treatment are safe for both the fetus and the mother. Studies of treated versus untreated pregnancies will monitor the safety of treatment, and enhance our understanding of the effects of prenatal steroid exposure to the human brain. Long-term studies underway in our group will evaluate long-term safety. Prenatal treatment of 21OHD CAH is the first instance of successful treatment of an inborn metabolic disorder. At present, there are very few genetic disorders for which prenatal treatment is effective in improving the phenotypic outcome, as well as future prognosis.

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