Prenatal genetic testing and treatment for congenital adrenal hyperplasia

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Couples at risk for autosomal recessive congenital adrenal hyperplasia often request anticipatory guidance and genetic counseling. Initially, hormones in amniotic fluid were measured to distinguish affected female fetuses from unaffected fetuses. With the molecular era, more-targeted approaches became possible. Prenatal genetic diagnosis via amniocentesis or chorionic villus sampling was used to determine the need for continuing fetal therapy (dexamethasone), allowing cessation if the fetus was unaffected. Newer methods now allow diagnosis earlier in gestation, further shortening the treatment time for unaffected female fetuses who will not develop genital ambiguity. Preimplantation genetic testing permits transfer only of an unaffected female or male fetus. Analysis of maternal cell-free DNA based on quantitative differences in the amount of allele parental DNA permits affected pregnancies to be differentiated from unaffected pregnancies. (Fertil Steril® 2019;111:21–3. ©2018 by American Society for Reproductive Medicine.)
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Couples at risk for the inherited disorder congenital adrenal hyperplasia (CAH) often request anticipatory guidance and genetic counseling. Initially, hormones in amniotic fluid were measured to distinguish affected from unaffected fetuses. With the arrival of the molecular era, more-targeted approaches became possible. Prenatal genetic diagnosis via amniocentesis or chorionic villus sampling (CVS) could then be used to determine the need for fetal therapy.

In an ongoing pregnancy, the traditional utilization of CVS or amniocentesis has now been supplanted by noninvasive prenatal testing involving cell-free DNA from maternal blood. An alternative approach applicable before clinical pregnancy involves preimplantation genetic testing (PGT), selecting only genetically normal embryos for transfer. With cell-free DNA or PGT, diagnosis can evolve into therapy.

CAH GENOTYPE

In more than 90% of cases, CAH is caused by autosomal-recessive mutations in the *CYP21A2* gene that encode the steroidogenic enzyme 21-hydroxylase, which impairs the activity of the steroidogenic 21-hydroylase (1, 2). Three clinical CAH phenotypes of varying severity exist. Salt-wasting and simple virilizing are both considered "classic CAH." The third phenotype is nonclassic CAH, which is usually of adult or adolescent onset and has an incidence of 1 in 27 live births in the Eastern European Jewish population (see the article in this Views and Reviews by New et al. on nonclassic CAH) (3). Genotype–phenotype correlations have been shown for 1,507 CAH patients (4). Computational studies have further modeled approximately 150 known mutations that predict the mechanism and extent of functional loss in 21-hydroxylase for a given mutation (5).

CAH EMBRYONIC AND FETAL PHENOTYPE

The prototypic clinical sign of classic CAH is genital ambiguity. This occurs only in affected females; genital ambiguity does not occur in males. Genital ambiguity in females leads to psychological and psychosexual issues in adult life (2). The pathogenesis involves excessive androgen production from fetal adrenal glands, beginning by 9 weeks of gestation. The phenotype includes clitoral enlargement of variable degrees, labioscrotal fusion, and a urogenital sinus. Urinary incontinence and

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sexual dysfunctions are manifested later in life. Surgical interventions to correct genital ambiguity are not universally successful (6–9).

The accepted protocol for prenatal treatment of CAH was the administration of dexamethasone to all pregnant mothers whose offspring were at risk for female genital masculinization. Without accurate prenatal genetic detection, one would not have been able to distinguish affected from unaffected fetuses. The likelihood for parents at risk was that one in four female offspring would be affected, or one in eight fetuses overall. The rationale here was to suppress fetal androgen production during the period of urogenital organogenesis, which begins by the ninth week of gestation. Without binding to corticosteroid-binding globulin or being metabolized by placental 11β-hydroxysteroid dehydrogenase, dexamethasone crosses the placenta, suppresses fetal androgen production, and prevents in utero virilization. Importantly, prenatal treatment must be initiated before the ninth week of gestation. Forest, Morel, and colleagues were the first to show that fetal hyperandrogenemia and genital ambiguity can be prevented by treating these pregnant mothers with low-dose oral dexamethasone (20 μg/kg/d of prepregnancy weight, with a maximum daily dose of 1.5 mg/d) (10, 11). Successful outcomes have been reported in a cohort of more than 500 patients (12).

Two major problems exist with the strategy described. Therapy is not required in seven of eight fetuses. This includes all male (four of eight) along with unaffected female or heterozygous female (three of eight) fetuses, whose genitalia are predicted to develop normally. With this approach, unaffected fetuses thus receive dexamethasone therapy until their gender and genotype for CAH become known and therapy can be stopped. Until recently, this was possible only after CVS at 10–12 weeks or after amniocentesis at 15–16 weeks. Thus, unaffected heterozygote females received unnecessary treatment for 5–10 weeks. A second potential problem may be due to dexamethasone, because it has been shown in animal studies to result in reduced fetal birth weight and possibly other problems (13–16). However, much higher doses of dexamethasone (100 μg/kg/d) were administered in animals than used for human fetal CAH therapy (20 μg/kg/d). Nonetheless, animal studies showed low birth weights and impaired renal, pancreatic β-cell, and brain development (17). Likewise, high-dose dexamethasone administration in human pregnancy when used to delay preterm labor or promote fetal lung maturation has been reported to be associated with low birth weight, impaired development of the hypothalamic–pituitary–adrenal axis, and aberrant fetal programming (18–20). Reflecting these concerns, a moratorium was placed on prenatal low-dose dexamethasone treatment in Sweden (15), whereas the Endocrine Society recommended that use be restricted to institutional review board–approved research settings (21).

Yet the rationale for prenatal treatment remains sound. The surgical alternative of genitoplasty during childhood can result in urinary and sexual dysfunction in adults (6–8). Longitudinal studies of females who underwent genitoplasty during infancy or early childhood have shown impaired genital sensitivity, sexual dysfunction, decreased frequency of intercourse, and urinary incontinence (9). Other complications of surgery include strictures, recurrent fibrotic scarring, fistula, and urinary infections (22). Prenatal fetal prevention is surely preferable to surgical treatment of affected individuals.

(NONINVASIVE) CELL-FREE DNA ANALYSIS

A more recent approach in prenatal genetic diagnosis is a method that eschews invasive CVS or amniocentesis but still requires a clinical pregnancy. Noninvasive prenatal diagnosis has long been pursued, initially selecting intact fetal cells (23). However, recovery was inconsistent, and these cells can remain in maternal circulation, presumably in niches (24, 25). This could potentially confound prenatal genetic analysis in subsequent pregnancies. A different approach for noninvasive prenatal diagnosis began in 1997, when Lo and colleagues successfully detected cell-free fetal DNA in maternal plasma (26, 27). Some monogenic disorders could be detected by merely identifying a paternal mutation in the pregnant mother's blood, indicating an affected fetus. This immediately led to the detection of sex and Rh(D) in Rh-negative women. Cell-free fetal DNA also does not persist after delivery.

Of relevance to CAH, Lo and colleagues more recently developed a correlative method by which inheritance of a *CYP21A2* mutation could be established through targeted massively parallel sequencing (MPS) of cell-free DNA plasma drawn from an expectant mother (28). This technology is more complicated than merely detecting specific *CYP21A2* mutations; the basic strategy requires targeted MPS of genomic DNA from the trio of both parents and the affected proband. Informative single-nucleotide polymorphisms (SNPs) on both sides of the *CYP21A2* locus allow one to construct haplotype blocks that are needed to determine paternal and maternal allelic inheritance. Full diagnostic concordance between this noninvasive method and invasive diagnostic procedures or postnatal genetic testing was initially demonstrated in 14 families (28). Determination of fetal sex is straightforward and based on identification of a Y sequence in maternal plasma by MPS (28) that complements traditional polymerase chain reaction using an SRY probe. Results can be available within hours and be used to avoid unnecessary treatment of mothers carrying male fetuses. Diagnosis can be made as early as 6 gestational weeks.

The essential strategy is the ability to determine SNPs on a quantifiable basis. Dosage analysis determines the amount of DNA inherited from the father and the mother, taking into account the mother's own alleles as well as those she transmitted to the fetus. If the fetus has inherited a CAH-linked haplotype, a linked SNP will show more DNA than if not.

PREIMPLANTATION GENETIC TESTING

Preimplantation genetic testing and the transfer of unaffected embryos would prevent the need to treat an unaffected embryo. Preimplantation genetic testing–monogenic (PGT-M) diagnosis allows a couple to avoid clinical termination of an affected pregnancy. In a couple at risk for CAH, assisted reproductive technology produces embryos that can be

distinguished as affected or unaffected by molecular techniques. No longer is it necessary to wait until clinical pregnancy to establish the diagnosis of CAH, thus avoiding the necessity for dexamethasone treatment to unaffected embryos, because analytical validation of PGT is well established.

Preimplantation genetic testing–monogenic can be carried out for any single gene disorder whose chromosomal location is known. This is possible even if the causative nucleotide mutation is not known. Thus, PGT-M is readily applicable for CAH. Reproductive Genetics Innovations has performed PGT for 600 monogenic disorders, including CAH (29). Preimplantation genetic testing–monogenic for single gene disorders should be accompanied by PGT–aneuploidy testing. This increases the pregnancy rate by some 20% over PGT-M alone. Details on PGT-M are provided elsewhere by one of the authors.

The preferred approach for PGT-M is blastocyst (trophectoderm) biopsy, which has superseded the cleavage-stage (blastomere) biopsy. By 5–6 days, the embryo has expanded in cell number (approximately 120 cells). The inner cell mass that will develop into the embryo per se can be distinguished from the trophectoderm per se. Biopsy of the trophectoderm usually requires 5–10 cells.

The embryo biopsy necessary for PGT may be associated with some damage that may preclude survival. However, any damage seems to be either lethal or overcome by surviving potential cells. The anomaly rate in live births does not seem to be increased (30).

In conclusion, new methods of prenatal genetic diagnosis exist for CAH. Preimplantation genetic diagnosis with transfer of unaffected embryos allows only the one in four females who will be affected to be treated with dexamethasone. This approach is a great advance for those who wish to avoid having an unaffected child and avoid a clinical abortion. An alternative noninvasive method also exists, requiring only plasma from the pregnant mother. Using MPS, quantitative differences in the amount of inherited parental DNA can be used to distinguish affected from unaffected embryos.

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