

Prenatal Exclusion of Recessively Inherited Disorders: Should Maternal Plasma Analysis Precede Invasive Techniques?

Since the presence of large amounts of circulating fetal DNA in maternal plasma and serum samples was first reported by Lo et al. in 1997 (1), a multitude of clinical applications for the noninvasive diagnosis of complications of pregnancy have been reported [reviewed in Ref. (2)]. These applications have largely focused on the quantification of male fetal DNA in maternal plasma/serum samples and its associated increase in conditions such as fetal trisomy 21, maternal hyperemesis gravidarum, preeclampsia, invasive placenta, and preterm labor (3–7). In addition, qualitative detection of uniquely fetal DNA sequences in maternal plasma/serum has facilitated the noninvasive prenatal diagnosis of Rhesus D genotype, myotonic dystrophy, and achondroplasia (8–10). Both of these approaches are feasible because fetal and maternal DNA can be distinguished from each other. This same approach, however, will not work in the noninvasive prenatal diagnosis of autosomal recessive disorders, in which a fetus shares a mutant allele with its mother.

In this issue of *Clinical Chemistry*, Chiu et al. (11) describe a novel strategy to exclude the diagnosis of a recessive condition by identifying polymorphic markers that distinguish the uniquely paternal mutant and wild-type alleles. They amplified cell-free fetal DNA in maternal plasma at 11 and 17 weeks of gestation, and by comparison with DNA from the couple's first affected child, were able to determine that the current pregnancy was unaffected.

As a model system, these investigators chose the disease congenital adrenal hyperplasia (CAH), but the approach is potentially warranted for other recessive disorders. Noninvasive prenatal exclusion of an autosomal recessive disorder can work only when there is a known positive family history of a specific disorder, the mutant alleles have been molecularly characterized in the affected family member, and the affected individual is a compound heterozygote. In other words, the mother and the father of the prospective fetus must have different mutant alleles that can be distinguished from each other in a PCR-based assay. Thus, prenatal exclusion of CAH was feasible in the couple described in this report, but for example, prenatal exclusion of β -thalassemia would not be feasible in a geographically isolated ethnically homogeneous population in Sardinia because of expected homozygosity at the molecular level.

CAH is of particular interest in the field of prenatal diagnosis because it is relatively common and in utero treatment is available to prevent virilization of an affected female fetus. CAH refers to a group of inherited disorders characterized by abnormal synthesis of cortisol in the adrenal cortex. Defects in the enzymes 21-hydroxylase, 11- β -hydroxylase, and 3- β -hydroxysteroid dehydrogenase lead to overproduction of adrenal androgens, with physical and psychological virilization of the affected female fetus. As in the case described by Chiu et al. (11),

21-hydroxylase deficiency accounts for >95% of all cases. The worldwide incidence of the more severe, salt-wasting form is between 1 in 10 000 and 1 in 15 000 live births (12, 13), whereas the milder nonclassical form has a disease frequency of 1 in 100 in a heterogeneous New York City population (13).

Prenatal diagnosis for CAH was first performed biochemically, by detection of increased 17-hydroxyprogesterone and Δ^4 -androstenedione in second-trimester amniotic fluid samples. Although this technique was accurate, the amniocentesis was performed too late in gestation to prevent masculinization of a female fetus by maternal administration of dexamethasone. The current standardized approach to prenatal diagnosis and treatment of CAH in families at risk is to administer dexamethasone to a pregnant woman no later than 9 weeks of gestation, before fetal gender or mutation status are known. For definitive determination of fetal genotype, a chorionic villus biopsy (CVS) is performed at 11 weeks or an amniocentesis at 16 weeks. Treatment is then discontinued for unaffected fetuses of both genders and for affected male fetuses in whom virilization is not a clinical problem. Interestingly, the report by Chiu et al. (11) did not comment on whether the family they studied underwent conventional prenatal diagnosis or treatment before the exclusion of CAH in the fetus.

A recent review summarized one center's experience in the prenatal diagnosis and treatment of 532 pregnancies at risk for CAH (13). In this study, 281 pregnant women received prospective prenatal treatment with dexamethasone. Of the 532 pregnancies, 116 produced fetuses that were diagnosed with classic 21-hydroxylase deficiency. Of these, 61 were female; 49 of 61 received antenatal treatment. Maternal dexamethasone treatment was effective in reducing fetal virilization. Treated mothers, however, had increased weight gain, edema, and striae. In this study, no significant side effects were seen in the treated newborns. A Swedish study of long-term outcome in prenatally treated affected and unaffected children noted cases of growth failure and developmental delay, a case of liver steatosis, and two males with multiple congenital anomalies (12).

Although the standard prenatal diagnosis and treatment protocol has been very effective in the treatment of affected females, a major concern is that seven of eight fetuses will have unnecessary exposure to dexamethasone during an early, and presumably vulnerable, period of gestation.

How, then, can maternal plasma DNA analysis improve on the standard prenatal diagnostic and treatment protocol that has been in widespread clinical use for 15 years? As a start, maternal plasma could be obtained in the first trimester and analyzed for the presence of SRY to determine fetal gender. This approach was first suggested by Rijnders et al. (14), but was more recently refined by

Honda et al. (15). This latter group demonstrated a 100% sensitivity of fetal gender detection by 7 weeks, using analysis of cell-free fetal DNA in maternal serum. Thus, if the fetus were shown to be male, no treatment would be needed. If no male DNA was detected, maternal dexamethasone could begin. Another sample could then be analyzed at 9 or 11 weeks of gestation. If still negative for the presence of male DNA, the fetus would be presumed to be female, and, assuming that the familial polymorphisms and mutations were known, fetal DNA could be analyzed in parallel to exclude the paternal mutation. If the disease was excluded, treatment could stop. If any uncertainty existed about the DNA results, there would still be adequate time to offer conventional invasive prenatal diagnosis. The advantages of this two-stage approach are that no male fetuses would be exposed to dexamethasone and that unaffected female fetuses would have a briefer exposure than at present to this drug. Furthermore, this approach might decrease the risk of procedure-related miscarriage by reducing the need for CVS or amniocentesis.

The proposed amended protocol is feasible, but is it practical? Gender determination by real-time PCR is accurate and relatively inexpensive once the necessary equipment is in place. The major limitation is likely to be in the molecular characterization of the mutations in families at risk. However, for conventional prenatal diagnosis to be offered, mutation analysis must be performed. Families in whom the mutations are already well characterized might serve as an initial subject population for the further study of an amended protocol.

With the current report by Chiu et al. (11), the potential clinical applications for the analysis of fetal cell-free DNA in maternal plasma and serum expand, but it is still unclear how the fetal DNA is produced or metabolized in the pregnant woman. It is remarkable that the pregnant woman is chronically exposed to such large amounts of foreign DNA without apparent adverse effects. It would be indeed exciting if fetal cell-free DNA analysis could enhance prenatal diagnostic options while simultaneously teaching us something about the biology of the fetomaternal unit. Stay tuned. This is a field that is evolving rapidly.

References

1. Lo YMD, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of fetal DNA in maternal plasma and serum. *Lancet* 1997;350:485-7.
2. Pertl B, Bianchi DW. Fetal DNA in maternal plasma: emerging clinical applications. *Obstet Gynecol* 2001;98:483-90.
3. Lo YMD, Lau TK, Zhang J, Leung TN, Chang AM, Hjelm NM, et al. Increased fetal DNA concentrations in the plasma of women carrying fetuses with trisomy 21. *Clin Chem* 1999;45:1747-51.
4. Sekizawa A, Sugito A, Iwasaki M, Watanabe A, Jimbo M, Hoshi S, et al. Cell-free fetal DNA is increased in plasma of women with hyperemesis gravidarum. *Clin Chem* 2001;47:2164-5.
5. Zhong XY, Burk MR, Troeger C, Jackson LG, Holzgreve W, Hahn S. Fetal DNA in maternal plasma is elevated in pregnancies with aneuploid fetuses. *Prenat Diagn* 2000;20:795-8.
6. Sekizawa A, Jimbo M, Saito H, Iwasaki M, Sugito Y, Yukimoto Y, et al. Increased cell-free fetal DNA in plasma of two women with invasive placenta. *Clin Chem* 2002;48:353-4.
7. Leung TN, Zhang J, Lau TK, Hjelm NM, Lo YMD. Maternal plasma fetal DNA concentrations as a marker for preterm labour. *Lancet* 1998;357:1904-5.
8. Lo YMD, Hjelm NM, Fdler C, Sargent IL, Murphy MF, Chamberlain PF, et al. Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. *N Engl J Med* 1998;339:1734-8.
9. Amicucci P, Gennarelli M, Novelli G, Dallipiccola B. Prenatal diagnosis of myotonic dystrophy using fetal DNA obtained from maternal plasma. *Clin Chem* 2000;46:301-2.
10. Saito H, Sekizawa A, Morimoto T, Suzuki M, Yanaiharu T. Prenatal diagnosis of a single-gene disorder from maternal plasma. *Lancet* 2000;356:170.
11. Chiu RWK, Lau TK, Cheung PT, Gong ZQ, Leung TN, Lo YMD. Noninvasive prenatal exclusion of congenital adrenal hyperplasia by maternal plasma analysis: a feasibility study [Technical Brief]. *Clin Chem* 2002;48:778-80.
12. Lajic S, Wedell A, Bui TH, Ritzen EM, Holst M. Long-term somatic follow-up of prenatally treated children with congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 1998;83:3872-80.
13. New MI, Carlson A, Obeid J, Marshall I, Cabrera MS, Goseco A, et al. Prenatal diagnosis for congenital adrenal hyperplasia in 532 pregnancies. *J Clin Endocrinol Metab* 2001;86:5651-7.
14. Rijnders RJ, van der Schoot CE, Bossers B, de Vroode MA, Christiaens GC. Fetal sex determination from maternal plasma in pregnancies at risk for congenital adrenal hyperplasia. *Obstet Gynecol* 2001;98:374-8.
15. Honda H, Miharu N, Ohashi Y, Samura O, Kinutani M, Hara T, Ohama K. Fetal gender determination in early pregnancy through qualitative and quantitative analysis of fetal DNA in maternal serum. *Hum Genet* 2002;110:75-9.

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