

MASTER CLASS

The Quest for Prenatal Evaluation

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It has eventuated into the well-established field of prenatal diagnosis.

The focus, for the most part, has been on gleaning information during the early and midtrimester periods of pregnancy. Such evaluation has been found most useful in providing reassurance to parents when anomalies are excluded, or—under

The quest for fetal and embryonic evaluation has been of great interest to many scientists, physicians, parents, and members of the lay public.

those uncommon circumstances when a diagnosis is made prenatally—in allowing parents and physicians to create appropriate medical and social strategies to deal with these diagnoses.

Despite the benefits afforded by prenatal diagnosis, there remains a subset of the potentially reproductive population for whom conceiving and delivering an abnormal child is not a rare event, but may in fact have a high degree of predictability. These aspiring parents have had to choose among high-risk pregnancy, nonconception, adoption, or egg or sperm donors.

Most recently, however, these patients and their physicians have been able to exploit the new technology of preimplantation genetic diagnosis. This is a rapidly expanding field that has the potential to

make a significant difference in the lives of patients who could not otherwise be anywhere near certain that they would deliver a healthy, normal child.

The technology affords us a wide range of possibilities, both now and in the future, but it also presents a number of challenges, including ethical issues, financial coverage issues, and issues concerning the actual availability of the services to select populations and individuals.

This all makes preimplantation genetics quite a complex topic, with great positive potential and a great many implications and potential hurdles and obstacles, all of which must be discussed and deliberated.

It is for this reason that we have invited Dr. Mark R. Hughes, a leading international scholar in the area of preimplanta-

tion diagnosis and one of the pioneers of this technology, to serve as the guest author of this month's Master Class.

Currently, Dr. Hughes is the director of the Genesis Genetics Institute in Detroit. He previously served on the faculty of the schools of medicine at Baylor College of Medicine, Georgetown University, and Wayne State University, and was a member of the founding group of the Human Genome Institute at the National Institutes of Health.

DR. REECE, who specializes in maternal-fetal medicine, is vice president for medical affairs, John Z. and Akiko K. Bowers Distinguished Professor, and dean, School of Medicine, University of Maryland. He is the medical editor of this column.

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BY MARK R.
HUGHES, M.D.

Preimplantation Genetic Diagnosis and Screening

The development of preimplantation genetics over the past 2 decades has helped to alleviate the frustration I used to feel as an obstetrician when I would leave my lab in the medical school and walk over to the hospital to visit the parents of a newborn with a terrible inherited condition.

It was my job to explain to the baby's parents what the disorder was and how it happened. Many of the babies I saw had an autosomal recessive disorder, and it was necessary to explain to the baby's mother and father how this awful disease suddenly appeared in their child, and tell them that they faced a 25% risk of its happening again should they decide to have more children. Their options for the future, I would tell them, would be to adopt, to elect not have any more children, to use an anonymous donor for eggs or sperm, or—as many couples do—to carefully roll the genetic dice again with hopes of a better outcome.

In the last scenario, I would explain, the parents would have the opportunity to have a chorionic villus sampling or amniocentesis. But it goes without saying that the 12-15 weeks that unfold before such testing is done are often filled with anxiety: first, about having the test, and second, about the decision to be made if the results are not favorable.

Today, obstetricians have good reason to present such parents with another option—preimplantation genetic diagnosis (PGD)—and to be aware of its capabilities and limitations. Developments in PGD mean that we, also, in all of our preconceptional obstetrical work, have good reason to be cognizant of ethnicity-based risks for genetic disorders and to advise patients, when indicated, to have genetic screening themselves.

We can use PGD today, in conjunction with in vitro fertilization, to test for over 250 serious diseases and conditions caused by mutations or chromosomal abnormalities. Parents who choose the technology—usually couples who know they carry mutations or who have had another baby or a family member with a serious inherited illness—can learn that an embryo is free of the disease that their family is prone to, and can thus start a pregnancy with a commitment to continuing it.

The Genetics, the Process

As obstetricians, we are not trained geneticists. Therefore, it is helpful for us and for our patients if we understand the basic genetics behind PGD, and appreciate how PGD pushes diagnostic technology to its absolute limits—both its theoretical limits and its practical limits.

Every cell taken from an embryo (or any cell in our

body) contains all the genes needed to make a new, complete individual. Each cell's DNA contains just four letters of the genetic alphabet: A, T, G, and C. The way in which these letters are strung together, just as letters are put together to make a book, will tell that cell what to do.

We can think of chromosomes as books of the gigantic encyclopedia of life, and genes as paragraphs within these chromosome books. Each chromosome has thousands of genes, most of which contribute something unique to the story of who we are, just as most paragraphs in a novel or encyclopedia contribute a unique element to the story or knowledge base. Some genes do not appear to be as important to our health as others, just as some paragraphs seem like "filler" in a story.

All genes have a defined beginning and a defined end, and just as a paragraph has an indentation, a gene has a promoter. Genes are made up of little bits called exons, just as paragraphs are made up of sentences. Some genes are gigantic; the gene whose mutation causes muscular dystrophy, for instance, would be equivalent to a paragraph about 158 pages long. Other genes are tiny, similar to a short phrase.

We all carry hundreds and hundreds of typographical errors in our personal encyclopedia, some of them inactivating the gene paragraph that contains them. Fortunately, the errors we inherit from our mothers are generally not matched by the errors we inherit from our fathers. Every once in a while, though, we choose a mate with a gene mutation in the same paragraph. When this occurs, the baby does not have a "backup" copy of the intact gene paragraph, and a recessive disease can occur.

Such unfortunate pairings happen more often in couples of similar ethnicity because many gene mutations are ancestral in a given ethnic population. Thus, one of our roles in preconceptional counseling is to think about the possibility that a patient who wants to discontinue birth control and start a family might carry a gene mutation for an inherited disorder common to his or her ethnic background.

In couples of Northern European ancestry, we think first of cystic fibrosis (with a carrier frequency of about 1 in 29 in the United States) and spinal muscular atrophy (1 in 5). In African Americans, we worry about sickle cell anemia (1 in 22).

If our patients have Southeast Asian ancestry, we should consider α -thalassemia (1 in 30), and for patients with Mediterranean ancestry, β -thalassemia. For patients of Ashkenazi Jewish ancestry, genetic screening is performed routinely for the ancestral mutations causing Tay-Sachs disease, familial dysautonomia, Gaucher's disease type 1, Niemann-Pick disease, Bloom



A human 8-cell embryo produced routinely in an IVF laboratory is undergoing the biopsy of one blastomere (cell) for testing.

syndrome, Canavan disease, and cystic fibrosis.

When we perform PGD, the testing is done overnight. Couples follow the same process that any infertile couple undergoing in vitro fertilization (IVF) would follow, but before implantation, a single cell is taken for analysis from each embryo in its day-3, eight-cell stage. The single-cell samples are sent by courier to a reference laboratory for overnight testing, and a report is electronically sent to the reproductive endocrinologist. Couples are notified of the results in time for embryo transfer on day 5.

In the future, we may be able to relax the timeline and allow more time for embryo transfer by performing the biopsies when the embryo is 5 days old. In this procedure, cells would be taken from the trophectoderm—the outer layer of the embryo that ultimately develops into the placenta—and the embryos would be frozen via a rapid freezing process called vitrification.

Ice crystals do not form in this method, so concerns about damage to the cells is alleviated. Women could then undergo embryo transfer the next month. For now, however, we follow a 5-day deadline for embryo transfer.

Regardless of what advances are made, we must appreciate the fact that this technology pushes medical diagnostics to its limits. PGD involves the testing of one single cell (the smallest unit of life) and one gene (the smallest unit of inheritance), for one typographical error in 3.3 billion DNA letters, and all of this occurs overnight.

Its Value and Accuracy

The nomenclature of what we have simply and rather loosely called PGD is actually changing a bit. Following

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Europe's lead, U.S. experts are beginning to use the term PGD to refer specifically to the actual diagnosis of a particular disease. PGS (preimplantation genetic screening) is exactly what its name implies as well—screening, largely for abnormal numbers of chromosomes—and not the actual disease diagnosis. Together, the terms fall under the general rubric of preimplantation genetics.

The differentiation is being made because everything about the two procedures—the technology; the people and issues involved; the risks and benefits; and importantly, the accuracy of the procedure—is different. All told, the error rate for preimplantation genetics is in the range of about 2%-4%. Analyzing chromosomes, however, is quite different from analyzing genes, just as counting books of the encyclopedia is quite different from opening a book and finding a letter error.

In analyzing chromosomes, we have to worry about the possibility of complex chromosomal mosaicism having occurred. This is a process by which chromosomes segregate unevenly to cells as the cells are dividing, and if it has occurred, some of the cells we biopsy may appear normal even though the rest of the cells are not.

Experts are increasingly concerned that the chromosome analysis component of preimplantation screening may not really be improving parents' chances of having a healthy baby. However, although the prognostic value of what we now should call PGS is unclear and confusing, there are no such doubts associated with PGD. Telling parents that their baby has a clear 25% chance of having a serious disease is quite different from telling parents that their baby may—or may not—have a chromosomal abnormality.

This is not to say, however, that PGS is without value. I would advise it in cases in which the woman already needs IVF and if she has had recurrent miscarriages.

Ethical Issues and the Future

The real issue with preimplantation genetics, I believe, is whether there are limits to when the technology can and should be used. We must continue, of course, to consider and address the questions associated with PGS and its value. But beyond this, we face numerous questions emanating not as much from a scientific or technologic perspective as from an ethical perspective.

For instance, couples who already have a child with a genetic disease and do not want it to happen again can test their embryos not only to learn which ones carry the genetic defect, but also to learn whether any of their embryos are an identical stem-cell match with their child who is ill.

At the time of delivery, then, they will have not only a healthy baby, but also a baby who can donate identically human leukocyte antigen (HLA)-matched cord blood for stem-cell transplantation to the sibling. Such testing for HLA matching happens daily in the United States.

It is also possible to screen embryos for genes that raise the risk of cancer—primarily breast, ovarian, and colon—in adulthood. We know this is being done in England today, where regulators ruled last year to allow it. The questions are a bit different with this issue, as I see it, because

genotype in this case does not accurately predict phenotype. Having the BRCA1 or BRCA2 gene mutation does not mean, for instance, that a person will develop breast or ovarian cancer. So the question really is whether we should be testing embryos for a disease that may never occur.

As in other ethical debates, we must listen to all points of view. Many couples have watched multiple family members die from colon cancer or breast cancer and have decided that enough is enough, whereas other couples who are testing for HLA matching have a child with an incurable, often fatal disease. These couples know there is no such thing as a perfect baby. All they

want is to have the A and the G and the T and the C in the right places, or to save their child while having the chance to have another healthy baby to love as well.

Most clinics have ethics teams to develop policies that address these issues and to describe which indications for preimplantation genetics are acceptable and which are not. Most clinics allow the use of technology for finding cancer genes and for HLA matching, for instance, but not for selecting gender.

Studies following children after IVF and preimplantation genetics that have been done in Europe—where the type of medical system allows investigators to effectively track patients for longer-term out-

comes—are better than those done in the United States. Clearly, safety and good outcomes have been demonstrated. Thousands and thousands of babies have been born after having undergone IVF and PGS or PGS, with no evidence of birth defects.

Still, experts in the United States have been designing a database—a prospective registry of sorts—that, when implemented, will collect data on the use of preimplantation genetics, primarily regarding how much is being done and for what ends the technology is being used. Such data will help us to further understand and guide this fast-growing facet of reproductive medicine. ■

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