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Review Article

Prenatal Diagnosis and Treatment of Steroid 21-Hydroxylase Deficiency

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Abstract. Steroid 21-hydroxylase deficiency (21-OHD) accounts for 90–95% of congenital adrenal hyperplasia (CAH) cases. It is classified into three distinct clinical phenotypes: the salt-wasting (SW), simple virilizing (SV) and nonclassical forms (NC). As girls with the SW and SV forms of 21-OHD are exposed to high systemic levels of adrenal androgens during fetal life, they show genital ambiguity. To ameliorate the degree of genital virilization, prenatal dexamethasone treatment has been performed for more than two decades, although mainly in the USA and Europe. This treatment has proven to be effective in preventing or reducing genital virilization. Some data also show that prenatal diagnosis and treatment are safe for the mother and fetus. However, prenatal treatment is still controversial for the following reasons. First, the risk of having an affected female fetus is only one in eight when both parents are known carriers of the autosomal recessive trait. Therefore, seven of eight fetuses will receive dexamethasone unnecessarily, and this raises ethical questions. Furthermore, maternal side effects such as excessive weight gain and hypertension have been observed. Finally, the long-term safety and outcome for dexamethasone-exposed children have not been established. In Japan, prenatal diagnosis and treatment has rarely been reported because of these reasons. Therefore, we must be cautious, and this treatment should be carried out in special centers with the approval of their ethical committees, that are capable of performing chorionic villus sampling (CVS) and subsequently determining the karyotype and genotype of 21-OHD.

Key words: prenatal diagnosis, prenatal treatment, dexamethasone, steroid 21-hydroxylase

Introduction

Congenital adrenal hyperplasia (CAH) is a congenital disorder caused by a defect in one of the enzymes of the steroidogenic pathway leading to synthesis of glucocorticoid. Steroid 21-hydroxylase deficiency (21-OHD) accounts for 90–95% of CAH cases.

In this review, we present a brief description of the pathophysiology and molecular basis of 21-OHD. We further concentrate on aspects of prenatal diagnosis and treatment of this disease.

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Pathophysiology

In 21-OHD, decreased glucocorticoid production results in increased pro-

opiomelanocortin and adrenocorticotropin secretion from the pituitary and subsequent hyperplasia of the adrenal cortex. It is classified into three distinct clinical phenotypes: the salt-wasting (SW), simple virilizing (SV) and nonclassical (NC) forms (1–3). The SW form is characterized by a defect of cortisol and aldosterone and increased adrenal androgen secretions. Patients with the SV form do not synthesize cortisol efficiently, but aldosterone secretion remains (1–3). The precursors to the 21-hydroxylase defect are shunted into the androgen pathway, and subsequently excessive androgen can cause virilization of external genitalia in females with the SW and SV forms (1–3). The degree of virilization is classified into five stages defined by Prader, ranging from a simple an enlarged clitoris, to complete fusion of the labial folds and a penile appearance of the clitoris like that of normal male genitalia.

Molecular Basis

The gene encoding the 21-hydroxylase enzyme is located on the short arm of chromosome 6p21 (1, 4). There are two genes, *CYP21A2* and *CYP21A1P*, each of which is located adjacent to one of the two genes for the fourth component of complement, C4A and C4B, in the class III region of the HLA complex. Of the two genes, *CYP21A2* encodes for active 21-hydroxylase and *CYP21A1P* is an inactive pseudogene (1, 4). *CYP21A2* and *CYP21A1P* have 98% nucleotide homology (1, 4). More than 90% of mutations of active *CYP21A2* are generated by recombination between the active and inactive genes. Unequal crossing over during meiosis can result in deletion of the gene and gross conversion transfers deleterious point mutations from the pseudogene to the active gene, causing either complete or partial deficiency of 21-hydroxylase activity (4–7). Thus, about 75% are defective mutations found in the pseudogene that are transferred to *CYP21A2*. About 20% of the mutant alleles are deletions of a 30 kb gene segment that includes the 3' end of *CYP21A1P*.

To date, more than 100 different mutations are listed in the Human Gene Mutation Database. As mentioned, most mutations are transferred from *CYP21A1P*; however some rare and new mutations in the *CYP21A2* gene have occurred independently from the pseudogene (3).

CYP21A2 is one of the most polymorphic human genes. Tuie-Luna *et al.* (8) have reported that spontaneous recombinations between *CYP21A2* and *CYP21A1P* occur in 1 in 1000 to 1 in 100,000 cells in sperm. This can presumably explain how 1 to 2% of affected alleles arise *de novo* in patients (1, 8, 9).

The Phenotype-genotype Correlation

It is known that the degree of enzymatic impairment caused by the different mutations *in vivo* generally correlates with the clinical severity of 21-OHD (1, 6, 7, 10, 11). Mutations that totally abolish enzymatic activity cause the severe form of 21-OHD, SW. These mutations consist of deletions, large gene conversions or nonsense mutations. The I172N mutation has a small amount of enzymatic activity compared with the normal enzyme (1–2%), so this mutant can synthesis aldosterone. This mutation is often associated with the SV form. Mutations (P30L, V281L, P453S) result in about 20–50% of wild-type enzymatic activity and are usually identified in the NC form. However, several investigators have shown that the genotype is not always related to the phenotype (10, 11). For example, a mutation in the second intron, which causes aberrant splicing of mRNA, is most frequently identified in 21-OHD patients. This mutation is found in both SW and SV forms. *In vitro* expression studies indicate that a small percentage of mRNA from this intronic mutation is normally spliced (12, 13) Thus, this leakiness of splicing would influence the genotype of the mutation.

In addition, it has been clarified that adrenomedullary function is impaired in 21-OHD

mice and affected patients (14, 15). The degree of impairment of adrenomedullary function in patients with 21-OHD may also affect the phenotype (15, 16). Finally, unidentified genetic modifications, genetic background and sensitivity to glucocorticoid may influence clinical manifestation.

Prenatal Diagnosis and Treatment of 21-OHD

In 1965, Jeffcoate *et al.* first reported a successful prenatal diagnosis of 21-OHD based on elevated levels of 17-ketosteroids and pregnanetriol in amniotic fluid (17). In 1972 suppression of the fetal adrenal by maternally administered dexamethasone was reported (18). Thus, prenatal dexamethasone treatment to prevent or reduce virilization of an affected female fetus was first introduced by David and Forest in 1984 (19). Since then, treatment of pregnant women carrying fetuses at risk of virilization with dexamethasone has been carried out for more than two decades, although mainly in the USA and Europe (20–23).

Theoretically, dexamethasone is used because it is not inactivated by placental 11β-hydroxysteroid dehydrogenase. Thus, dexamethasone crosses the placenta from the mother to the fetus and presumably suppresses the fetal hypothalamic-pituitary-adrenal axis, resulting in reduced secretion of adrenal androgens; however, the exact mechanism is still unknown.

New *et al.* (20) proposed an algorithm for prenatal diagnosis and treatment of 21-OHD (Fig. 1). As previously mentioned, virilization of the external genitalia in affected females starts by 8 wk of gestation. Therefore, dexamethosone (20 μg/kg/day) should be started as soon as pregnancy is confirmed. Furthermore, chorionic villus sampling (CVS) or amniocentesis should be performed as early as possible. If the fetus is male or an unaffected female, treatment should be stopped. For an affected female, treatment is continued throughout pregnancy. In the study of New *et al.* (20), results were reported 532 cases prenatally diagnosed using amniocentesis or CVS between 1978–2001 in the USA. In their study, 61 of the fetuses were female, 49 of whom were treated prenatally with dexamethasone. Of these 49 fetuses, 25 affected female fetuses were administered dexamethasone at or before 9 wk of gestation. Of these 25 fetuses, 11 fetuses were born with entirely normal female genitalia, and

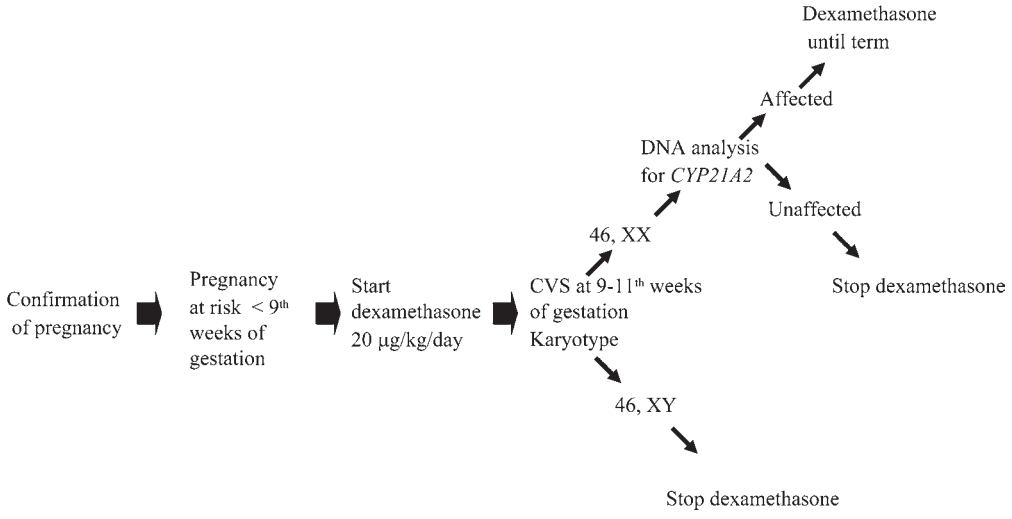

Fig. 1 Algorithm for prenatal diagnosis and treatment of 21-OHD.

11 were born with significantly milder symptoms than their untreated siblings. Therefore, about 85% of prenatally treated female infants are born with normal or slightly virilized genitalia. The authors reported that early cessation of therapy, late start of treatment, suboptimal dosing or poor compliance could result in treatment failure. In the above study, no significant side-effects were noted in the mothers or fetuses.

Lajic *et al.* (23) reported the Scandinavian experience with prenatal treatment of 21-OHD during the period of 1985–1995. Of 44 pregnancies, 37 were treated short term because the fetus was either unaffected or an affected male. Seven patients were treated from the 6th wk of pregnancy until term. In four of five cases exhibiting severe 21-OHD, virilization of external genitalia was significantly reduced compared with that in elder siblings. The majority of the 44 dexamethasone-treated fetuses demonstrated normal pre/post natal growth compared with matched controls. The incidences of fetal abnormalities and fetal death were not increased, although some adverse events were observed among cases treated both short and full term. It is difficult to determine whether or not these evens are caused by dexamethazone. They also analyzed maternal side-effects and complications. Significant weight gain was observed during early pregnancy when treatment was initiated, but this initial rapid weight gain declined when dexamethasone was discontinued.

While these studies may suggest treatment is safe and effective, others have reported problems with this treatment (24–28). In regard to this autosomal recessive condition, the risk of having an affected female fetus is only one in eight when both parents are known carriers, and therefore seven of eight fetuses will receive dexamethasone treatment unnecessarily. This issue raises ethical concern. Furthermore, there is a varied incidence of maternal side effects as mentioned previously. Finally, there is little long term human data available concerning treatment of prenatal children with dexamethasone as discussed in the next section.

Follow-up Data for Individuals Subjected to Prenatal Dexamethasone Treatment

Findings in animals suggest that repeated doses of steroids can interfere with growth and development of the immature brain (28–30). In addition, prenatal dexamethasone exposure alters cardiometabolic and hypothalamic-pituitary-adrenal axis function and increases food intake (28, 31, 32). However, studies in animals may not be applicable to humans, and these experiments used excessive glucocorticoid in dosages 5–10 times the human dose.

In regard to humans, some observations suggest that antenatal dexamethasone may negatively affect the child's neuropsychological development (28–30, 33). Furthermore, recent research is now focused on uncovering the mechanisms by which glucocorticoids are involved in programming the fetus for its future life, such as hypertension, diabetes and stress responses (30, 32–34).

Therefore, the pre-and postnatal growth and psychomotor development of treated children must be carefully followed. In particular, the development of unaffected children subjected to short-term treatment with dexamethasone should be monitored because these children receive unnecessary treatment.

Meyer-Bahlbunrg *et al.* (35) reported the cognitive and motor development of children after early prenatal dexamethazone treatment in the USA. The mothers of 174 prenatally dexamethasone-exposed children (including 48 CAH) and 313 unexposed children (including 185 with CAH) completed four standardized developmental questionnaires about the children. The data in this study shows that there are no marked adverse effects on cognitive or motor development. However, because the treated children were not tested directly, we must carefully interpret the results. Recently,

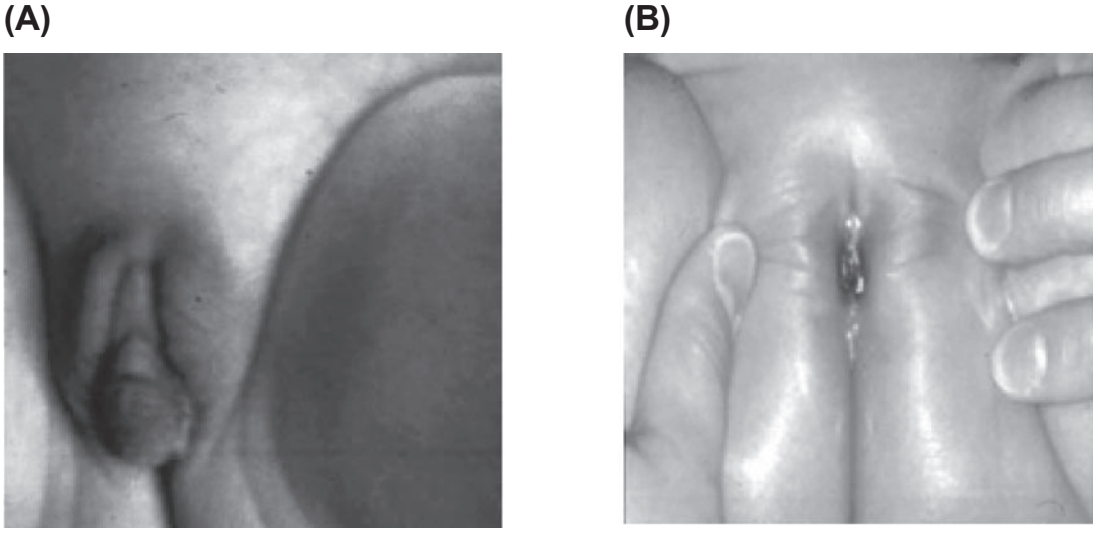

Fig. 2 (A) An elder sister showed virilization of the external genitalia. (B) Her younger sister, who had prenatal treatment, had normal female genitalia.

Hirvikoski *et al.* (36) reported the neuropsychological function and scholastic performance of children treated in Sweden. This is the first report of long-term direct testing results for dexamethasone-exposed children. Their study included 26 children at risk for 21-OHD who were treated prenatally with dexamethazone. Four affected females were treated until term. Furthermore, seven unaffected boys, ten unaffected girls and five CAH-affected boys were treated only in the first trimester. They were 7 to 17 yr of age. According to their study, the 21-OHD unaffected, short-term treated children had significantly poorer results in verbal working memory and increased social anxiety compared with the control group, but the children with 21-OHD and even the girls treated until term did not. All other test parameters including school performance were comparable for the treated and control groups. Because the sample size was very small and 21-OHD affected patients without prenatal therapy were not evaluated as a control group, a definitive conclusion could not be drawn; however, this puzzling result again emphasizes the necessity of retrospective randomized study of a large number of patients.

Current Conditions in Japan

In Japan, prenatal diagnosis and treatment of this disease has rarely been reported. Previously, we reported the results of prenatal diagnosis and treatment using karyotype and genotype determination using CVS (37). CVS samples were obtained at 10 to 11 wk of gestation from two females carrying fetuses at risk of 21-OHD. Prenatal diagnosis was successful in both cases. One affected female was treated with dexamethasone to term. The female infant was born with normal female genitalia, and surgical intervention was not required (Fig. 2). In the other case, treatment was withdrawn at an early stage when testing revealed a normal male fetus.

To analyze the status of prenatal diagnosis and treatment of 21-OHD in 2002 in Japan, Kinoshita *et al.* (38) sent a nationwide questionnaire to all members of the Japanese Society for Pediatric Endocrinology. Out of 954 questionnaires sent out, 371 were utilized in their analysis. According to their study, from 1995 to 2002, 13 patients in 9 hospitals received this treatment. Among these 13 patients, two

were affected females, and treatment continued to term and was effective. However, 25% of the respondents opposed prenatal diagnosis and treatment due to the above-mentioned reasons. To minimize unnecessary treatment, non-invasive testing of free fetal DNA in maternal blood may be useful. Honda *et al.* (39) reported that fetal gender can be determined by analyzing maternal serum taken as early as the 7th gestational wk. Thus, it could be used prior to CVS to warrant early withdrawal of unnecessary treatment.

Another problem in Japan concerns determination of the genotype of *CYP21A2*. A laboratory company is able to analyze defects of *CYP21A2*; however, the cost is very expensive in Japan. Furthermore, prenatal genetic testing of inherited diseases is not generally accepted by commercial laboratories. Therefore, ideally, specific centers that perform CVS could also analyze the karyotype and genotype of *CYP21A2* from CVS samples. Alternatively, an expert hospital, which perform CVS sampling and follow-up for the mothers and fetuses, could collaborate with specific research centers for analysis of the karyotype and genotype of *CYP21A2*. In any circumstances, the issues related to the accuracy of genetic diagnosis and the cost of genetic testing must be resolved.

Summary

In summary, we have provided an overview of both the benefits and risks of prenatal diagnosis and treatment of 21-OHD. The therapy is effective; however, the reported studies of long-term outcome are non-randomized, poorly controlled trials, and therefore, a proper large prospective randomized control trial is necessary to evaluate dexamethasone-exposed children. There is also an issue concerning where prenatal genetic diagnosis of 21-OHD should be done in Japan. Finally, the ethical burden is heavy. Therefore, until these problems are solved, we must be cautious. This therapy should be administered only to selected parents who have a clear understanding of the possible risks and benefits, strong desire for the treatment and the ability to be followed-up with carefully throughout pregnancy in a specific center, whose ethical committee approves and regulates the therapy.

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