pregnancy, treating such women seems reasonable, although further studies are definitely warranted. One could also argue for just giving low-dose ASA, which is inexpensive and very safe to all women, although it is argued that compliance improves if women are recognized to be high risk. In any event, it seems that either these authors have finally solved the riddle and found the truly beneficial utility, or we will once again fall into the pit of aspirin despair.—MEN)

Noninvasive Prenatal Diagnosis for Cystic Fibrosis: Detection of Paternal Mutations, Exploration of Patient Preferences, and Cost Analysis

Melissa Hill, Philip Twiss, Talitha I. Verhoef, Suzanne Drury, Fiona McKay, Sarah Mason, Lucy Jenkins, Stephen Morris, and Lyn S. Chitty

North East Thames Regional Genetics Service (M.H., P.T., S.D., F.M., S. Mason, L.J., L.S.C.), and Genetics and Genomic Medicine, UCL Institute of Child Health and Great Ormond Street Hospital for Children NHS Foundation Trust (M.H., L.S.C.); and Research Department of Applied Health Research, University College London (T.I.V., S. Morris), London, United Kingdom

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ABSTRACT

Cystic fibrosis (CF) is a severe, autosomal recessive, multisystem condition affecting the respiratory and digestive systems. It is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. Prenatal diagnosis of CF currently requires an invasive test to obtain fetal genetic material and carries a small risk of miscarriage. Noninvasive prenatal diagnosis (NIPD) based on analysis of cell-free fetal DNA in maternal plasma has been reported to exclude the paternal mutation in couples carrying different CF mutations. This study describes the development of a next-generation sequencing assay designed to detect or exclude 10 of the most common CF mutations, for use when each parent carries a different CFTR mutation, and the paternal mutation is one of the 10 included in this panel. A cost analysis of NIPD for CF is reported to inform implementation strategies. Normal and heterozygous genomic DNA (gDNA) control samples with known CFTR mutations were used to assess test performance, before testing on maternal plasma samples collected, as part of a larger program designed to develop standards for NIPD from women undergoing invasive diagnostic prenatal testing because of a risk of CF. The presence of fetal DNA was confirmed by the detection of paternal CFTR sequences, ZFY, or paternal HLA-type sequences in the maternal plasma. The total test-related costs of 3 different clinical pathways (the current invasive testing only pathway, NIPD for the paternal CF mutation, and NIPD for direct diagnosis) were estimated to assess the economic consequences of implementing NIPD for CF. Total test-related costs were estimated for the current care pathway and were compared with those incorporating NIPD by using uptake data from a study exploring views on NIPD for CF. All 8 mutations in the gDNA samples from CF carriers were reliably detected at an allele frequency of 50%. A questionnaire-based study of stakeholder views and preferences was undertaken to estimate the uptake of invasive testing and NIPD with detailed results published elsewhere. Most participants (n = 130; 94.9%) said that they would choose NIPD for CF, and 90% would be prepared to pay for it, with 49.2% prepared to pay up to £50, 39.0% prepared to pay £100 to £200, and 10.3% prepared to pay more than £200. The total cost for this pathway per 100 women was £57,185. Using these potential uptake data, the incremental costs of NIPD over invasive testing per 100 pregnancies at risk of CF are £9025 for paternal mutation exclusion and £26,510 for direct diagnosis. The authors have successfully developed a next-generation sequencing assay to allow NIPD to be used for risk stratification in a significant proportion of CF families. Consideration of stakeholders' views and cost-effectiveness alongside test development indicates that introduction of NIPD for CF would be welcomed and uptake is likely to be high. These findings may have
implications for NIPD for other conditions and highlight the need for prospective consideration of the ethical and economic issues that may arise as more tests are developed.

EDITORIAL COMMENT

(The introduction of cell-free DNA (cfDNA) screening for fetal disorders has been an exciting development in the field of prenatal diagnosis. Testing for fetal aneuploidy using cfDNA has had tremendously rapid uptake over the 4 years since this test was introduced. The test initially focused on Down syndrome, with rapid subsequent addition of other aneuploidies and the sex chromosomes. In some laboratories, microdeletion testing has also been added. It is inevitable that cfDNA testing for additional conditions, including single gene disorders such as CF, will be introduced clinically as the technology evolves.

In this abstracted article, the authors describe a study in which they developed an assay for cfDNA testing for a few of the more common CF mutations and tested this assay in a number of "spiked" maternal samples as well as 4 maternal samples in which the fetus was known to have a paternal CF mutation that was different than the maternal mutation. They also surveyed families with children affected with CF to determine how they would feel about noninvasive versus invasive testing for fetal CF and used these data to conduct a cost analysis of clinical pathways in which at-risk couples had the option of (1) invasive diagnostic testing with chorionic villus sampling or amniocentesis, (2) cfDNA testing for the presence of a paternal mutation (which can decrease the risk if the paternal mutation is absent, but cannot make a definitive diagnosis if the paternal mutation is present because the presence of the maternal mutation is unknown), or (3) direct diagnosis using cfDNA (essentially "complete" prenatal diagnosis using cfDNA for CF, a technique that is not currently available). Using a small number of samples, they found that the assay was able to detect a mutation in a carrier parent and that they were able to identify the presence of a mutation using "spiked gDNA" into a maternal sample in 10 cases. They were also able to identify a paternal mutation that had been inherited by the fetus in 3 maternal samples. In the patient survey part of their study, they found that patients would prefer to have prenatal diagnosis for CF noninvasively, if that was possible. The results of the study are interesting, although limited by the very small number of cases. However, almost more interesting is how the study was conducted, how the hypothetical options were presented to the families, and consideration of how such a test might be utilized in actual clinical practice. With regard to the assay, a complication is that each mutation must be developed, validated, and then tested independently. Cystic fibrosis is made complicated by the fact that there are now nearly 2000 mutations that have been implicated as causing the disorder. There are a number of mutations that are very common and many that are very rare. With the common mutations, it is likely in many cases that both parents will carry the same common mutation, for example, the F508 mutation is responsible for more than half of all mutations in northern European populations. If both parents carry the same mutation, this assay cannot be used to tell if the fetus has inherited a unique paternal allele. For this reason, the authors estimate that only 29.7% of carrier parents would be able to take advantage of this approach.

In their cost analysis, the authors presumably asked patients if they would prefer invasive (risky, painful) prenatal diagnosis procedures or a noninvasive blood draw to obtain the same information. It seems silly to even ask such a question; a true evaluation of patient preferences would be far more nuanced and would have to consider how much they value accuracy, and the value of knowing that an individual's risk has increased from 25% (the risk with carrier parents) to 50% (the risk to carrier parents if it is known that the fetus inherited the paternal allele, but without available information about the maternal allele). For parents that would not consider invasive prenatal diagnosis, it is unclear that information about this amount of risk increase would be helpful. It would be helpful and reassuring for the 50% of families in whom the fetus appears not to have inherited the paternal mutation, but this gets into the final consideration, which is the real benefit to the health system and to families, of such a test for risk stratification or alleviation of anxiety. If a test has no
cost, or minimal cost, that may be reasonable, but for an expensive test that is helpful to a small number of families, it is unclear how much true clinical utility is provided by this approach. Nevertheless, perhaps these are initial small steps toward a time when cfDNA testing has further advanced and developed such that it is more accurate, and better able to distinguish affected from unaffected fetuses for a variety of disorders.—MEN

Diagnostic Utility of Microarray Testing in Pregnancy Loss

J. A. Rosenfeld, M. E. Tucker, L. F. Escobar, N. J. Neill, B. S. Torchia, L. D. McDaniel, R. A. Schultz, K. Chong, and D. Chitayat

Signature Genomic Laboratories, PerkinElmer, Inc, Spokane, WA (J.A.R., N.J.N., B.S.T., L.D.M., R.A.S.); Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX (J.A.R., N.J.N.); St Vincent Hospital, Indianapolis, IN (M.E.T., L.F.E.); and The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada (K.C., D.C.)

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ABSTRACT

Miscarriage is associated with 10% to 15% of all recognized pregnancies in the United States, and stillbirth occurs in approximately 1 in 165 births. Approximately 50% of recognized first-trimester miscarriages and 6% to 17% of stillbirths are chromosomally abnormal; therefore, diagnosis of a chromosomal abnormality as a cause of pregnancy loss provides important information for recurrence-risk counseling and helps identify familial chromosomal rearrangements. Chromosomal microarray testing can be performed on DNA extracted from direct fetal samples and does not require culturing of live cells. It can detect significant chromosomal abnormalities below the resolution of karyotyping, which is 5 to 10 megabases, or higher with poor fetal chromosome morphology. The authors of this study analyzed over 500 pregnancy losses across all gestational ages with clinical microarray-based comparative genomic hybridization (aCGH) testing to evaluate the performance of chromosomal microarray testing in the setting of pregnancy loss. Clinical aCGH testing was performed using a variety of platforms; the majority of samples were studied using whole-genome, oligonucleotide-based arrays with the densest coverage available (n = 467 samples). Among 242 fetal demise specimens received between January 2012 and December 2013, 8.3% (n = 20) failed to yield microarray results, 8 specimens (3.3%) were not tested, and an additional 12 specimens (5.0%) were tested with aCGH but yielded uninterpretable results, 9 due to poor DNA quality and 3 with maternal cell contamination (MCC). Among 136 samples received in 2012 to 2013 with information on karyotype available, 15 (11.0%) had failed karyotypes; microarray was successful in 9 (60.0%) of these cases. An additional 5 samples (3.7%) failed to yield microarray results but had known karyotypes. Among the 515 fetal samples yielding aCGH results, 16 cases were referred with abnormal karyotypes to provide better characterization of the karyotypic abnormality, these cases were removed from the calculations. Overall, aCGH testing identified clinically significant abnormalities in 12.8% (64/499) of cases. The highest detection rate was found among first-trimester losses (P < 0.0001). Clinically significant abnormalities were found in 29.2% of miscarriage specimens referred for recurrent pregnancy loss. Excluding first-trimester miscarriages, clinically significant abnormalities were found in 11.1% (32/287) of cases referred with fetal anomalies. Among the subset of cases tested after a normal karyotype, 6.9% (20/288) had clinically significant findings on aCGH. Of the 107 samples that underwent additional single nucleotide polymorphism analysis, abnormalities of potential clinical significance undetectable by aCGH (such as trisomy) were detected in 6.5% (7/107) of cases. Isolated variants of uncertain clinical significance were identified in 3.0% (15/499) of all cases without abnormal karyotypes known at referral. The study shows higher detection rates in miscarriages than stillbirths. The increased detection rate of chromosomal microarrays and their ability to test direct fetal samples are likely to make this a more sensitive, reliable test for fetal chromosome analysis; microarray testing should be considered for pregnancy losses of all gestational ages.

EDITORIAL COMMENT

(Miscarriage is surprisingly common in human pregnancies and is the outcome of approximately 15% of pregnancies that are recognized, and probably occurs even more often when considering very early miscarriages. It is thought that most early miscarriages are chromosomally abnormal,