Placental, maternal, fetal, and technical origins of false-positive cell-free DNA screening results

Placental, maternal, fetal, and technical origins of false-positive cell-free DNA screening results

Yvette Raymond, BMedSc (Hons); Shavi Fernando, MBBS (Hons); BMedSc (Hons), PhD; Melody Menezes, BSc (Hons) GDipGenetCouns, PhD; Ben W. Mol, MD, PhD, MSc; Andrew McLennan, BSc, MBBS (Hons); Fabricio da Silva Costa, MD, MSc, PhD; Tristan Hardy, MBBS (Hons) MRMed, PhD; Daniel L. Rolnik, MD, MSc, PhD, MPH

The introduction of noninvasive prenatal testing has resulted in substantial reductions to previously accepted false-positive rates of prenatal screening. Despite this, the possibility of false-positive results remains a challenging consideration in clinical practice, particularly considering the increasing uptake of genome-wide noninvasive prenatal testing, and the subsequent increased proportion of high-risk results attributable to various biological events besides fetal aneuploidy. Confined placental mosaicism, whereby chromosome anomalies exclusively affect the placenta, is perhaps the most widely accepted cause of false-positive noninvasive prenatal testing. There remains, however, a substantial degree of ambiguity in the literature pertaining to the clinical ramifications of confined placental mosaicism and its potential association with placental insufficiency, and consequentially adverse pregnancy outcomes including fetal growth restriction. Other causes of false-positive noninvasive prenatal testing include vanishing twin syndrome, in which the cell-free DNA from a demised aneuploidy-affected twin triggers a high-risk result, technical failures, and maternal origins of abnormal cell-free DNA such as uterine fibroids or unrecognized mosaicisms. Most concerningly, maternal malignancies are also a documented cause of false-positive screening results. In this review, we compile what is currently known about the various causes of false-positive noninvasive prenatal testing.

Key words: cancer in pregnancy, cell-free DNA screening, confined placental mosaicism, genome-wide screening, maternal malignancy, noninvasive prenatal testing, placental insufficiency, prenatal screening, rare autosomal trisomy, segmental copy number variation, uterine fibroids, vanishing twin syndrome

Introduction

The detection of placenta-derived cell-free DNA (cfDNA) in maternal plasma by Lo et al, and the subsequent development of prenatal screening technologies to analyze this genetic material revolutionized prenatal screening for fetal chromosome anomalies.[1] The introduction of cfDNA screening, commonly termed noninvasive prenatal testing, provided substantial improvements to the accuracy of prenatal screening.[2] This is of particular importance because invasive diagnostic testing is typically offered as a consequence of high-risk screening results. These diagnostic investigations bring with them small but significant risks of procedure-related pregnancy loss, estimated to be approximately 1 in 500 and 1 in 1000 for chorionic villus (CVS) and amniocentesis, respectively.[3]

Noninvasive prenatal testing has a much lower false-positive rate than alternative methods of prenatal screening, with the rate for targeted screening panels being approximately 0.13%.[2] Comparatively, the next most accurate screening investigation, combined first trimester screening, has a false-positive rate of 3% to 5%.[2,4] However, recent expansions to screening panels to analyze the entire fetal genome (as opposed to exclusively targeting chromosomes 21, 18, or 13), have resulted in an increase in the number of women obtaining high-risk results despite carrying a euploid fetus, prompting the U.S. Food and Drug Administration to issue a statement of caution regarding the interpretation of high-risk screening results.[5–7] In addition, the emerging literature detailing observations of adverse pregnancy outcomes associated with high-risk results even after fetal aneuploidy exclusion has raised interest in the possible cause of these false-positive results, and whether additional interventions are warranted to monitor these pregnancies.[8,9] In this article, we review the scientific literature regarding the documented causes of

From the Department of Obstetrics and Gynaecology, Monash University, Melbourne, Australia (Ms Raymond and Drs Fernando, Mol, and Rolnik); Monash Women's, Monash Health, Melbourne, Australia (Drs Fernando, Mol, and Rolnik); Monash Obstetrics, Melbourne, Australia (Dr Fernando); Monash Ultrasound for Women, Melbourne, Australia (Drs Menezes and Rolnik); Department of Paediatrics, The University of Melbourne, Melbourne, Australia (Dr Menezes); Monash IVF Group, Melbourne, Australia (Drs Menezes and Hardy); Centre for Women's Health Research, The University of Aberdeen, Aberdeen, UK (Dr Mol); Sydney Ultrasound for Women, Sydney, Australia (Dr McLennan); Discipline of Obstetrics, Gynaecology and Neonatology, The University of Sydney, Sydney, Australia (Dr McLennan); Maternal Fetal Medicine Unit, Gold Coast University Hospital, Queensland, Australia (Dr da Silva Costa); School of Medicine and Dentistry, Griffith University, Gold Coast, Queensland, Australia (Dr da Silva Costa); and Repromed Adelaide, Dulwich, Australia (Dr Hardy).

Received Sept. 5, 2023; revised Nov. 5, 2023; accepted Nov. 16, 2023.

The authors report no conflict of interest.

Corresponding author: Yvette Raymond, BMedSc (Hons). Yvette.raymond@monash.edu

0002-9378

https://doi.org/10.1016/j.ajog.2023.11.1240

Click Supplemental Materials and Video under article title in Contents at ajog.org

false-positive noninvasive prenatal testing results.

Confined placental mosaicism

Contribution to false-positive noninvasive prenatal testing results

Confined placental mosaicism (CPM) refers to a situation where the placenta is affected by genetic anomalies in a mosaic distribution, but the fetus is euploid. CPM is classified into types 1, 2, or 3 depending on the cellular lines involved in aneuploidy (Table). Type 1 CPM describes aneuploidy exclusively in the cytotrophoblast, type 2 CPM involves aneuploidy exclusively in the mesenchymal layer, whereas type 3 involves aneuploidy in both the cytotrophoblast and mesenchyme.[10]

CPM is the most widely-recognized cause of false-positive noninvasive prenatal testing (NIPT) results, because the cfDNA analyzed is of placental, not fetal, origin.[11] Given that most nonmosaic fetal trisomies are incompatible with life (other than the common trisomies of chromosomes 21, 18, and 13 which have well-documented phenotypical syndromes), it is accepted that these anomalies are more likely confined to placental tissues when detected by NIPT after 10 weeks' gestation.[12,13] In addition, CPM is not a rare phenomenon, estimated to affect up to 2% of all pregnancies.[14]

A recent meta-analysis by Acreman et al investigating the diagnostic accuracy of NIPT for rare autosomal trisomies (RATs, defined as any autosomal trisomy excluding 21, 18, or 13) revealed that approximately 90% of fetuses screened as high-risk for these anomalies are unaffected by trisomy, with these results potentially being attributable to CPM instead.[15] Van Opstal et al observed CPM in all (10/10) term placentas biopsied from pregnancies with a false-positive trisomy screening result; however, NIPT in these women was performed following a high-risk first trimester combined screening test result, which includes placental biomarkers in the risk algorithm.[16] Because trisomy may disturb placental functioning and thereby derange these biomarkers, the frequency of CPM observed in this study may not be comparable to that in pregnancies receiving false-positive results when NIPT is used as a first-line investigation.[17] It is worth noting that though this review will focus on CPM involving trisomy, CPM of segmental copy number variants may also generate false-positive NIPT results, although documentation of this phenomenon occurring is significantly less robust.

Mechanism of aneuploidy and degree of placental involvement

Aneuploidy involved in CPM may arise from either meiotic or mitotic errors (Figure 1). In mitotic errors, there is nondisjunction during mitosis causing uneven division of chromosomes into daughter cells, with one cell becoming trisomic and the other monosomic. This can theoretically occur at any point during development. Therefore, CPM resulting from these errors may feature either large proportions of mosaicism from early nondisjunction events persisting throughout subsequent cell cycles, or only small portions resulting from mitotic errors later in development. Type 1 and type 2 CPM usually result from mitotic errors occurring after differentiation of the cytotrophoblast and mesenchymal layers.[10,18] NIPT analyzes DNA arising only from the cytotrophoblast, and therefore type 2 CPM is unlikely to generate a high-risk NIPT result (Figure 2).[19]

Meiotic errors occur when there is a nondisjunction event in meiosis causing 1 gamete to receive 2 copies of the same chromosome, resulting in a trisomic zygote following fertilization. For aneuploidy of this origin to cause CPM, trisomy rescue must occur early in embryonic life after differentiation of the inner cell mass from the trophectoderm and, subsequently, uniparental disomy is a common occurrence among these cases.[18] Placentas affected by meiotic errors generally have a high proportion of trisomic cells because the error is present from fertilization and will persist through all cellular divisions unless rescue occurs. These contribute to a high proportion of type 3 CPM, the most concerning subtype.[10,18] Previous studies have revealed association between the percentage of mosaic cells and the extent of placental functional impairment.[20–22]

Patterns of placental mosaicism by respective chromosomal trisomy

Several studies have sought to establish patterns regarding the chromosome involved in CPM, and the mechanism by which trisomy likely occurred. This is of clinical importance because the mechanism of trisomy may predict the proportion of mosaicism in the

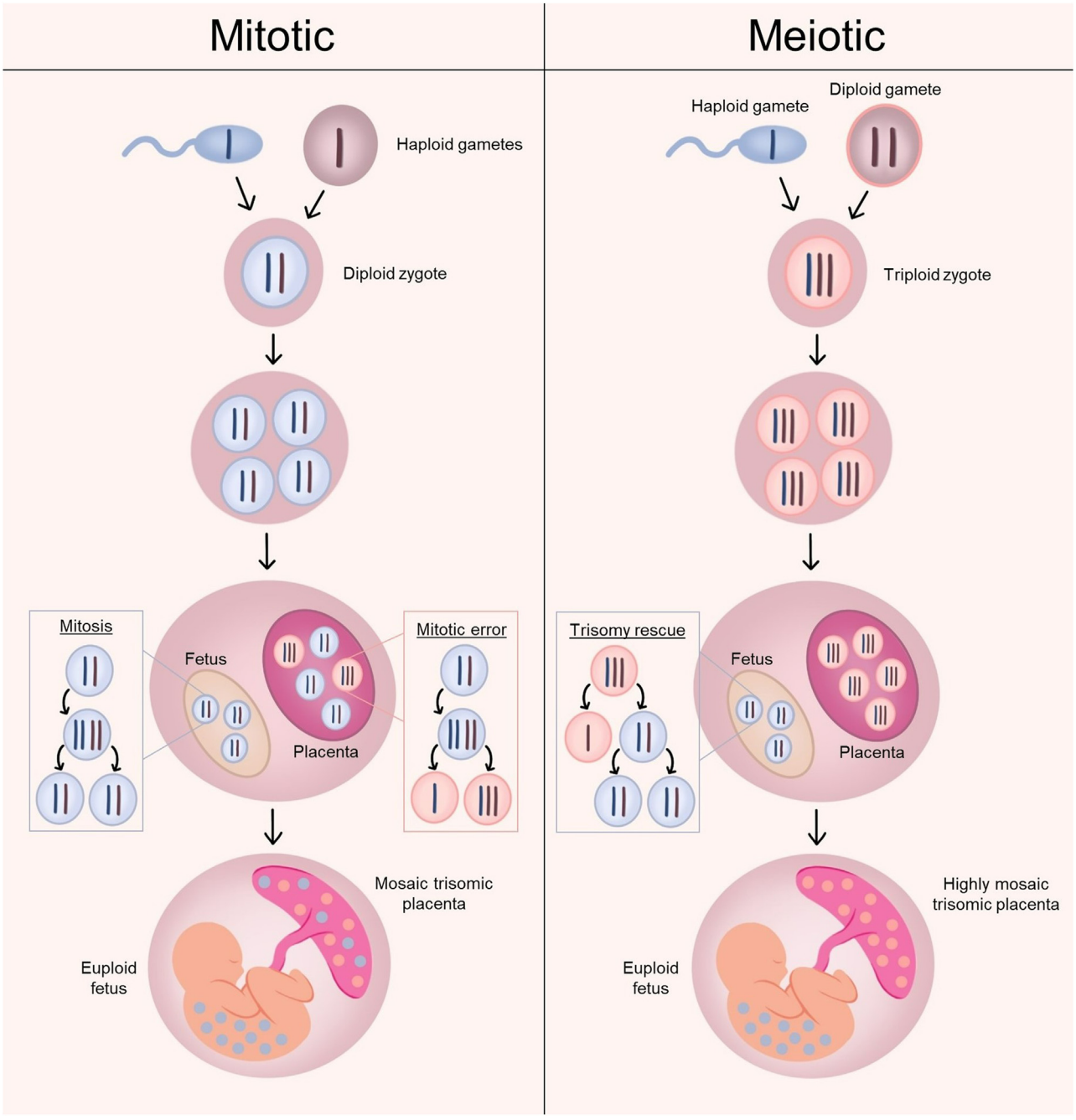
TABLE
Characteristics of different types of confined placental mosaicism

Characteristics	Type 1	Type 2	Type 3
Layers involved	Cytotrophoblast	Mesenchyme	Cytotrophoblast and mesenchyme
Likely origin	Mitotic errors		Meiotic errors
Commonly involved chromosomes	2, 3, 7, 8, 10, 12		14, 15, 16, 22
Expected NIPT result	High-risk	Low-risk	High-risk

NIPT, noninvasive prenatal testing.

Raymond. Origins of false-positive cell-free DNA screening results. Am J Obstet Gynecol 2023.

FIGURE 1
Mitotic vs meiotic development of confined placental mosaicism



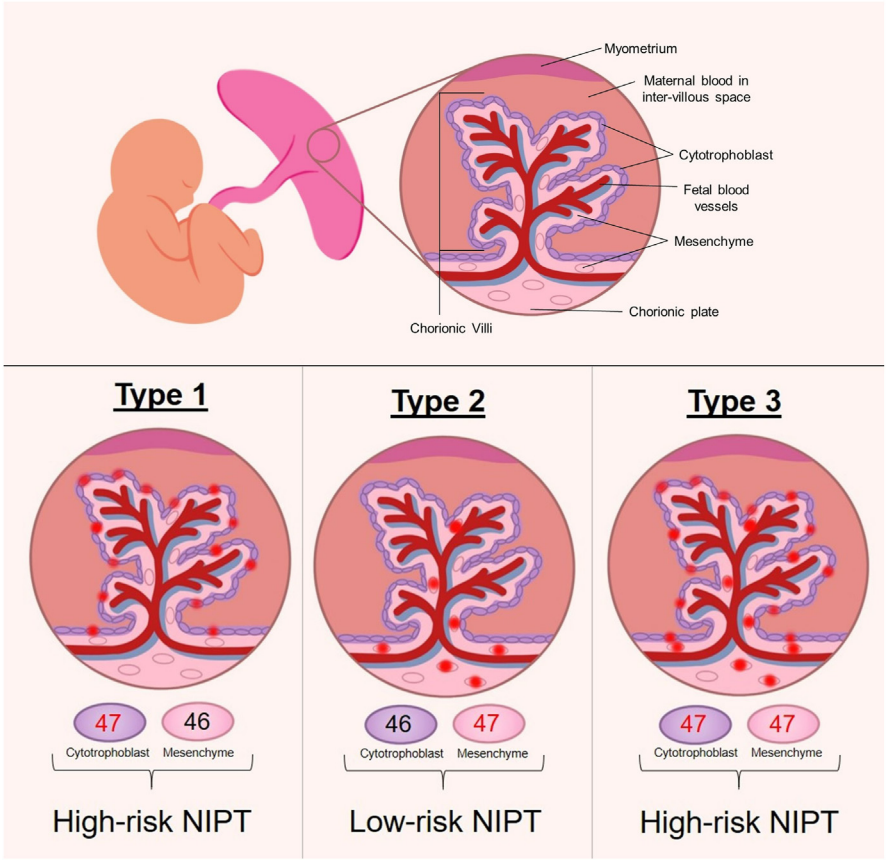
Raymond. Origins of false-positive cell-free DNA screening results. Am J Obstet Gynecol 2023.

placenta, and in turn the extent of functional impairment. CPM involving trisomy of chromosomes 2, 3, 7, 8, 10, or 12 tends to arise from mitotic nondisjunction, whereas trisomies of chromosomes 14, 15, 16, or 22 are more likely resultant from meiotic errors.[18,23,24] These represent trends rather than definitive mechanisms and there is a degree of ambiguity in the literature. An exception to this is trisomy 16, which almost exclusively results from meiotic errors causing type 3 CPM.[25]

Confined placental mosaicism and placental insufficiency

Given the strong correlation between type 3 CPM and T16, it is unsurprising that CPM involving this trisomy has been strongly correlated with adverse pregnancy outcomes including fetal

FIGURE 2
Confined placental mosaicism types involving trisomy and expected NIPT result



NIPT, noninvasive prenatal testing.

Raymond. Origins of false-positive cell-free DNA screening results. Am J Obstet Gynecol 2023.

growth restriction and stillbirth as a result of placental insufficiency.[26–28] For other trisomies and chromosome anomalies, predicting the extent of placental involvement and in turn the likelihood of placental insufficiency is more complex.

A 2022 meta-analysis found that CPM not involving T16 corresponded to a 3-fold increase in the risk of a small for gestational age infant, compared with a control cohort with euploid placentas.[28] The results of this analysis were generated by findings of CPM on CVS, and did not include cases of suspected CPM based on NIPT (high-risk NIPT with a typical fetal genome revealed by amniocentesis), unless direct cytogenetic analyses on placental tissue were also conducted. This is important because NIPT is theorized to be more sensitive than CVS in the detection of CPM, given abnormal cfDNA may be released into maternal plasma by even small areas of mosaicism, compared with CVS in which low-level mosaicism may be missed if not involved in the biopsy site.[29] Therefore, the results of this meta-analysis may not be applicable in the clinical setting of assumed CPM based on NIPT findings alone, because they represent the risks associated with CPM involving a higher proportion of trisomic cells than what may be observed in NIPT-detected cases.

A small number of studies have sought to investigate the outcomes of pregnancies with suspected CPM based on NIPT results; however, the findings have been limited by incomplete follow-up introducing the possibility of attrition bias, as well as failure to confirm CPM with analysis of placental tissue after confirmation of an euploid fetus.[6,8,9] Given that CPM is not the only cause of false-positive NIPT, failure to confirm placental aneuploidy may overestimate the risk profile of NIPT-detected CPM by accounting for complications resulting from other biological explanations (such as uterine fibroids), or inversely, could undermine the associated risks by diluting results with observations from euploid placentas.[30]

Persistence of placental mosaicism throughout pregnancy

Another consideration for suspected CPM following a high-risk NIPT result is the possibility of resolution, either by trisomic rescue or by selective advantage of normal cell line growth diluting the aneuploidy-affected site with increasing gestation. Prior studies in preimplantation genetics have revealed that there is selection during the blastocyst stage against proliferation of cells affected by chromosomal abnormalities, and it is conceivable that this process may persist into later development.[31] In a study investigating placental karyotypes of pregnancies with high-risk NIPT results, Van Opstal et al revealed evidence of multiple trisomic rescue events, when previously this was considered to be confined to a single occurrence during early embryogenesis.[16] By these mechanisms, it is plausible that abnormal placental cell lineages are diluted and eliminated with increasing gestation, culminating in a mostly euploid placenta at term. It is possible that were NIPT repeated at a later gestation, initially high-risk results may resolve to low-risk with increasing development of the placenta. The proportion of pregnancies with suspected CPM, based on first trimester NIPT findings, that have placentas affected by aneuploidy at the end of pregnancy is currently unknown and is another area warranting further research.[16]

Vanishing twin syndrome

Prevalence of vanishing twin syndrome

Vanishing twin syndrome (VTS) denotes early demise of a twin in a multiple

pregnancy, most often during the first trimester, with another fetus remaining viable. The detection of VTS has increased with more frequent use of early pregnancy ultrasonography and the incidence has also increased with rising rates of assisted reproductive technology and delayed childbearing contributing to a higher frequency of multiple pregnancy conceptions.[32] Currently, VTS is thought to occur in 15% to 35% of twin pregnancies and in up to 10% of all IVF pregnancies resulting in a singleton birth.[33,34] Although VTS is observed more frequently in IVF pregnancies, this may in part be due to these pregnancies generally having earlier ultrasounds to confirm pregnancy viability, and the frequency in spontaneous pregnancies is potentially similar.[33]

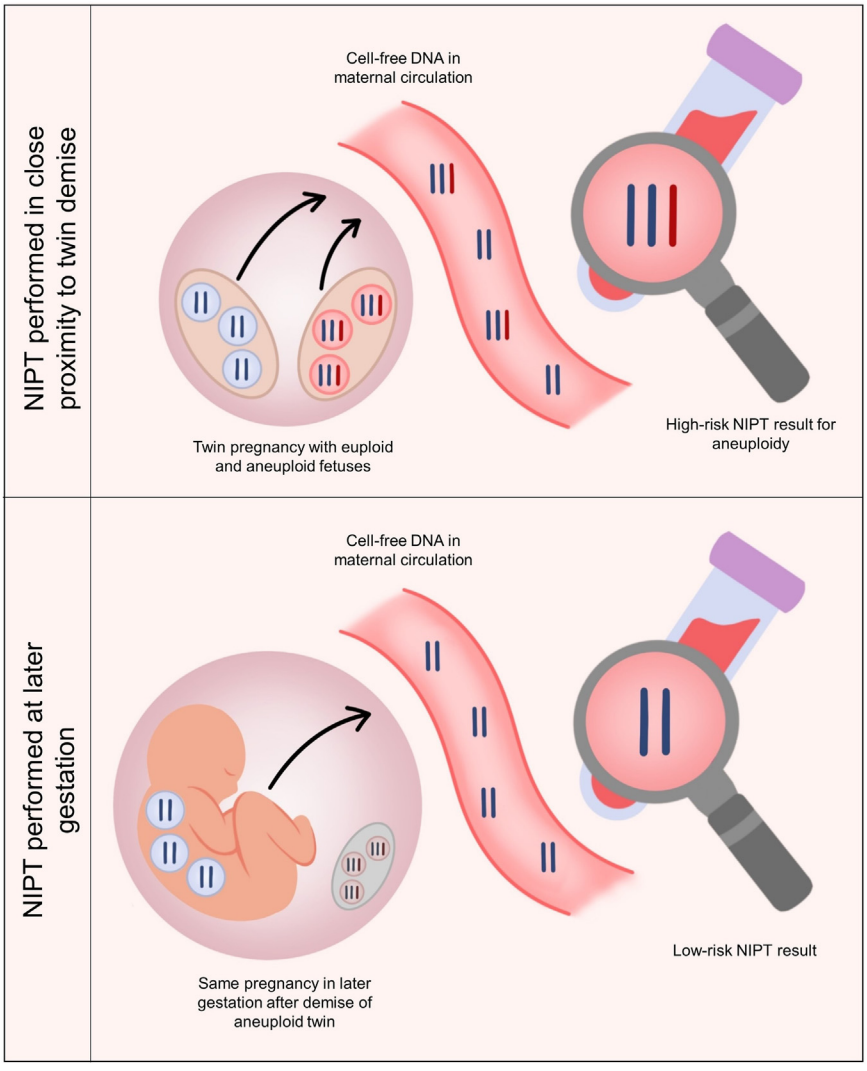
The classification of VTS is made difficult by the lack of clinical indicators, with the only recognizable symptom being early pregnancy bleeding, although this presentation is nonspecific and only occurs in 25% of VTS cases.[35] Routine first-trimester ultrasonography may miss VTS if not performed before 7 weeks gestation because early embryo demise commonly results in resorption of the products of conception.[33]

Vanishing Twin Syndrome and noninvasive prenatal testing

Fetal aneuploidy is a major cause of twin demise in VTS and consequently may cause an abnormal NIPT result that is discordant with the genome of the surviving fetus (Figure 3). A demised twin may release cfDNA for up to 15 weeks post demise; however, the likelihood of this cfDNA being detected by NIPT decreases with time, such that co-twin demise is an uncommon cause of false-positive results received after 14 weeks' gestations.[36]

A 2013 study found that VTS accounted for 15% of false-positive NIPT results which is likely to be an underestimate, given the inherent difficulties in identifying VTS.[33,37] Furthermore, this study was conducted before the introduction of genome-wide screening panels and given the lethality associated with RATs and many other rare chromosomal anomalies, it is likely that the frequency of false-positive NIPT results generated by VTS is even greater today.[12] The most frequent implication of VTS on NIPT is fetal sex discordance, whereby the Y chromosome from a demised male twin is detected in a pregnancy carrying a phenotypically female fetus.[11] Single nucleotide polymorphism (SNP)–based NIPT platforms are able to distinguish the genome of a demised twin but counting–based NIPT methods (including all currently available genome-wide screening platforms) cannot.[32]

FIGURE 3
Mechanism of false-positive NIPT result by demise of aneuploidy-affected twin



NIPT, noninvasive prenatal testing.

Raymond. Origins of false-positive cell-free DNA screening results. Am J Obstet Gynecol 2023.

The evidence regarding the clinical consequences of VTS is conflicted. This is partially attributable to inconsistency in the literature regarding the definition of VTS. Studies that include twin losses beyond the first trimester reveal an association between increasing gestational age at vanishing and worsening pregnancy outcomes.[38,39] These late losses represent the minority of cases; however, with most instances of VTS occurring early in pregnancy, including those associated with false-positive NIPT results. Adverse outcomes for the

surviving twin in these instances are far less frequent.[33,40]

Maternal origins of false-positive noninvasive prenatal testing

Maternal mosaicism

Most commercially available NIPT platforms (excluding those which utilize SNP-based methods) are unable to distinguish between maternal and placental sources of cfDNA; thus, anomalies of maternal origin may trigger false-positive NIPT screening results (Figure 4).[32] A 2017 study by Zhou et al investigating the causes of false-positive screening results for trisomies 21, 18, and 13 revealed that 8.1% were attributable to maternal segmental duplications affecting the flagged chromosome.[41] Benign copy number variants exceeding 500 Kb are thought to be present in as many as 10% of the general population, which could increase false-positive NIPT results, although most genome-wide screening panels are only able to detect those exceeding 7 Mb.[42] More rarely, unrecognized mosaic maternal autosomal trisomies may generate a high-risk result, with several documented instances of mosaic T8 and T18 revealed by NIPT in asymptomatic pregnant women.[43]

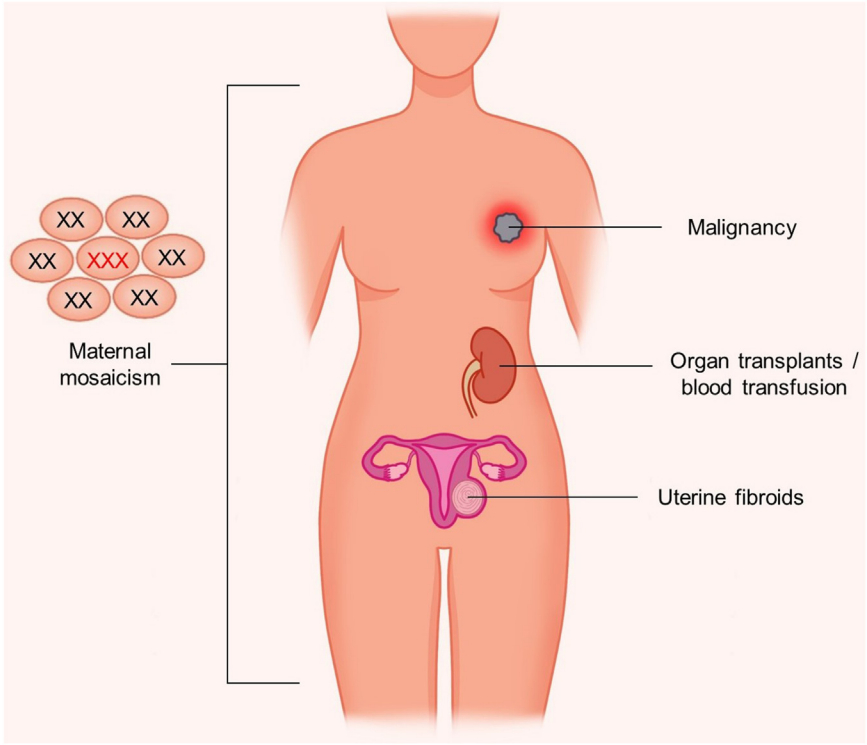
Sex chromosome aneuploidies (SCAs) are the most common screening results found to be attributable to maternal mosaicism, with 1 study finding that 8.6% of all high-risk SCA results were due to an abnormal X chromosome karyotype in the mother.[44] Many SCAs, particularly triple X, present no distinct phenotypical features, especially when mosaic. Triple X is the most common chromosomal anomaly in females, with a birth incidence of 1 in 1000; however, only 10% are diagnosed.[45] Another cause of high-risk SCA results attributable to a maternal origin is the age-related loss of an X chromosome in maternal blood cells, resulting in a positive monosomy X screen for a euploid fetus.[42,46] Russell et al[47] found that after the age of 25 years, X chromosome loss increased with increasing age. Finally, maternal blood transfusions or organ donations from a male donor may also cause discordant assessment of the sex chromosomes. More rarely, a donor may have a mosaic autosomal chromosome anomaly; however, in the case of a blood transfusion this is only likely to cause a false result for NIPT if performed within 4 weeks of the transfusion.[48]

Uterine fibroids

Uterine leiomyomas (fibroids), are the most common female pelvic tumors and are present in an estimated 11% of pregnant women.[49] Fibroids are characteristically monoclonal, comprising cells with the same genome irrespective of fibroid size or plurality. Approximately half of all fibroids, most notably large fibroids, possess karyotypically detectable chromosome anomalies.[30] The genetic alterations observed in fibroids tend to be similar, affecting genes responsible for regulating cell growth, hormonal responses, and apoptosis.

Abnormal cfDNA originating from fibroids provide another cause of false-positive NIPT results because genetic anomalies confined to fibroid cells may be identified during genome-wide NIPT screening.[50] A 2022 study by Scott et al found that the risk of receiving a false-positive NIPT result was significantly higher in women with fibroids and the risk ratio significantly increased with increasing number and total volume of fibroids, although this was only true for results indicating rare chromosome anomalies such as RATs, segmental copy number changes or multiple anomalies, and not for SCAs or trisomies 21, 18, or 13. It is however worth noting that most women with fibroids will not receive a discordant NIPT result, with the same study observing that the absolute false-positive rate of NIPT among women with fibroids remained low, being only 2%.[30] Still, given that the false-positive rate of NIPT among women without fibroids is approximately 0.5%, it is

FIGURE 4
Maternal origins of false-positive noninvasive prenatal testing results



Example of triple X is given for maternal mosaicism.

Raymond. Origins of false-positive cell-free DNA screening results. Am J Obstet Gynecol 2023.

reasonable that a higher clinical index of suspicion of a false-positive result be employed for women with fibroids, particularly when there are no other indications of fetal aneuploidy, or NIPT indicates an anomaly known to be associated with fibroids, such as chromosome 7q deletions.[30,51]

Maternal malignancies

Undoubtedly, the most alarming consideration of false-positive NIPT results has been the discovery that cfDNA released by maternal malignancies may be a causative agent. Most circulating cfDNA in the plasma of pregnant women is derived from maternal tissues (~85%–90%) rather than placental tissue, and this concentration increases substantially in the presence of cfDNA-secreting malignancies.[52,53] A 2014 study by Bettegowda et al[53] found that among nonpregnant patients with known metastatic and localized cancers, 80% and 50%, respectively were found to have abnormal cfDNA on plasma analysis. The genomic profile of cfDNA released by malignant tumors tends to be grossly abnormal, with multiple chromosomal aberrations.[54,55] This abnormal circulating cfDNA may trigger a high-risk genome-wide NIPT result, or may cause test failure for targeted screening panels due to failure of the bioinformatic algorithm.[52,56]

Overall, false-positive NIPT results due to maternal cancer are rare; these are thought to occur once in every 10,000 screening tests performed.[55] Given the inherent challenges in identifying malignancy in pregnancy due to often benign symptoms being misattributed to those of normal pregnancy, the capacity of NIPT to identify cancers has been regarded by many as having potential, especially in light of ongoing developments of cfDNA screening tools for malignancies in oncology.[54,57] In the TRIDENT-2 study, among 231,896 screening results, 51 were interpreted as being suspicious of malignancy, from which maternal cancers were subsequently detected in 18 (35%), most of which were hematopoietic in origin.[54] The overall cancer incidence in the study was 0.0096%, which given the prevalence of malignancy in pregnancy is approximately 0.1%, suggests that 85% to 90% of cancers in this cohort were not detected.[52] In addition, it remains unknown whether earlier detection of malignancy via NIPT translates to better clinical outcomes. Therefore, further development and validation seems warranted before adoption of NIPT as a screening tool for maternal cancers is seriously considered.[54]

In the absence of universal guidelines, the management of false-positive NIPT results that are potentially suggestive of maternal malignancy, particularly those involving multiple chromosome anomalies, poses a significant challenge to clinicians. A survey of over 300 certified genetic counselors found that whereas 77% indicated they would inform patients of the implications of these findings when detected, over half would feel uncomfortable or very uncomfortable counseling families with these results.[58] Although precise management pathways remain unclear, there is general consensus that given the potentially grave consequences of ignoring these results, further investigations are warranted unless patient preference dictates otherwise.[58,59] Several proposals for the workup of these patients have been published, with suggested investigations generally encompassing medical history, clinical examination, complete blood panels, and weighted consideration of imaging studies such as X-ray or positron emission tomography scans.[54,59,60] It bares mentioning, however, that these management protocols are largely formulated on opinion, and to date there have been no concise guidelines put forward by any professional obstetrical organizations.[61]

Other causes and unexplained false-positive noninvasive prenatal testing results

In addition to the biological reasons discussed above, there are technical causes of false-positive NIPT. As is the case with all laboratory investigations, inaccuracies may result from rare technical errors.[48] A major technical parameter regarding the accuracy of NIPT is "fetal" fraction, the proportion of pregnancy-derived cfDNA in the maternal plasma. Fetal fraction is influenced by various factors, including gestational age, placental mass, and maternal body mass index (BMI). High maternal BMI is associated with lower fetal fraction values due to a dilution effect caused by increased circulating cfDNA in obese women, resulting in an increased frequency of failed or "no-call" NIPT results.[62] Prior studies have found that low fetal fraction values, generally accepted as those <4%, are associated with an increased frequency of inaccurate NIPT results. False-negative results are more common in these instances than false-positive ones.[63–67] False-positive results may also be attributable to random probability because the cutoff for a high-risk result is set at a z-score of 3. Each respective chromosome screened is subject to the same potential error. Thereby the likelihood of false-positive results attributable to probability alone is much higher in the context of genome-wide screening. Given that these errors can only be suspected by exclusion of other biologic causes, quantifying their attribution to false-positive results remains difficult.[48]

Finally, the inherent difficulties in identifying various causes of false-positive results, including CPM and VTS, means that a significant proportion of inaccuracies receive no explanation. A 2017 systematic review by Hartwig et al found no obvious biological or technical reason in 67% of cases of discordant NIPT results, highlighting the need for further research into these instances.[68]

Conclusion and future directions

Although the introduction of NIPT has undoubtedly offered improvements to prenatal screening practices, the ramifications of discordant results warrant attention, particularly with the expansion of screening panels and subsequent increases in false-positive results. Although there are several documented causes of false-positive NIPT results as outlined in this review, there is insufficient evidence available to quantify the contribution of each to the overall number of discordant results. Further research is required to understand both

the frequency and risk profile of CPM in the context of NIPT, as well as other causes of false-positive results, including VTS and maternal origins, to guide the development of management protocols. Consequently, the authors of this review are currently coordinating a prospective cohort study of women with false-positive NIPT which aims to understand what proportion are attributable to CPM vs other biological causes and examine the outcomes of these pregnancies. While awaiting further evidence, it is important that families opting for NIPT are informed of the limitations of the screening test, including the possibility of false-positive results, to facilitate informed choice. ■

REFERENCES

1. Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485–7.

2. Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol 2017;50:302–14.

3. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 2015;45:16–26.

4. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn 2011;31:7–15.

5. Wise J. Non-invasive prenatal screening tests may give false results, warns US regulator. BMJ 2022;377:o1031.

6. Van Opstal D, Van Maarle MC, Lichtenbelt K, et al. Origin and clinical relevance of chromosomal aberrations other than the common trisomies detected by genome-wide NIPS: results of the TRIDENT study. Genet Med 2018;20:480–5.

7. Di Renzo GC, Bartha JL, Bilardo CM. Expanding the indications for cell-free DNA in the maternal circulation: clinical considerations and implications. Am J Obstet Gynecol 2019;220:537–42.

8. van Prooyen Schuurman L, Sistermans EA, Van Opstal D, et al. Clinical impact of additional findings detected by genome-wide non-invasive prenatal testing: follow-up results of the TRIDENT-2 study. Am J Hum Genet 2022;109:1140–52.

9. Pertile MD, Halks-Miller M, Flowers N, et al. Rare autosomal trisomies, revealed by maternal plasma DNA sequencing, suggest increased risk of feto-placental disease. Sci Transl Med 2017;9:eaan1240.

10. Toutain J, Goutte-Gattat D, Horovitz J, Saura R. Confined placental mosaicism revisited: impact on pregnancy characteristics and outcome. PLoS One 2018;13:e0195905.

11. Bianchi DW, Chiu RWK. Sequencing of circulating cell-free DNA during pregnancy. N Engl J Med 2018;379:464–73.

12. Rolnik DL, Carvalho MH, Catelani AL, et al. [Cytogenetic analysis of material from spontaneous abortion]. Rev Assoc Med Bras (1992) 2010;56:681–3.

13. Benn P, Grati FR. Aneuploidy in first trimester chorionic villi and spontaneous abortions: windows into the origin and fate of aneuploidy through embryonic and fetal development. Prenat Diagn 2021;41:519–24.

14. Malvestiti F, Agrati C, Grimi B, et al. Interpreting mosaicism in chorionic villi: results of a monocentric series of 1001 mosaics in chorionic villi with follow-up amniocentesis. Prenat Diagn 2015;35:1117–27.

15. Acreman ML, Bussolaro S, Raymond YC, Fantasia I, Rolnik DL, Da Silva Costa F. The predictive value of prenatal cell-free DNA testing for rare autosomal trisomies: a systematic review and meta-analysis. Am J Obstet Gynecol 2023;228:292–305.e6.

16. Van Opstal D, Diderich KEM, Joosten M, et al. Unexpected finding of uniparental disomy mosaicism in term placentas: is it a common feature in trisomic placentas? Prenat Diagn 2018;38:911–9.

17. Eckmann-Scholz C, Mallek J, von Kaisenberg CSv, et al. Chromosomal mosaicisms in prenatal diagnosis: correlation with first trimester screening and clinical outcome. J Perinat Med 2012;40:215–23.

18. Robinson WP, Barrett IJ, Bernard L, et al. Meiotic origin of trisomy in confined placental mosaicism is correlated with presence of fetal uniparental disomy, high levels of trisomy in trophoblast, and increased risk of fetal intrauterine growth restriction. Am J Hum Genet 1997;60:917–27.

19. Van Opstal D, Srebniak MI. Cytogenetic confirmation of a positive NIPT result: evidence-based choice between chorionic villus sampling and amniocentesis depending on chromosome aberration. Expert Rev Mol Diagn 2016;16:513–20.

20. Kalousek DK, Howard-Peebles PN, Olson SB, et al. Confirmation of CVS mosaicism in term placentae and high frequency of intrauterine growth retardation association with confined placental mosaicism. Prenat Diagn 1991;11:743–50.

21. Wolstenholme J, Rooney DE, Davison EV. Confined placental mosaicism, IUGR, and adverse pregnancy outcome: a controlled retrospective U.K. collaborative survey. Prenat Diagn 1994;14:345–61.

22. Grau Madsen S, Uldbjerg N, Sunde L, Becher N. Danish Fetal Medicine Study Group, Danish Clinical Genetics Study Group. Prognosis for pregnancies with trisomy 16 confined to the placenta: a Danish cohort study. Prenat Diagn 2018;38:1103–10.

23. Hahnemann JM, Vejerslev LO. European collaborative research on mosaicism in CVS (EUCROMIC)—fetal and extrafetal cell lineages in 192 gestations with CVS mosaicism involving single autosomal trisomy. Am J Med Genet 1997;70:179–87.

24. Wolstenholme J. Confined placental mosaicism for trisomies 2, 3, 7, 8, 9, 16, and 22: their incidence, likely origins, and mechanisms for cell lineage compartmentalization. Prenat Diagn 1996;16:511–24.

25. Xiang J, Li R, He J, et al. Clinical impacts of genome-wide noninvasive prenatal testing for rare autosomal trisomy. Am J Obstet Gynecol MFM 2023;5:100790.

26. Grati FR, Ferreira J, Benn P, et al. Outcomes in pregnancies with a confined placental mosaicism and implications for prenatal screening using cell-free DNA. Genet Med 2020;22:309–16.

27. Brandenburg H, Los FJ, In't Veld P. Clinical significance of placenta-confined nonmosaic trisomy 16. Am J Obstet Gynecol 1996;174:1663–4.

28. Spinillo SL, Farina A, Sotiriadis A, et al. Pregnancy outcome of confined placental mosaicism: meta-analysis of cohort studies. Am J Obstet Gynecol 2022;227:714–27.e1.

29. Van Opstal D, Eggenhuizen GM, Joosten M, et al. Noninvasive prenatal testing as compared to chorionic villus sampling is more sensitive for the detection of confined placental mosaicism involving the cytotrophoblast. Prenat Diagn 2020;40:1338–42.

30. Scott F, Menezes M, Smet ME, et al. Influence of fibroids on cell-free DNA screening accuracy. Ultrasound Obstet Gynecol 2022;59:114–9.

31. Eggenhuizen GM, Go A, Koster MPH, Baart EB, Galjaard RJ. Confined placental mosaicism and the association with pregnancy outcome and fetal growth: a review of the literature. Hum Reprod Update 2021;27:885–903.

32. Curnow KJ, Wilkins-Haug L, Ryan A, et al. Detection of triploid, molar, and vanishing twin pregnancies by a single-nucleotide polymorphism–based noninvasive prenatal test. Am J Obstet Gynecol 2015;212:79.e1–9.

33. Batsry L, Yinon Y. The vanishing twin: diagnosis and implications. Best Pract Res Clin Obstet Gynaecol 2022;84:66–75.

34. Pinborg A, Lidegaard O, la Cour Freiesleben N, Andersen AN. Consequences of vanishing twins in IVF/ICSI pregnancies. Hum Reprod 2005;20:2821–9.

35. McNamara HC, Kane SC, Craig JM, Short RV, Umstad MP. A review of the mechanisms and evidence for typical and atypical twinning. Am J Obstet Gynecol 2016;214:172–91.

36. Balaguer N, Mateu-Brull E, Serra V, Simón C, Milán M. Should vanishing twin pregnancies be systematically excluded from cell-free fetal DNA testing? Prenat Diagn 2021;41:1241–8.

37. Futch T, Spinosa J, Bhatt S, de Feo E, Rava RP, Sehnert AJ. Initial clinical laboratory

experience in noninvasive prenatal testing for fetal aneuploidy from maternal plasma DNA samples. Prenat Diagn 2013;33:569–74.
38. Zhou L, Gao X, Wu Y, Zhang Z. Analysis of pregnancy outcomes for survivors of the vanishing twin syndrome after in vitro fertilization and embryo transfer. Eur J Obstet Gynecol Reprod Biol 2016;203:35–9.
39. Weitzner O, Barrett J, Murphy KE, et al. National and international guidelines on the management of twin pregnancies: a comparative review. Am J Obstet Gynecol 2023;229:577–98.
40. Li YX, Sun TZ, Lv MQ, et al. Is vanishing twin syndrome associated with adverse obstetric outcomes of ART singletons? A systematic review and meta-analysis. J Assist Reprod Genet 2020;37:2783–96.
41. Zhou X, Sui L, Xu Y, et al. Contribution of maternal copy number variations to false-positive fetal trisomies detected by noninvasive prenatal testing. Prenat Diagn 2017;37:318–22.
42. Brison N, Van Den Bogaert K, Dehaspe L, et al. Accuracy and clinical value of maternal incidental findings during noninvasive prenatal testing for fetal aneuploidies. Genet Med 2017;19:306–13.
43. Bianchi DW. Cherchez la femme: maternal incidental findings can explain discordant prenatal cell-free DNA sequencing results. Genet Med 2018;20:910–7.
44. Wang Y, Chen Y, Tian F, et al. Maternal mosaicism is a significant contributor to discordant sex chromosomal aneuploidies associated with noninvasive prenatal testing. Clin Chem 2014;60:251–9.
45. Yao H, Zhang L, Zhang H, et al. Noninvasive prenatal genetic testing for fetal aneuploidy detects maternal trisomy X. Prenat Diagn 2012;32:1114–6.
46. Sandow R, Scott FP, Schluter PJ, et al. Increasing maternal age is not a significant cause of false-positive results for monosomy X in non-invasive prenatal testing. Prenat Diagn 2020;40:1466–73.
47. Russell LM, Strike P, Browne CE, Jacobs PA. X chromosome loss and ageing. Cytogenet Genome Res 2007;116:181–5.
48. Samura O, Okamoto A. Causes of aberrant non-invasive prenatal testing for aneuploidy: a systematic review. Taiwan J Obstet Gynecol 2020;59:16–20.
49. Coutinho LM, Assis WA, Spagnuolo-Souza A, Reis FM. Uterine fibroids and pregnancy: how do they affect each other? Reprod Sci 2022;29:2145–51.
50. Dharajiya NG, Namba A, Horiuchi I, et al. Uterine leiomyoma confounding a noninvasive prenatal test result. Prenat Diagn 2015;35:990–3.
51. Vanharanta S, Wortham NC, Laiho P, et al. 7q deletion mapping and expression profiling in uterine fibroids. Oncogene 2005;24:6545–54.
52. Lannoo L, Lenaerts L, Van Den Bogaert K, et al. Non-invasive prenatal testing suggesting a maternal malignancy: what do we tell the prospective parents in Belgium? Prenat Diagn 2021;41:1264–72.
53. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra24.
54. Heesterbeek CJ, Aukema SM, Galjaard R-JH, et al. Noninvasive prenatal test results indicative of maternal malignancies: a nationwide genetic and clinical follow-up study. J Clin Oncol 2022;40:2426–35.
55. Bianchi DW, Chudova D, Sehnert AJ, et al. Noninvasive prenatal testing and incidental detection of occult maternal malignancies. JAMA 2015;314:162–9.
56. Lenaerts L, Brison N, Maggen C, et al. Comprehensive genome-wide analysis of routine non-invasive test data allows cancer prediction: a single-center retrospective analysis of over 85,000 pregnancies. EClinicalmedicine 2021;35:100856.
57. Cisneros-Villanueva M, Hidalgo-Pérez L, Rios-Romero M, et al. Cell-free DNA analysis in current cancer clinical trials: a review. Br J Cancer 2022;126:391–400.
58. Giles ME, Murphy L, Krstić N, Sullivan C, Hashmi SS, Stevens B. Prenatal cfDNA screening results indicative of maternal neoplasm: survey of current practice and management needs. Prenat Diagn 2017;37:126–32.
59. Dow E, Freimund A, Smith K, et al. Cancer diagnoses following abnormal noninvasive prenatal testing: a case series, literature review, and proposed management model. JCO Precis Oncol 2021;5:1001–12.
60. Benn P, Plon SE, Bianchi DW. Current controversies in prenatal diagnosis 2: NIPT results suggesting maternal cancer should always be disclosed. Prenat Diagn 2019;39:339–43.
61. Turriff AE, Annunziata CM, Bianchi DW. Prenatal DNA sequencing for fetal aneuploidy also detects maternal cancer: importance of timely workup and management in pregnant women. J Clin Oncol 2022;40:2398–401.
62. Rolnik DL, Yong Y, Lee TJ, Tse C, McLennan AC, da Silva Costa F. Influence of body mass index on fetal fraction increase with gestation and cell-free DNA test failure. Obstet Gynecol 2018;132:436–43.
63. Scott FP, Menezes M, Palma-Dias R, et al. Factors affecting cell-free DNA fetal fraction and the consequences for test accuracy. J Matern Fetal Neonatal Med 2018;31:1865–72.
64. Palomaki GE, Kloza EM, Lambert-Messerlian GM, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genet Med 2011;13:913–20.
65. Wright D, Wright A, Nicolaides KH. A unified approach to risk assessment for fetal aneuploidies. Ultrasound Obstet Gynecol 2015;45:48–54.
66. Ashoor G, Syngelaki A, Poon LC, Rezende JC, Nicolaides KH. Fetal fraction in maternal plasma cell-free DNA at 11−13 weeks' gestation: relation to maternal and fetal characteristics. Ultrasound Obstet Gynecol 2013;41:26–32.
67. Yang J, Wu J, Wang D, et al. Combined fetal fraction to analyze the Z-score accuracy of noninvasive prenatal testing for fetal trisomies 13, 18, and 21. J Assist Reprod Genet 2023;40:803–10.
68. Hartwig TS, Ambye L, Sørensen S, Jørgensen FS. Discordant non-invasive prenatal testing (NIPT) − a systematic review. Prenat Diagn 2017;37:527–39.

000 Placental, maternal, fetal, and technical origins of false-positive cell-free DNA screening results

Yvette Raymond; Shavi Fernando; Melody Menezes; Ben W. Mol; Andrew McLennan; Fabricio da Silva Costa; Tristan Hardy; Daniel L. Rolnik, on behalf of the TARGET Consortium*