UPDATE

Louise Wilkins-Haug, MD, PhD

Medical Director, Center for Fetal Medicine, Brigham and Women's Hospital, and Associate Professor and Division Director, Maternal-Fetal Medicine and Reproductive Genetics, Department of Obstetrics, Gynecology and Reproductive Biology, Harvard Medical School, Boston

FAST TRACK

The prevalence of fragile X premutations in women is relatively high

PRENATAL COUNSELING

• Screening for fragile X, the most common cause of mental retardation • Fetal RhD genotyping using maternal plasma

Genetic medicine is fast gaining recognition as an essential component of clinical care. Since this Update last year, 2 areas have seen notable progress:
- **New guidelines** were issued for fragile X screening in women of reproductive age, and
- Research on **free fetal DNA** in the maternal circulation deepened our understanding of its diagnostic potential.

Clinical applications are emerging in both avenues, and there will be further investigation and refinement. Be sure to check this column again next year!

New guidelines on who to test for mental retardation marker

Sherman S, Pletcher BA, Driscoll DA. Fragile X syndrome: diagnostic and carrier testing. Genet Med. 2005;7:584–587.

McConkie-Rosell A, Finucane B, Cronister A, Abrams L, Bennett RL, Petterson BJ. Genetic counseling for fragile X syndrome: updated recommendations of the National Society of Genetic Counselors. J Genet Couns. 2005;14:249–270.

Fragile X syndrome is the most common inherited cause of mental retardation. The condition can occur in both males and females and is characterized by a range of behavioral changes consistent with autism spectrum, mental retardation, and developmental delay, as well as a facial phenotype that tends to become more recognizable as the individual ages.

▮ Test is not for everybody

New guidelines issued by the American College of Medical Genetics recommended general population screening only within the constructs of research protocols. In selected populations, however, screening should be considered (**TABLE 1**). Among preconception and prenatal patients, directed interrogation of the family history for findings suggestive of fragile X syndrome can be guided by these recommendations.

Prevalence. ObGyns should be aware of the increasing spectrum of full and premutation fragile X phenotypes and the relatively high prevalence of premutations among women.

Anatomy of fragile X

Changes in a specific region of the X chromosome known as the *fragile X mental retardation-1* (*FMR-1*) gene are

TABLE 1

Fragile X syndrome: Diagnostic and carrier testing guidelines

Both women and men with
• mental retardation
• or developmental delay
• or autism . . . *especially with:*
    Physical or behavioral characteristics of fragile X
    or family history of fragile X
    or a relative with undiagnosed mental retardation

Persons seeking reproductive counseling who have
• family history of fragile X syndrome
• or a relative with undiagnosed mental retardation

Fetuses of carrier mothers

Affected individuals or relatives in whom the diagnosis was made
by cytogenetic studies

Women with elevated follicle-stimulating hormone, *especially with*
family history of
• premature ovarian failure
• or fragile X syndrome
• or relative of either sex with undiagnosed mental retardation

Men or women with late-onset intention tremor or ataxia . . . *especially with*
family history of
• movement disorders
• or fragile X
• or undiagnosed mental retardation

Source: Sherman et al.

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All sons who inherit an expanded, full mutation will have fragile X features. In daughters, prognostication is limited

responsible for the syndrome. Elongation of an unstable CGG repeat sequence at the 5´ end of *FMR-1* leads to hypermethylation, impaired translation, and altered production of the fragile X mental retardation protein. Investigations of knock-out mice reveal that this protein plays an important role in prenatal and postnatal brain development, especially in the area of dendrite maturation.

Among Caucasians, the characteristic features of fragile X syndrome occur in approximately 1 in 4,000 males and 1 in 8,000 females and are associated with elongation of the *FMR-1* gene to more than 200 CGG repeats (a full mutation). Initial studies of other races suggest a similar range of full mutations in males and females.

❚ Which offspring will inherit the gene?

In the general population, the *FMR-1* region has variable lengths.[1] In most individuals, 40 or fewer CGG repeats are present and the region remains stable when passed from either parent to the child. Occasionally, however, individuals inherit expansions of this repeat region—either slight (41–60 repeats, intermediate range) or larger (61–200, premutation range). Repeats in the premutation range are car-

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TABLE 2

Number of CGG repeats influences mutation status

MATERNAL REPEATS	PERCENT RISK OF EXPANSION TO FULL MUTATION (>200 REPEATS)			
	NOLIN, 1996[2]	PESSO, 2000[3]	TOLEDANO-ALHADEF, 2001[4]	NOLIN, 2003[5]
55–59	13 (3/22)	0 (0/11)	0 (0/22)	4 (1/27)
60–69	21 (7/34)	12 (1/8)	10 (2/20)	5 (6/113)
70–79	58 (59/102)	50 (1/2)	17 (1/6)	31 (28/90)
80–89	73 (78/107)	50 (1/2)	—	58 (81/140)
90–99	94 (83/88)	100 (1/1)	—	80 (89/111)
100–200	99 (177/179)	75 (3/4)	—	98 (194/197)

Values presented as percent (n/N).
Data modified from Nolin et al.[5]

ried by 1 in 700 to 1,000 males and 1 in 113 to 350 females.

Expansion of the premutation to a full *FMR-1* mutation depends on the sex of the transmitting parent, the length of repeats, and the frequency of AGG interspersion. Only the first 2 criteria are available for clinical interpretation. *FMR-1* expansion occurs only in the X originating from the maternal cell line. The larger the premutation, the more likely it will expand to a full mutation (**TABLE 2**).

Timing of the maternal *FMR-1* expansion can vary, with meiotic, postzygotic, and mitotic instability of CGG length all reported.

- Typically, all sons who inherit an expanded, full mutation exhibit features of fragile X syndrome.
- In daughters, however, a full mutation causes a range of features. In daughters with a full mutation, prognostication is limited. Studies indicate that at least 50%, and in some series 75%, have IQs in the borderline or mentally retarded range.

Longitudinal studies of asymptomatic females with full mutations have not been reported. Fathers with premutations pass the *FMR-1* gene in a stable fashion to all offspring, occasionally with contraction to a smaller repeat size.

Note that the complex inheritance pattern, with premutations transmitted through both sexes but expansion limited to the maternal X chromosome, can confound interpretation of family histories of mental retardation or developmental delay.

▮ Screening populations

Testing for the *FMR-1* gene by determining the expansion size is possible via DNA analysis. Most labs utilize both Southern analyses, to measure the degree of methylation, and polymerase chain reaction, to discriminate at a more refined level the subtle differences in repeat sizes that distinguish intermediate and premutation sizes.

Screening the general population for premutations of the *FMR-1* gene is not yet the standard of care. However, several authorities advocate fragile X screening among prenatal and preconception populations, given the relatively high rate of the carrier state (1 in 113 to 350), the sensitivity of testing, and the implications for mental retardation and disability in offspring.

In the United States, even assuming a relatively conservative premutation rate of 1 in 300 and an expansion rate of only 11.3%, such testing would be cost-effective, ranging from $99 to $300 per test.[6]

What are the risks?

Concerns include the implications of intermediate expansions and the substantial patient education needed to convey the risk of expansion to premutation (but not full mutation). No child with a full mutation has been born to a mother with 59 or fewer repeats. Also needing study is the variability of fragile X syndrome in women with a full mutation. These women are at substantial risk for learning impairment, but the degree of disability varies unpredictably.

Prenatal diagnosis requires DNA from amniocytes or chorionic villus sampling. If the latter, follow-up amniocentesis may be needed because methylation begins at variable times during placental development.[7]

Preimplantation genetic assessment for fragile X premutation carriers has been reported using a system of closely linked markers, circumventing the need to assess onset of methylation abnormalities.

▮ Spectrum of symptoms

Previously, individuals with premutations were considered clinically asymptomatic. However, we now know that phenotypic expression of expansion sizes occurs along a spectrum.

Recent data indicate 2 phenotypes associated with premutations:

- **In women, premature ovarian failure,** defined as menopause before the age of 40 years, occurs in 13% to 24% of those with premutations of the *FMR-1* gene, among families with fragile X syndrome. Conversely, among women with premature ovarian failure, premutations are found in 2% and 14% of sporadic and familial cases, respectively. Further, the size of the premutation may be directly correlated to the risk of premature ovarian failure.

- **In men with premutations, a neurologic syndrome of tremors and ataxia** is a newly described phenomenon. The fragile X-associated tremor/ataxia syndrome (FXTAS) is a progressive, neurodegenerative process with Parkinsonism and peripheral neuropathy, and penetrance appears to increase with age.[8] The frequency of this diagnosis among older men with premutations is under study.

Fetal RhD genotyping now possible using maternal plasma

Gautier E, Benachi A, Giovangrandi Y, et al. Fetal RhD genotyping by maternal serum analyses: a two-year experience. Am J Obstet Gynecol. 2005;192:666–669.

Moise K. Fetal RhD typing with free DNA in maternal plasma. Am J Obstet Gynecol. 2005;192:663–665.

Fetal RhD typing using free fetal DNA (ffDNA) is routine in the United Kingdom but not yet in this country. Since 1997, when Lo identified ffDNA in maternal plasma,[9] numerous studies have focused on the physiology, timing, and clinical application of fetal RhD typing using ffDNA. Previously, the focus was detection of fetal cells in maternal circulation.

Unlike intact fetal cells, ffDNA fragments are present in the maternal plasma in sufficient quantities to allow extensive investigation. While most maternal free DNA is composed of longer DNA fragments, shorter DNA fragments of fetal origin appear as the pregnancy advances and in some studies are first detectable as early as 32 days after conception.

Free fetal DNA increases throughout gestation, representing 3% of total DNA in maternal plasma during the second trimester, and increasing to 6% in the third trimester. Free fetal DNA fragments are cleared rapidly by the renal system,

with a half-life of 16 minutes and no discernable levels as soon as 2 hours after delivery.

We now understand that ffDNA fragments are continuously deposited in the maternal circulation from early in pregnancy, perhaps even before fetal circulation develops. We also know that maternal levels of ffDNA depend on 2 forces: rate of deposition and rate of removal.[10]

Trophoblastic origin?
A placental source is suggested by evidence that ffDNA can be retrieved from maternal plasma prior to the development of fetal circulation. A trophoblastic origin is supported by identification in maternal plasma of fetal mRNA with specificity for genes expressed by the placenta. Moreover, ffDNA has been detected in maternal circulation as early as 14 days after conception, corroborating a trophoblastic origin, with programmed apoptosis of placental cells a likely mechanism.

Further increases in ffDNA throughout gestation may reflect direct contributions from the fetal circulation that are transferred to maternal circulation via the placenta. In later gestation, destruction of fetal cells within the maternal circulation may contribute to the pool of ffDNA in maternal plasma. The exact proportions of each contribution are unknown.[11]

▮ ffDNA may help diagnose these disorders
Placental abnormalities
Recent work suggests sufficient quantities of ffDNA can be obtained for both quantitative and qualitative assessments.[12] Used quantitatively, ffDNA reflects placental integrity, an active area of investigation. **Autosomal trisomies, preeclampsia, and fetal growth restriction** are conditions thought to involve abnormal placental function. Increased levels of ffDNA have been found in these entities. Increases have been documented even before onset of preeclampsia.[10]

Gene defects
Use of ffDNA to identify specific gene defects is also under study. Sensitive microarray technology will likely be needed to assess fetal chromosome aneuploidy from maternal plasma.

The detection of single gene defects from ffDNA has been reported for paternally inherited myotonic dystrophy, Huntington disease, and achondroplasia.

For autosomal recessive disorders, genetic testing of ffDNA may be a first step to exclude inheritance of a paternal allele. For this application, discordant parental alleles will be needed so that exclusion of the paternal mutation in the ffDNA signifies an unaffected fetus or a heterozygotic carrier of the maternal allele. If the paternal allele is detected by ffDNA, further genetic testing by chorionic villus sampling or amniocentesis would be needed to differentiate heterozygotic carriers of the paternal mutation from homozygotic, affected fetuses.

RhD genotyping
Since 2001, ffDNA has been used clinically in the United Kingdom for fetal blood group genotyping in isoimmunized gravidas with heterozygous partners, through the International Blood Group Reference Laboratory (part of the National Blood Service), which brings us to the highlighted study. Gautier and colleagues added data affirming that the RhD genotype can be detected through ffDNA with high sensitivity and specificity. Among 285 RhD-negative women, the fetal RhD genotype was determined in 283. In 2 cases, the maternal RhD-negative phenotype did not result from a complete gene deletion; thus, the genotypes of fetus and mother could not be differentiated. Among the women with RhD-negative genotypes, all fetuses were accurately genotyped through ffDNA.

This study differs from prior investigations in its use of RhD-negative women who were not already sensitized, and suggests that ffDNA genotyping in RhD-negative women is sensitive enough to be

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Preeclampsia, autosomal trisomies, and fetal growth restriction are thought to involve placental abnormalities

incorporated into the distribution of Rh immune globulin.

2 problems

As Moise points out in an editorial accompanying the study, a robust, automated system for ffDNA assessment prior to administration of Rh immune globulin likely would be cost-effective. The Moise editorial also points out these 2 concerns:

False positives are a real possibility, as the 2 cases in the Gautier study illustrate. Free fetal DNA analysis for RhD genotyping assumes that the serologic finding that indicates RhD-negative status (lack of RhD on the fetal red blood cells) is due to deletion of the RhD locus. Thus, when RhD DNA fragments are detected in maternal plasma, they are presumed to be fetal in origin. However, we now know that pseudogene regions of the RhD locus occur with relatively high frequency—in particular, in more than half of African Americans, who serologically type as RhD-negative. Such pseudogenes cause a stop codon that effectively diminishes production of RhD antigen. Serologic typing of such individuals indicates an RhD-negative phenotype. Because the most common pseudogenes are within exon 4, inclusion of primers that assess multiple exons can reduce these false positives.

False negatives have graver clinical implications. Misidentification of an RhD-positive fetus as RhD-negative could prevent that fetus from receiving appropriate surveillance and intervention. False-negative assessments from ffDNA are probably caused by poor amplification of the test sample.

Safeguards have been used in most protocols, including tracer mouse DNA as an internal control to assure amplification. Simultaneous SRY gene testing assures amplification of male fetal DNA. For females, incorporation in the amplification assessment of highly polymorphic markers different from those of the maternal sample may verify fetal DNA amplification.

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Free fetal DNA assessment prior to administration of Rh immune globulin would probably be cost-effective

What is ahead?

Protocols to refine use of ffDNA for RhD genotyping are likely. Meanwhile, techniques are being modified to assure extraction of sufficient quantities of fetal DNA.

Future research will focus on quantitative changes in ffDNA as a marker for pregnancy complications, and development of noninvasive prenatal assessment of specific genes. Successful development of a noninvasive ffDNA diagnostic test will enhance prenatal evaluations without the risk of pregnancy loss currently associated with amniocentesis and chorionic villus sampling. ■

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