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## ***Cherchez la femme*: maternal incidental findings can explain discordant prenatal cell-free DNA sequencing results**

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### **Abstract**

Circulating DNA fragments in a pregnant woman's plasma derive from three sources: placenta, maternal bone marrow, and fetus. Prenatal sequencing to noninvasively screen for fetal chromosome abnormalities is performed on this mixed sample; results can therefore reflect the maternal as well as the fetoplacental DNA. Although it is recommended that pretest counseling include the possibility of detecting maternal genomic imbalance, this seldom occurs. Maternal abnormalities that can affect a prenatal screening test result include disorders that affect the size and metabolism of DNA, such as B12 deficiency, autoimmune disease, and intrahepatic cholestasis of pregnancy. Similarly, maternal tumors, both benign and malignant, can release DNA fragments that contain duplications or deletions. Bioinformatics algorithms can subsequently interpret the raw sequencing data incorrectly, resulting in false-positive test reports of fetal monosomies or test failures. Maternal sex-chromosome abnormalities, both constitutional and somatic, can generate results that are discordant with fetal ultrasound examination or karyotype. Maternal copy-number variants and mosaicism for autosomal aneuploidies can also skew interpretation. A maternal etiology should therefore be considered in the differential diagnosis of prenatal cell-free DNA test failures, false-positive and false-negative sequencing results. Further study is needed regarding the clinical utility of reporting maternal incidental findings.

### **Keywords**

cell-free DNA sequencing; copy-number variant; incidental finding; prenatal screening; sex-chromosome aneuploidy

## **INTRODUCTION**

As any fan of detective novels knows, the phrase “*cherchez la femme* (look for the woman)” is often used cynically to suggest that a woman is the reason for an unresolved situation.<sup>1</sup> The phrase was first used in 1864 by Alexandre Dumas père in the book *Les Mohicans de Paris*,<sup>1</sup> but one could claim that it has newfound relevance to the field of noninvasive prenatal screening (NIPS) for aneuploidy.

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### **DISCLOSURE**

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Maternal plasma DNA sequencing has been offered as an alternative to biochemical and sonographic screening tests for fetal aneuploidy since 2011. The circulating cell-free DNA fragments in the peripheral blood sample of a pregnant woman derive from three tissues of origin: placenta, maternal bone marrow, and the fetus (or fetuses). Most of the circulating DNA (~70–90%) derives from maternal apoptotic hematopoietic cells.<sup>2,3</sup> Of the hematopoietic DNA, 70% derives from the white-cell lineage and 30% from the erythroid lineage.<sup>3</sup> Because of the significant presence of maternal DNA in a plasma sample, NIPS cannot be diagnostic. Although it is recommended that pretest counseling include the possibility of detecting maternal genomic imbalances,<sup>4,5</sup> in practice this seldom occurs. In fact, in a 2015 review of consent forms from commercial providers of prenatal DNA sequencing, only a few were found to mention the chance of detecting a maternal abnormality.<sup>6</sup>

A false-positive result occurs when the NIPS result is abnormal, but discordant with the results of a diagnostic fetal or neonatal karyotype or chromosome microarray (CMA). Although initially there were concerns that technical aspects of the sequencing and/or bioinformatics analyses were the reason for the false-positive results,<sup>7</sup> over time it has become clear that a significant proportion of cases have an underlying biological etiology. Potential explanations include confined placental mosaicism, true fetal mosaicism, co-twin demise, and maternal pathology. A limitation of the published cases of discordance, however, is that they have incomplete clinical outcome information, for example, lacking placental biopsies or long-term information on the child.

Recently, Hartwig et al.<sup>8</sup> performed a systematic review of NIPS literature published between 1 January 1997 and 1 March 2016, looking specifically for evidence of discordant results. Only studies involving autosomes were reviewed. The investigators identified reports that together comprised 206 discordant cases, of which 182 (88%) had false-positive and 24 (12%) had false-negative results. Thirteen (54%) of the 24 false-negative cases had a biological or technical reason documented. Most of the cases were due to true fetal or confined placental mosaicism with a euploid cell line present. Sixty (33%) of the 182 false-positive cases also had a biological or technical reason found. The remainder of the false-positive cases (122, 67%) were unexplained, probably owing to a lack of comprehensive follow-up studies. Of the 60 false-positive cases, the great majority (40, or 67%) were explained by a maternal finding.

The purpose of this review, therefore, is to *cherchez la femme*, i.e., to focus on the (pregnant) woman as the source of unexpected DNA abnormalities in order to educate providers regarding the ever-expanding list of conditions that have been incidentally detected and reported in the NIPS literature. The review is divided into the different types of maternal conditions that can cause discordant NIPS results: medical problems and their treatments, cancer, sex-chromosome abnormalities, autosomal mosaicism, and copy-number variants (CNVs). After reading this review, it is hoped that practitioners will be better informed and thus able to provide improved posttest counseling in the setting of a positive NIPS result.

## MATERNAL MEDICAL CONDITIONS THAT CAN AFFECT INTERPRETATION OF TEST RESULTS

Several maternal medical conditions have been shown to either directly affect the interpretation of test results or contribute to test failure. These include vitamin B12 deficiency,<sup>9</sup> autoimmune disease,<sup>10-14</sup> and intrahepatic cholestasis of pregnancy (ICP).<sup>15</sup> These diseases and/or their treatments affect either the quantity or the quality of the circulating maternal DNA fragments, which comprise the majority of the blood sample.

### Vitamin B12 deficiency

A NIPS test in a 38-year-old woman at 12 weeks was positive for trisomy 21 but also showed a complex chaotic pattern, with overrepresentation of chromosomes 2, 3, 4, 5, 7, and 8 and underrepresentation of 14, 15, 17, 21, and 22.<sup>9</sup> Amniocentesis showed a normal male fetus (46, XY). Repeat tests at 16 and 20 weeks consistently showed the chaotic DNA patterns. Further medical workup of the woman demonstrated a hemolytic macrocytic anemia and severe vitamin B12 deficiency (levels 0.50 pmol/L). She was diagnosed with pernicious anemia and treated with weekly intramuscular injections of 1,000 µg vitamin B12. At 31 weeks, after correction of the vitamin B12 deficiency, a fourth blood sample was obtained for NIPS; this showed that the abnormal DNA patterns were no longer present. In this case, the maternal B12 deficiency resulted in intramedullary hemolysis that reversed with treatment.

### Autoimmune disease

Maternal autoimmune disease affects the size and the metabolism of circulating DNA fragments and can also be a reason for false-negative or failed test results. Using massively parallel genomic and methylomic sequencing, Chan et al.<sup>10</sup> analyzed the biological characteristics of nonpregnant individuals with systemic lupus erythematosus (SLE) versus healthy controls. The plasma of individuals with active SLE had a greater proportion of shorter DNA fragments and lower methylation densities. In a case report, a 41-year-old pregnant woman had two NIPS test failures due to a fetal fraction below 4%.<sup>11</sup> This was reversed with steroid treatment.

In addition, low-molecular-weight heparin, one component of treatment for autoimmune disease as well as hypercoagulability, is associated with low fetal fractions.<sup>12-14</sup> This occurs because heparin exerts a direct effect on the trophoblast by reducing apoptosis.<sup>14</sup>

### Intrahepatic cholestasis of pregnancy

Another example is ICP, which manifests as pruritus but is associated with fetal complications, including preterm delivery, fetal hypoxia, and stillbirth.<sup>15</sup> In a study in 15 pregnant women with ICP and 19 age- and gestation-matched controls, the amount of short-fragment nuclear DNA was twice as high in the women with ICP.

## TUMORS (BENIGN AND MALIGNANT)

Clinical laboratories that perform whole-genome, as opposed to targeted, sequencing may occasionally have samples that produce nonreportable or unusual test results (such as monosomy) due to the presence of genome-wide imbalance. An important cause of genome-wide imbalance is the presence of a tumor with cytogenetic instability that sheds DNA fragments into the plasma.

### Uterine leiomyomas

A common benign gynecological condition that has been demonstrated to cause discordant results is uterine leiomyoma.<sup>16</sup> The overall prevalence of leiomyomas, as detected by first trimester endovaginal sonography, is 10.7%, with significant variation according to race.<sup>17</sup> African-American women are much more commonly affected, with a prevalence among them of 18%.<sup>17</sup>

In a well-documented study with complete follow-up, a 40-year-old nulliparous woman underwent NIPS at 14.5 weeks of gestation. The result was abnormal with a very negative z-score for chromosome 13, suggesting the presence of monosomy 13. In-depth analysis of the entire genome sequencing results showed monosomy 13, as well as decreased numbers of sequence tags mapping to chromosomes 1p, 14, and Xq. At 19 weeks, an ultrasound examination showed normal fetal and placental anatomy and the presence of a 7-cm subserosal uterine leiomyoma. Amniocentesis revealed a normal female fetus (46, XX). At the elective cesarean section delivery, a uterine leiomyomectomy was also performed. Whole-genome single-nucleotide-polymorphism analysis of the leiomyoma biopsy showed multiple deletions involving parts of chromosomes 1, 13, 14 and X, consistent with the NIPS results. A postpartum maternal blood sample was normal, providing further validation that the source of the genome-wide abnormalities was the leiomyoma. This same clinical laboratory observed unreportable DNA results due to confirmed leiomyomas in 15 cases out of ~ 400,000 samples processed, giving a prevalence of around 3.75/100,000.<sup>16</sup> Not all uterine leiomyomas result in NIPS test failures, because many are small with limited blood supply, and the most common cytogenetic changes are balanced translocations. Furthermore, sequencing methods that target only chromosomes 13, 18, and 21 will not detect most leiomyomas.

### Malignant tumors

Whereas leiomyomas are very common in women of reproductive age, cancer is not. The most common malignancies observed in pregnant women include breast, ovarian, cervical, and colorectal cancers, lymphomas (Hodgkin and non-Hodgkin), malignant melanoma, and leukemia.<sup>18</sup> The first (retrospective) recognition that a maternal malignant tumor could be the source of circulating DNA that affected prenatal screening test results was reported in 2013.<sup>19</sup> A 37-year-old, seemingly healthy woman opted for NIPS at 13 weeks of gestation for advanced maternal age. Her test results were abnormal, with normalized chromosome values of +10.9 for chromosome 13, and – 10.7 for chromosome 18. This was repeated on the same specimen and eventually reported out as “trisomy 13 and monosomy 18.” Amniocentesis revealed a normal male (46, XY) fetus. A second maternal blood sample was

drawn at 17 weeks and sent to the laboratory that performed the original test. The results again showed aneuploidies of chromosomes 13 and 18, with even more markedly abnormal normalized chromosome values. In the third trimester, the patient had vaginal bleeding and a mass was noted on her cervix, with a clinical diagnosis of a prolapsed leiomyoma. Treatment was deferred until after delivery. At 36 weeks another maternal sample was drawn and sent to a different laboratory. The results were “nonreportable,” with a suggestion of monosomies for chromosomes 13 and 18. The vaginal delivery was uneventful and the baby was born healthy with normal Apgar scores. Postpartum the patient complained of pelvic pain. Her evaluation included a pelvic radiograph that demonstrated pathologic fractures in the right superior and inferior pubic rami. A fine-needle bone biopsy of the affected areas was diagnostic for a metastatic neuroendocrine carcinoma. The primary site was later shown to be in the vagina. Subsequent fluorescent in situ hybridization (FISH) studies revealed an increased number of chromosome 13 signals relative to chromosome 18, indicating that the tumor was the source of the aberrant circulating DNA profiles.

Subsequently, a Belgian study documented three cases of maternal cancer detected prospectively in a NIPS research study involving 4,000 pregnant women.<sup>20</sup> All had abnormal quality scores and reproducible abnormal genome-wide representation profiles. With ethical approval, subsequent management included whole-body diffusion-weighted magnetic resonance imaging. In all three pregnant women, cancer was found. One was a case of metastatic bilateral ovarian carcinoma, one was due to follicular lymphoma, and one was a nodular sclerosing form of Hodgkin lymphoma. In the last patient, initiation of chemotherapy resulted in normalization of circulating cell-free DNA (cfDNA) profiles within 6 weeks.<sup>21</sup>

Using cases of maternal cancer retrospectively identified from a large clinical laboratory database ( $n = 125,426$  samples), Bianchi et al.<sup>22</sup> showed that examining the entire genome-wide sequencing profile provided a biological explanation for why their NIPS results were discordant with the fetal karyotype. Although the women were all asymptomatic at the time of their initial NIPS, cancer was diagnosed between 13 and 39 weeks (mean = 16 weeks) later. Their clinical presentations varied from early-stage to widely metastatic disease, and included three cases of non-Hodgkin lymphoma, one case of Hodgkin lymphoma, one of anal cancer, one of colorectal cancer, one of neuroendocrine origin, and one of acute T-cell lymphoblastic leukemia. All of the babies were healthy, although three were preterm (one was electively delivered early to facilitate maternal treatment). In this study, the participants were all known to have cancer because their physicians voluntarily reported the information to the laboratory. These women were then contacted to participate in a research study in which additional clinical details were sought, and the sequencing results were rereviewed for all chromosomes. In all eight cases analyzed, there were multiple copy-number gains and losses across many different chromosomes, but the clinical laboratory’s bioinformatics algorithm masked results on chromosomes other than 13, 18, and 21. Furthermore, the proprietary bioinformatics program interpreted the test to reference chromosome ratio as a “monosomy,” when the real issue was the excess of sequences in the tumor relative to the reference chromosome(s). Review of the genome-wide profiles showed exaggeration of DNA abnormalities with advancing disease and resolution of these patterns with successful treatment.

Another case report in the literature is that of a 25-year-old woman who underwent NIPS as a first-tier screening test and was shown to have deletions on 9q and 22q in her plasma DNA.<sup>23</sup> A single-nucleotide-polymorphism array performed on her leukocytes and follow-up FISH studies showed the presence of a t(9; 22)(q34;q11.2) translocation (the Philadelphia chromosome), consistent with a diagnosis of chronic myelogenous leukemia. The patient had hepatomegaly, a slightly elevated platelet count, and leukocytosis. She was started on aspirin to prevent thrombosis. At 36 weeks she underwent elective cesarean delivery of a healthy male infant. Postpartum she underwent a bone-marrow biopsy that showed granulocyte proliferation and the presence of “dwarf” megakaryocytes. She began treatment with the tyrosine kinase inhibitor dasatinib and had a rapid clinical response.

In another case, the outcome was not as positive.<sup>24</sup> With the transfer of two frozen embryos, a 37-year-old G2P1001 woman conceived by in vitro fertilization. An initial cfDNA test, performed at 12 weeks, suggested full or partial monosomies for chromosomes 13, 18, 21, and X. Ultrasound examination revealed only a singleton fetus, and no anomalies were detected. Amniocentesis at 18 weeks showed a normal female fetus (46, XX) with a normal CMA. Owing to the concern about maternal malignancy, the clinicians requested a deeper analysis of the whole-genome sequencing results; this demonstrated multiple areas of imbalance. The patient was then referred to an oncologist and had a normal physical examination and unremarkable laboratory values. Whole-body magnetic resonance imaging without contrast at 23 weeks identified multiple T2 hyperintense and T1 hypointense hepatic lesions. The differential diagnosis included benign hepatic adenomas, primary hepatocellular carcinoma, or a secondary metastasis. At 28 weeks she had an invasive radiology-guided embolization procedure. A repeat DNA analysis showed a similar, but more exaggerated pattern of genome-wide imbalance, consistent with her enlarging hepatic lesions. At 32 weeks the woman had an elective cesarean-section delivery and open-liver biopsies to facilitate her medical management. One biopsy specimen showed poorly differentiated adenocarcinoma. Postpartum, computerized and positron emission tomography scans showed stage IV colon cancer. Although the neonate did well, the mother died 10 months after delivery.

### **Clinical management of genome-wide results suggestive of malignancy**

At present, the clinical utility of disclosing NIPS results that suggest malignancy is unproven and somewhat controversial. Two groups in Belgium have collectively reported on four cases of maternal cancer detected in asymptomatic women via NIPS under a research protocol.<sup>20,21,23</sup> They argue strongly in favor of disclosing the incidental findings. In the case of chronic myelogenous leukemia, no invasive procedures were performed on the mother or fetus. The authors stated that being aware of the diagnosis allowed monitoring of what can be a very rapid transition from an indolent phase to a blast crisis.<sup>23</sup> Similarly, a woman with the nodular sclerosing form of Hodgkin disease<sup>20,21</sup> was successfully treated during pregnancy, and a woman with metastatic bilateral ovarian carcinoma was successfully treated postpartum.<sup>20</sup> An additional woman with a slow-growing form of follicular lymphoma did not require treatment. A medical oncologist who specializes in the care of lymphoma patients expounded a contrasting opinion.<sup>25</sup> He reviewed the histories of the first four cases of maternal malignancy detected by NIPS<sup>19–21</sup> and concluded that

there was no evidence of improved clinical outcomes and that they may have experienced “net harm” due to unnecessary procedures.

The current reality, however, is that some NIPS laboratories report on the presence of multiple aneuploidies, or genome-wide imbalances that are suggestive of malignancy.<sup>26</sup> It is mainly the genetic counselors, therefore, who have the initial responsibility of explaining the unusual results and guiding subsequent management. Giles et al.<sup>27</sup> performed an anonymous online survey of 367 US-based board-eligible/board-certified genetic counselors regarding the potential for NIPS to detect genome-wide imbalance suggestive of maternal malignancy. Ninety-five percent of survey respondents were aware of this possible test result. There was a discrepancy, however, in the percentage of counselors who routinely discuss this (if results are suggestive) in pretest (29%) versus posttest (77%) counseling. Sixty-nine percent of respondents stated that the findings should be documented on the test report as well as discussed by a laboratory director. Current management recommendations are highly variable, and most counselors expressed a need for national or institutional guidelines.

## SEX CHROMOSOMES

Fetal sex discordance occurs when the cfDNA test report indicates a fetal sex that differs from what is observed on ultrasound examination, or more rarely, with the results of a diagnostic test. Whereas twin demise is likely to be the most common explanation for this phenomenon, maternal reasons include transplant from a male donor and maternal disorder of sexual differentiation (Table 1). In one case, the NIPS result was XY, but repeated sonographic examinations demonstrated female fetal genitalia. A detailed review of the maternal medical history was significant for receiving a kidney transplant from a male donor.<sup>28</sup> Presumably, the circulating Y chromosome fragments derive from low-level chronic rejection of the transplanted kidney, resulting in cell death.<sup>29</sup> In another case,<sup>30</sup> a 36-year-old pregnant woman underwent NIPS for a positive first trimester combined biochemical/ nuchal translucency screening result. An extremely high proportion of Y chromosome signals (on the order of that expected for a normal adult male) was observed. The maternal medical history showed that 10 years before her pregnancy she had undergone an allogeneic bone-marrow transplant with a male donor, owing to aplastic anemia. On sonographic examination the fetus was seen to be female. It was therefore concluded that the source of the Y-chromosome material was the transplanted blood cells.

The use of egg donation is a clue that there may be an abnormality in the pregnant woman’s sex chromosomes (Table 1). Bagby et al.<sup>31</sup> described the case of a 37-year-old woman whose NIPS results showed Y-chromosome z-scores well outside the expected range for a male fetus. Upon further review of the maternal medical history, it was determined that she conceived with the assistance of an egg donor because she had complete gonadal dysgenesis due to Swyer syndrome. This disorder of sexual differentiation is characterized by a 46, XY karyotype with normal female external genitalia, a uterus, and fallopian tubes. Instead of ovaries, scar tissue is present. Affected individuals can conceive with assisted reproductive technology, as was done here. In this case, the Y-chromosomal material originated from both the mother’s hematopoietic cells and the male fetus’s placenta.

## Maternal sex-chromosome aneuploidies

False-positive cases of sex-chromosome aneuploidies (SCAs) can be due to maternal constitutional monosomy X (apparent full or mosaic) or somatic mosaicism involving a single or an extra X chromosome.<sup>28</sup> Monosomy X is the one SCA that is not associated with advanced maternal age. Bianchi et al.<sup>28</sup> compared the mean maternal ages in true positive (31.7 years) versus false-positive (36.7 years) NIPS results of 45, X, and found a significant difference ( $P < 0.001$ ). These results suggest that the majority of false-positive cases of 45, X are due to maternal somatic mosaicism as a result of age-related loss of a single X chromosome (Table 1).

Two studies in the older cytogenetic literature have shown an association between maternal aging and somatic loss of a single X chromosome in peripheral blood nuclei. Devi et al.<sup>32</sup> performed FISH studies using X-chromosome probes on lymphocytes from 15 women with premature ovarian failure, 20 age-matched controls, and 10 older women with normal reproductive histories. They demonstrated a linear correlation between the number of nuclei with a single X-chromosome signal and age. The rate of accumulation of cells with a single X chromosome was on the order of 700 per million cells per year. At age 40, there was a mean of 2.5% of nuclei demonstrating only one signal. There was no relationship observed between subject age and the number of nuclei with three X signals (47, XXX). Russell et al.<sup>33</sup> studied 19,650 metaphases from 655 females at different ages, ranging from birth to 80 years. The frequency of X chromosome loss was significantly related to age ( $P = 0.00001$ ), and ranged between 0.07% (at less than 16 years) to 7.3% (at over 65 years).

True maternal SCAs have been detected by NIPS, broadening our understanding of the reproductive potential of women affected by these disorders. Wang et al.<sup>34</sup> developed a rapid method of sequencing the maternal white-blood-cell fraction in the original sample used for plasma analysis. They applied this method to analyze the maternal sex chromosomes in 187 samples that demonstrated an SCA. Of these, 124 showed evidence of loss of material from the X chromosome. In 10 of 124 (8.06%) maternal white blood cell samples there was evidence of mosaicism for 45, X. In 63 samples there was evidence of gain of X chromosome signal. Six of 63 (9.52%) samples showed evidence of maternal mosaicism for 47, XXX. Bianchi et al.<sup>28</sup> reported on 204 maternal plasma samples in which sex-chromosome aneuploidies were detected by NIPS. All but one of these cases involved the X chromosome. Twelve of 38 samples with 47, XXX reported were discordant with the fetal karyotype. In 2 of 12, there was maternal mosaicism for 47, XXX. In this study, false-positive cases were shown to have a significantly lower mean maternal age (31.6 years) than the true positives (37.8 years) ( $P = 0.008$ ). Other reports of true maternal SCAs as the basis for discordance include Yao et al.,<sup>35</sup> who found that 1 of 33 cases was due to maternal mosaicism for 47, XXX; Reiss et al.,<sup>36</sup> who described a case of 13% maternal mosaicism for monosomy X; and McNamara et al.,<sup>37</sup> who reported on two newly diagnosed women with Turner syndrome mosaicism and one woman whose karyotype was a priori known to be 47, XXX.

Based on these data, it is suggested that a maternal peripheral blood karyotype be performed before an invasive fetal diagnostic procedure. Furthermore, pregnant women who are known



to have an SCA or who have had an organ or bone-marrow transplant should be counseled that NIPS would probably give uninterpretable results.

### Maternal mosaicism for autosomal aneuploidy

Rarely, maternal mosaicism for autosomal aneuploidy is the explanation for the discordant NIPS results. In a case that was reported as monosomy 13, the etiology was maternal mosaic trisomy 8.<sup>26</sup> In a study of rare autosomal trisomies as an explanation for discordant NIPS results, two other cases of maternal mosaic trisomy 8 were identified.<sup>38</sup> Another NIPS discordant result was due to maternal mosaicism for trisomy 18.<sup>39</sup>

### CNVs

Detection of CNVs is an issue mainly for centers that employ genome-wide, as opposed to targeted, sequencing. In a study of genome-wide plasma cfDNA sequencing in over 10,000 Belgian pregnant women, approximately 10% had nonrecurring CNVs exceeding 500 kb in size with no known disease associations.<sup>40</sup> In addition, approximately 0.4% of samples had recurrent CNVs detected that were associated with an increased risk for developmental disorders and late-onset conditions. They pose a dilemma for counseling, particularly when they appear to be maternal in origin. In Belgium, maternal CNVs are reported only if they pertain to highly penetrant single-gene disorders and have clinical utility.<sup>40</sup>

Although early NIPS bioinformatics algorithms called any excess sequence on a target chromosome (13, 18, or 21) “positive,” subsequent analyses have relied on analyzing smaller sections of each chromosome of interest, a technique known as “binning.”<sup>41,42</sup> This has the advantage of reducing false-positive trisomy calls due to benign CNVs that are present in only one part of the chromosome,<sup>43</sup> but has the disadvantage of identifying maternal, and in some cases, extended family CNVs.<sup>44</sup> Thus, there are different genetic counseling implications for a CNV detected by NIPS, in comparison with the other maternal medical conditions discussed in this review.

Snyder et al.<sup>42</sup> specifically investigated whether maternal CNVs could be the basis of false-positive NIPS results. They identified four women who were referred to their center for perinatal genetic counseling due to positive screening for trisomies 18 or 13. Two of the three women who were screen positive for trisomy 18 had duplications of chromosome 18p. Using a database of 112, 021 clinical samples, Zhou et al.<sup>45</sup> found 74 false-positive cases out of 781 positive screening results. In 6 of 74 cases (8.1%), CNV sequencing of maternal leukocytes identified a maternal CNV on chromosomes 13, 18, or 21. None of the variants were associated with any genetic syndromes. Modeling experiments showed a strong correlation between high *z*-scores and an increased size of the maternal CNV. By mathematically subtracting the maternal CNV tag counts, the revised *z*-scores moved into the normal reference range. These authors suggested that the first line of follow-up for a positive NIPS result could be sequencing maternal leukocytes from the buffy coat of the original sample. By doing this, they posit that 10% of false-positive cases could be resolved without performing invasive diagnostic procedures. Rather than sequencing all screen-positive samples, the authors suggest that unusually high *z*-scores for a given fetal fraction are most likely to be the result of maternal CNVs. Similarly, in a reanalysis of

the original 11 false-positive results in the general-risk Comparison of Aneuploidy Risk Evaluations (CARE) study,<sup>46</sup> an updated analytic algorithm determined that maternal CNVs accounted for three of them (one on chromosome 18p and two on chromosome 13q).<sup>43</sup>

In a large study ( $N = 175,393$  clinical samples) reporting on the use of an algorithm to detect subchromosomal events such as 5pdel, 22q11del, 15qdel, 1p36 del, 4pdel, 11qdel, and 8qdel, 55 microdeletions were detected.<sup>47</sup> The most commonly detected deletions were at 22q11.2 ( $n = 32$ ). Of the 32 deletions, 13 were confirmed and 6 were suspected of having at least a maternal component. Of the total 55 CNVs ascertained in this study, 25 (45%) had a suspected or confirmed maternal contribution because the deleted fraction of DNA exceeded the fetal fraction. In another study of 74,938 pregnant women, 6 were noted to be at high risk for maternal deletion of 22q11.2.<sup>48</sup> The knowledge that the mother has a 22q11.2 deletion may affect her care, as multiple reproductive-health issues have been identified in affected adults.<sup>49</sup>

Brison et al.<sup>40</sup> explored the accuracy and clinical utility of reporting out maternal CNVs in a study of 9,882 pregnant women who had NIPS performed by whole-genome shotgun sequencing. Five clinically actionable variants were reported, including haploinsufficiency for *RUNX1*, mosaicism for a segmental deletion of chromosome 13, an unbalanced chromosome translocation between the long arms of chromosomes Xq and 3q, and two cases of interstitial maternal X-chromosome deletions. In the woman with haploinsufficiency for *RUNX1*, NIPS results were significant for a chromosome 21 z-score of 3.3, consistent with a fetal diagnosis of monosomy 21. Visual inspection of the sequencing results showed a localized segmental monosomy suspected to be maternal in origin. The deletion was confirmed by array CGH and shown to be intragenic within the *RUNX1* gene. Haploinsufficiency for *RUNX1* is an autosomal dominant disorder that causes platelet abnormalities and an associated myeloid malignancy.

A clinically significant microdeletion was found in a 39-year-old woman who underwent cfDNA screening for advanced maternal age.<sup>50</sup> She was 5 feet tall and had a history of hypothyroidism and infertility secondary to increased follicle-stimulating hormone levels. cfDNA sequencing data showed markedly lower than normal levels of DNA fragments mapping to the Xp region; no Y-chromosome sequences were present. Maternal CMA analysis demonstrated a 39.5-Mb deletion of Xp22.33 to p11.4, consistent with a maternal diagnosis of variant Turner syndrome. Detection of this deletion had clinical and genetic counseling utility because women with partial deletions of Xp have an increased incidence of cardiovascular complications during pregnancy and therefore require ongoing cardiac surveillance.<sup>51</sup> In addition, there is likely to be a 50% recurrence risk, which may result in a female fetus with signs and symptoms of Turner syndrome, or lethality in a male fetus due to the large size of the deletion.

Flowers et al.<sup>52</sup> described a 38-year-old woman whose fetal sonogram at 20 weeks showed an isolated aberrant right subclavian artery. The woman was not referred for genetic counseling and independently sought NIPS. The results were nonreportable owing to an atypical finding outside the scope of the test. At amniocentesis her male fetus was shown to have a 16.1-Mb duplication of 18q12.1 to q21.1. Follow-up parental samples showed that

20% of the mother's cells demonstrated the same segmental duplication seen in the fetus. While the mother was asymptomatic, the presence of the full duplication in the fetus was associated with a cardiac anomaly. Furthermore, the maternal somatic mosaicism suggested a recurrence risk of 50%.

Important factors to consider regarding the likelihood of a CNV resulting in a false-positive call include the size of the maternal CNV, whether the fetus inherits the CNV, the percent fetal fraction, the depth of sequencing, and the coefficient of variation of sequence reads for the chromosome of interest.<sup>42,52</sup>

Finally, the numerous ethical and genetic counseling issues involved in incidental detection of maternal CNVs were illustrated in a case described by Meschino et al.<sup>44</sup> NIPS was performed for two soft anatomic markers in the fetus; the results were positive for trisomy 21. The report had a commentary section, which mentioned the observation of a duplication outside the Down syndrome critical region at 21q22 that included *APP*. This was important because there was a positive family history of early-onset Alzheimer disease in five paternal relatives spanning two generations. In addition, the pregnant woman had an identical twin sister. A follow-up amniocentesis with prenatal CMA demonstrated a 9.81-Mb duplication at 21q21.1 to q21.3 that included *APP*. Thus, as a result of the NIPS, as many as three people were identified as being at high risk for developing early-onset Alzheimer disease, and the pregnant woman's older child was found to have a 50% risk.

## SUMMARY AND CONCLUSIONS

In this review, the “woman was found.” The results of the many studies and individual cases described here provide a significant evidence base from which to conclude that abnormalities in maternal DNA can lead to false-positive NIPS test results. For this reason it is suggested that the possibility of finding a disorder in a pregnant woman's own DNA be addressed in pretest counseling discussions with her. Furthermore, following a positive NIPS result, additional workup may be considered, including analysis of maternal peripheral leukocytes before an invasive diagnostic fetal procedure is performed (Table 2). The question of whether to report secondary maternal results is far from settled, and different approaches are being taken in different parts of the world. The test is not designed to detect maternal abnormalities, and its clinical utility has not yet been proven. Given the increase in the number of tests being performed on an annual basis, and the rapidity with which NIPS studies are published, further evidence will continue to accrue. This will allow further analysis and updated clinical management recommendations.

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**Table 1**

Considerations with regard to discordant sex-chromosome results

<b>Questions to ask the pregnant woman</b>	<b>Diagnostic considerations</b>
Are you over 35 years old?	Somatic loss of X chromosome due to aging
Have you ever received a transplant of any kind?	Bone-marrow or organ source of Y-chromosomal DNA
Are you significantly shorter or taller than your parents and/or same sex siblings?	Full or partial sex-chromosome aneuploidy (45, X or 47, XXX)
Did you conceive via assisted reproductive technology?	
If yes, was a reason determined for your infertility?	Sex chromosome aneuploidy, X deletion
Has a karyotype ever been performed on you?	Sex-chromosome aneuploidy, X deletion
Did you need egg donation to become pregnant?	Disorder of sexual differentiation (e.g., Swyer syndrome)

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**Table 2****Suggested follow-up management of a maternal incidental finding after NIPS**

1. Obtain detailed maternal medical and family histories.
  2. Consider obtaining a separate maternal blood sample or sequence leukocytes (if available) from the original sample used for NIPS.
  3. Offer referrals as necessary to provide medical care to the mother (e.g., cardiology consultation if 45, X or a 22q11.2 deletion is detected).
  4. Consider whole-body magnetic resonance imaging if a genome-wide pattern of imbalance is detected, suggesting malignancy.
  5. Provide genetic counseling regarding the inheritance patterns and implications of maternal CNVs for the current pregnancy, as well as for prior and future children.
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CNV, copy-number variant; NIPS, noninvasive prenatal screening.

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