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ARTICLE

Performance of prenatal cfDNA screening for sex chromosomes

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ABSTRACT

Purpose: The aim of this study was to assess the performance of cell-free DNA (cfDNA) screening to detect sex chromosome aneuploidies (SCAs) in an unselected obstetrical population with genetic confirmation.

Methods: This was a planned secondary analysis of the multicenter, prospective SNP-based Microdeletion and Aneuploidy RegisTry (SMART) study. Patients receiving cfDNA results for autosomal aneuploidies and who had confirmatory genetic results for the relevant sex chromosomal aneuploidies were included. Screening performance for SCAs, including monosomy X (MX) and the sex chromosome trisomies (SCT: 47,XXX; 47,XXY; 47,XYY) was determined. Fetal sex concordance between cfDNA and genetic screening was also evaluated in euploid pregnancies.

Results: A total of 17,538 cases met inclusion criteria. Performance of cfDNA for MX, SCTs, and fetal sex was determined in 17,297, 10,333, and 14,486 pregnancies, respectively. Sensitivity, specificity, and positive predictive value (PPV) of cfDNA were 83.3%, 99.9%, and 22.7% for MX and 70.4%, 99.9%, and 82.6%, respectively, for the combined SCTs. The accuracy of fetal sex prediction by cfDNA was 100%.

Conclusion: Screening performance of cfDNA for SCAs is comparable to that reported in other studies. The PPV for the SCTs was similar to the autosomal trisomies, whereas the PPV for MX was substantially lower. No discordance in fetal sex was observed between cfDNA and postnatal genetic screening in euploid pregnancies. These data will assist interpretation and counseling for cfDNA results for sex chromosomes.

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Introduction

Sex chromosome aneuploidies (SCAs) are the most common abnormalities occurring in human reproduction.[1] Monosomy X (MX or 45,X), also known as Turner syndrome, is the most prevalent aneuploidy identified in miscarriage,[2] whereas the sex chromosome trisomies (SCTs), including 47,XXY (Klinefelter syndrome), 47,XXX, and 47,XYY, represent the most common aneuploidies in live births with an approximate prevalence of 1:400 with a male phenotype and 1:750 with a female phenotype.[1,3] Although SCTs are not typically associated with structural malformations, they are associated with a range of neurodevelopmental disabilities.[4-6]

Prenatal genetic screening has historically focused on autosomal trisomies, particularly trisomy 21. Prenatal cell-free DNA (cfDNA) screening based on sequencing of circulating cfDNA in maternal blood has introduced the potential to target any region of the genome, including the SCAs. Although cfDNA screening is increasingly used as the primary screening test for trisomies 13, 18, and 21, inclusion of the sex chromosomes and prediction of fetal sex is not universally available or accepted.[7-10] Several reports on the performance of cfDNA to detect MX have been published, typically showing a lower positive predictive value (PPV) than for trisomy 21.[11] This difference in performance is believed to be related to the common presence of MX mosaicism, which may be placental, fetal, or maternal in origin.[8,12,13]

Although the SCTs are the most prevalent aneuploidies in live births, these have not been universally included in available cfDNA screening tests. Studies reporting the performance of cfDNA screening for the SCTs have been limited by the absence of genetic confirmatory testing in the majority of screened pregnancies.[14-17] Because most infants with an SCT are not identified at birth, this may have resulted in significant underascertainment of SCTs in published prenatal and neonatal cohorts,[18,19] and the need for additional, well-designed studies to evaluate cfDNA screening performance has been recognized.[20] Given the lack of other available prenatal screening tests for SCTs and their generally normal phenotype at birth, most affected individuals will remain undiagnosed until later in life, if ever, and therefore will not have the opportunity to benefit from interventions to optimize health.[21,22]

The objective of this study was to determine the performance of single-nucleotide polymorphism (SNP)-based cfDNA screening for SCAs in a large, unselected cohort with complete confirmatory genetic testing. We also evaluated the prevalence of these disorders in the study cohort as well as the accuracy of fetal sex prediction.

Materials and Methods

This was a planned secondary analysis of the SNP-based Microdeletion and Aneuploidy RegisTry (SMART) study, a large multicenter prospective study that assessed the performance of prenatal cfDNA screening, including prenatal or postnatal confirmatory genetic testing, in an unselected cohort of >18,000 pregnancies.[23,24] SMART patient enrollment, study inclusion and exclusion criteria, confirmatory genetic testing, and quality assurance concordance check methods have been previously reported.[23,24] Eligible women had singleton pregnancies and chose to undergo cfDNA screening for autosomal trisomies, sex chromosomal aneuploidies, and the 22q11.2 deletion syndrome (22q11.2DS) at 21 perinatal centers.[23,24] Results of SNP-based cfDNA screening were reported to patients and used in clinical care decisions. Sites in Europe and Australia represented 45% of the participants, and the remainder were in the United States. All study sites received approval from their local institutional review board or ethics committee.

Results for sex chromosomes were included in cfDNA reports, and confirmatory genetic testing was performed based on local practice policies and patient choice. In some centers, screening for MX was routinely performed, whereas screening for SCTs or for fetal sex was not. The analyses presented were grouped into 3 categories of sex chromosome findings: (1) MX, (2) SCTs, and (3) fetal sex, and the size of the cohort of patients available for each analysis differed. The study cohort for each analysis included women who received results for that indication as well as participants who consented to have their deidentified samples used for additional research, including analyses that were not available clinically. Participants without genetic confirmation were excluded from this analysis.

cfDNA screening for MX

Screening results for MX were provided to all SMART study participants, and participation in the study included consent for confirmatory genetic testing for MX using fetal or newborn samples.[23,24] Participants with a high-risk result for another chromosomal abnormality were excluded from this analysis. Confirmatory testing was requested on all participants by postnatal microarray performed on newborn blood spots, cord tissue, or cord blood by the Center for Applied Genomics at the Children's Hospital of Philadelphia. This analysis was blind to cfDNA results and clinical information. Results of prenatal diagnostic genetic testing performed clinically were also collected and available for analysis in cases in which postnatal confirmatory testing was not available. Mosaic results limited to CVS samples were excluded.

cfDNA screening for SCTs

Screening results for SCTs were only provided to participants when a SCT was suspected; low-risk SCT results were collected but were not reported because of a lack of validation. Patients were therefore consented that they would only receive cfDNA results for SCT if they were found to be

at high risk. Postnatal confirmatory testing was performed only on patients who had had clinical screening for a given disorder or who had consented to future research. Three groups of women therefore had confirmatory genetic testing for SCTs: (1) women who consented to future research (n = 10,333), (2) women who declined future research but had cfDNA results indicating high risk for an SCT (n = 21), and (3) women who declined future research but had other genetic testing (eg, prenatal diagnostic testing or genetic testing on products of conception) that returned results for the SCT (n = 202).

cfDNA determination of fetal sex

Genetic fetal sex concordance with cfDNA results was evaluated in subjects with a confirmed euploid pregnancy and for whom cfDNA screening reported fetal sex. Reporting of fetal sex as a part of cfDNA screening differed by study sites; some declined reporting for all participants, some accepted for all participants, and some allowed the participant to determine whether fetal sex was reported. Participants were excluded from the analysis of fetal sex if the pregnancy resulted in miscarriage, and confirmatory genetic testing of the miscarriage sample indicated a normal female result but could not exclude maternal cell contamination.[25] Variables associated with the request for fetal sex reporting were evaluated for sites in which fetal sex reporting was at the patient's request.

Therefore, all cases in the cohort had confirmatory testing for MX, but cases only received confirmatory testing for SCT if they had a high-risk cfDNA result or if they consented to future research. To minimize bias that would occur by enriching for positive cases, only the cohort of participants who consented to future research were included in the performance analysis for SCT. Likewise, cases had analysis for fetal sex accuracy only if they had requested determination of fetal sex. This resulted in substantially different sized cohorts for MX analysis, the SCT analysis, and the analysis of the accuracy of fetal sex reporting.

The sensitivity, specificity, positive likelihood ratio, and PPV and negative predictive value (NPV) of cfDNA results were assessed for each SCA across the study cohorts. When appropriate, exact (Clopper-Pearson) 95% confidence intervals were reported. SAS Studio 9.04 software (SAS Institute) was used for analysis. MedCalc Software was used to calculate confidence intervals for the positive likelihood ratios.[24] Continuous variables were compared using the Wilcoxon test and categorical variables using the χ^2 test or Fisher's exact test. McNemar's test was used for paired analyses.

Results

A total of 20,193 patients were enrolled in the SMART study. Of these, 2339 (11.5%) had cfDNA results but did not have genetic confirmation for the child; another 37 (0.1%) were missing genetic confirmation for sex chromosomes. In addition, 277 (1.3%) cases lacked results from cfDNA screening; 199 of these had genetic confirmation and were unaffected with SCA, whereas 78 did not have genetic confirmation with postnatal microarray but had either invasive prenatal testing or testing on products of conception that provided results for trisomy 13/18/21 but not the sex chromosomes. There were 2 unusual cases (described in Supplemental Table 1) that were excluded. One case had high-risk results for MX and conflicting confirmatory results: mosaicism was detected on newborn clinical karyotype and fluorescence in situ hybridization but not on the research microarray, leaving some uncertainty as to "genetic truth." In the second case, there were complex X and Y copy number variants in a phenotypically normal male fetus with low-risk cfDNA results in which fetal sex determination was not requested.

Demographic and clinical characteristics of the 17,538 eligible SMART study cohort and the excluded cases (2655) are presented in Table 1. Excluded cases were younger, more likely to self-define as Black, had a higher maternal body mass index and lower fetal fraction, a higher frequency of major ultrasound abnormalities, and had a reduced likelihood of live birth (all $P < .001$).

cfDNA screening for MX

Of 17,538 patients who had genetic confirmation results for the common trisomies, 182 (1.0%) received a high-risk result for trisomy 21, 18, 13, or the SCTs, and 59 (0.3%) received a result for the autosomal trisomies but did not receive a cfDNA risk assessment for MX, leaving 17,297 patients eligible for analysis of the performance of cfDNA for MX (Table 2). In this group, a total of 22 had a high-risk result for MX for a screen positive rate of 0.13%. Confirmatory genetic testing identified MX in 6 pregnancies for a prevalence of 1:2882 (0.035%) live births. Three of these 6 were mosaic for a normal cell line. Five of the 6 affected cases, including the 3 mosaic cases, received a high-risk cfDNA result, yielding a sensitivity of 83.3%, a specificity of 99.90% (17,274 of 17,291), and a PPV of 22.7% (5 of 22) (Table 2). The undetected case had a fetal fraction of 5.7% compared with a mean of 9.3% in the entire cohort. Four of the 6 affected cases, including the 3 mosaic cases, had normal first trimester ultrasound findings, whereas 2 had cystic hygroma. Outcomes of the 59 samples that did not receive a cfDNA risk assessment for MX because of uninformative cfDNA patterns are presented in Supplemental Table 2. This cohort contained 52 normal females, 6 normal males, and 1 case of XXY. There were an additional 7 cases with high-risk results for MX that were excluded because of a lack of confirmatory genetic testing; the outcomes for these cases were lost to follow-up (n = 1), live birth (n = 2), spontaneous abortion (n = 2), therapeutic abortion (n = 1), and intrauterine fetal death (n = 1). Including the 2339 high- and low-risk cases without

Table 1 Demographics and pregnancy characteristics for eligible, excluded and included SMART study participants

Variable	SMART Eligible Cohort (n = 17,538)	Exclusions From SMART Cohort (n = 2655)	P Value
Mean maternal age (SD), y	33.5 (5.4)	32.9 (5.9)	<.001
Nulliparity, n (%)	7743 (44.3)	1115 (42.2)	.046
Mean gestational age at screening (SD), wk	13.3 (3.2)	13.6 (3.5)	.001
Mean maternal body mass index (SD), kg/m^2	26.3 (5.8)	27.4 (7.0)	<.001
Median fetal fraction (IQR), %	9.3 (7.0-12.2)	8.9 (6.1-12.1)	<.001
Race, n (%)[a]			<.001
Asian	1516 (8.6)	211 (8.0)	
Black	1503 (8.6)	360 (13.6)	
White	10,653 (60.7)	1542 (58.1)	
Latina	3267 (18.6)	437 (16.5)	
Other/unknown	599 (3.4)	105 (4.0)	
Ultrasound abnormality before cfDNA screening, n (%)	98 (0.6)	3 (0.1)	<.001
Ultrasound abnormality at any time, n (%)	667 (4.0)	180 (7.1)	<.001
Pregnancy outcome, n (%)			<.001
Live birth	17,341 (98.95)	1723 (83.48)	
Intrauterine fetal death/stillbirth	24 (0.14)	83 (4.02)	
Spontaneous abortion	26 (0.15)	171 (8.28)	
Pregnancy interruption	134 (0.76)	87 (4.22)	

cfDNA, cell-free DNA; *IQR*, interquartile range; *SMART*, SNP-based Microdeletion and Aneuploidy RegisTry.
[a]Race and ethnicity as reported by participants. If the participant did not report the information, the information from the medical record was used.

confirmatory testing, the overall screen positive rate was 29 of 19,636 (0.15%).

cfDNA screening for SCTs

Overall, 10,333 SMART participants consented to future research, received cfDNA results, and had confirmatory SCT genetic testing. An increased risk for an SCT was reported in 23 participants in this cohort, for a screen positive rate of 0.23%. Twenty-seven patients in the cohort were confirmed to have an SCT, with a prevalence of 1:382 or 0.26%. There were 5 cases of 47,XXX, for a prevalence of 1:2065 (0.05%), 8 of 47,XXY for a prevalence 1:1291 (0.08%), and 14 with 47,XYY for a prevalence of 1:737 (0.13%). In all, 19 of the 27 cases with any SCT received a high-risk result, for a sensitivity of 70.4%, a specificity of 99.96% (10,302 of 10,306), and a PPV of 82.6% (19 of 23). The sensitivities and specificities of the individual SCTs are provided in Table 3. Pregnancies with a confirmed SCT were more likely to have a major ultrasound abnormality (10.2% vs 4% in the entire cohort; P = .043), and 26% resulted in a termination of pregnancy. The 8 false-negative SCTs had fetal fractions ranging from 5.5% to 8.2% compared with a mean of 9.3% in the entire cohort. In the cohort of excluded cases, there were 3 cases with a high-risk cfDNA SCT result without confirmatory testing (1 liveborn infant with an XYY cfDNA result, 1 miscarriage with an XXY result, and 1 lost to follow-up case with an XXX result). Demographic and clinical information for pregnancies with a confirmed SCT is summarized in Supplemental Table 3.

The combined prevalence of any SCA, including the SCTs and MX, was 0.30%, and the likelihood of receiving a high-risk result for any SCA was 0.35%.

cfDNA screening and concordance with fetal sex

Overall, fetal sex reporting was requested for 14,660 of 17,538 (83.6%) participants. Of these, 14,486 participants had fetal sex reported by cfDNA and genetic confirmation

Table 2 Performance of cfDNA screening for MX

Cohort	Prevalence of MX, n/N (%)	High-Risk cfDNA Result, n/N (%)	Sensitivity cfDNA, n/N (%) [95% CI]	Specificity cfDNA, n/N (%) [95% CI]	PPV cfDNA n/N (%) [95% CI]	NPV cfDNA n/N (%) [95% CI]
N = 17,297	6/17,297 (0.03%)	22/17,297 (0.13%)	5/6 (83.3%) [53.5-100]	17,274/17,291 (99.90%) [99.85-99.95]	5/22 (22.7%) [5.2-40.2]	17,274/17,275 (99.99%) [99.98-100]

cfDNA, cell-free DNA; *MX*, monosomy X; *NPV*, negative predictive value; *PPV*, positive predictive value.

Table 3 cfDNA screening performance for sex chromosome trisomies

No. Affected	Prevalence, *n/N* (%)	High-Risk cfDNA Result, n/*N* (%)	Sensitivity, *n/N* (%) [95% CI]	Specificity, *n/N* (%) [95% CI]	PPV, *n/N*%) [95% CI]	NPV, *n/N* (%) [95% CI]
47,XXX *n* = 5	5/10,333 (0.05%)	5/10,333 (0.05%)	3/5 (60%) [17.1-100]	10,326/10,328 (99.98%) [99.95-100]	3/5 (60%) [17.1-100]	10,326/10,328 (99.98%) [99.95-100]
47,XXY *n* = 8	8/10,333 (0.08%)	6/10,333 (0.06%)	4/8 (50%) [15.4-84.7]	10,323/10,325 (99.98%) [99.92-100]	4/6 (66.7%) [29.0-100]	10,323/10,327 (99.96%) [99.92-100]
47,XYY *n* = 14	14/10,333 (0.14%)	12/10,333 (0.12%)	12/14 (85.7%) [67.4-100]	10,319/10,319 (100%) [99.96-100]	12/12 (100%) [73.5-100]	10,319/10,321 (99.98%) [99.95-100]
Any SCT *n* = 27	27/10,333 (0.26%)	23/10,333 (0.23%)	19/27 (70.4%) [53.2-87.5]	10,302/10,306 (99.96%) [99.92-100]	19/23 (82.6%) [67.1-98.1]	10,302/10,310 (99.92%) [99.87-99.98]

cfDNA, cell-free DNA; *PPV*, positive predictive value; *NPV*, negative predictive value; *SCT*, sex chromosome trisomy.

results available. Of the 174 cases that were excluded, 167 were high risk for a chromosomal aneuploidy, MX, or SCT. In the remaining 7 cases, sex was not reportable by prenatal cfDNA screening results because of suspected sex chromosomal mosaicism, including suspected maternal MX mosaicism ($n = 2$), maternal XXX ($n = 2$), maternal X copy number variant ($n = 1$), and fetal Y mosaicism ($n = 1$) as well as for undecipherable SNP patterns in 1 case. These 7 cases were all male by postnatal confirmatory testing. Confirmatory genetic testing identified 7215 females and 7271 males, and results were 100% (95% CI 99.97-100) concordant with cfDNA screening.

In some centers, patients were given the choice to receive fetal sex results. In 12,797 cases, patients were offered fetal sex determination, and 10,745 (84.0%) requested that fetal sex be reported. The rate of requested fetal sex reporting was significantly higher at the US sites (92.4%; range 69.1%-98.3%) compared with non-US sites (67.9%; range 64.8%-98.8 %) ($P < .001$). Comparison of additional demographic and clinical factors associated with the decision to request fetal sex reporting are provided in Supplemental Table 4. Those who requested fetal sex reporting were younger and less likely to self-identify as White.

Discussion

We report the performance of SNP-based cfDNA for the detection of MX, SCTs, and fetal sex in a large, prospectively ascertained cohort with confirmatory diagnostic genetic testing.[24] The sensitivity for MX was 83.3% and for the SCTs was 70.4%, with PPVs of 22.7% and 82.6%, respectively. Concordance of fetal sex prediction was 100%. These data add to the previously limited prospective data on the accuracy of cfDNA screening for sex chromosomal abnormalities and for the determination of fetal sex.

Performance metrics for MX measured in this study are similar to those reported by other investigators, using a variety of laboratory methodologies.[11,20] This study further supports the ability of cfDNA to identify pregnancies with MX, including cases with mosaicism, which was present in 3 of the 6 cases in this cohort. The single false-negative case had a fetal fraction of 5.7%. There were 59 cases with nonreportable results for MX; 58 of these had a normal outcome, whereas 1 had 47,XXY. Although reassuring that the likelihood of a fetal sex chromosomal aneuploidy in this setting is low, this relatively small number of cases limits our ability to determine whether this finding increases the risk for MX or another sex chromosomal abnormality.

The PPV for MX in this study was 22.7%, which is less than that reported for the autosomal trisomies[25] and lower than the PPV of 77.5% reported in a previous publication using SNP-based cfDNA screening.[26] This lower PPV likely reflects differences in the study cohorts because the prior report had a lower rate of follow-up and reported a higher prevalence of MX (1 of 663 vs 1 of 2882). A number of other publications featuring other cfDNA methods have reported prevalence and PPVs similar to this study.[27] A lower PPV could occur because of false-positive results associated with placental or maternal mosaicism,[28] and in this study, 6 cases were excluded because of suspected maternal sex chromosomal mosaicism by cfDNA, although confirmation with maternal karyotype was not available. Specific testing of the placentas for mosaicism after delivery was not feasible.

Given the lack of a suggestive phenotype at birth, data on the performance of cfDNA to detect fetal SCT have been limited. This study demonstrates that SNP-based cfDNA screening can detect SCTs with 70.4% sensitivity and 99.96% specificity. As with MX, the 8 false-negative SCTs had lower fetal fractions, ranging from 5.5% to 8.2%. It is interesting that there was a significantly higher rate of malformations in the SCT pregnancies because these are not typically thought to be associated with an increased risk of structural anomalies. It is possible that this reflects increased use of cfDNA screening in cases once a structural anomaly is identified, although most did not have a structural abnormality detected before screening. It is also possible that

the abnormal screening results led to a more comprehensive sonographic examination.

The benefit of screening for fetal sex chromosomal aneuploidy is debated because the phenotype is generally mild, but some features can potentially benefit from interventions if an SCA is recognized. Identification of girls with MX prenatally or in the immediate postnatal period allows evaluation for structural malformations, ongoing health surveillance for endocrine deficiencies,[29] and therapeutic interventions that can potentially improve outcomes. Treatment with growth hormone and the option of fertility preservation may also be pursued and could potentially benefit these individuals.[30] Individuals with SCTs have also been reported to benefit from early identification and a variety of interventions, including early recognition and management of learning differences, preservation of reproductive options through sperm and egg banking, screening for associated anomalies and endocrine differences, and avoidance of diagnostic odysseys.[31-36] Surveys of parents of children with sex chromosome abnormalities reveal support for the availability of prenatal screening using cfDNA to facilitate early diagnosis.[37]

In this study, cfDNA fetal sex reporting was requested by 84% of study participants who were given the option of screening, and cfDNA screening results were concordant in all cases with confirmatory genetic testing. When discrepancies between clinical and cfDNA sex determination are found, associated disorders of sexual development have been reported.[38,39] Whether inclusion of fetal sex using cfDNA screening is of benefit in detecting a disorder of sexual development warrants further investigation.

Although this was a large study that uniquely included genetic confirmation, there are limitations to this study that should be acknowledged. The SMART study was a registry study; therefore, individual study sites may have differed in the criteria for which participation was offered. Although the cohort was large, at nearly 20,000 participants, the number of confirmed sex chromosomal abnormalities was small, given the relatively low prevalence of these disorders, and for the SCTs, the cohort size was substantially smaller. Pregnancies in which an ultrasound abnormality had been identified before enrollment were eligible for inclusion, which could affect test performance assessment by enriching for chromosomal abnormalities. The mean maternal age of participants in the study was higher than the general obstetrical population, although most sex chromosome abnormalities are not associated with increasing maternal age. Other variables also differed between the eligible and excluded cohort. Most of these were related to low fetal fraction, which is associated with an increased no result rate for cfDNA screening, and was a cause of some exclusions. However, the overall differences were small and not expected to affect performance estimates. Maternal and placental karyotyping was not routinely performed, limiting our ability to determine the role of mosaicism in cfDNA false-positive and false-negative results. It is well known that sex chromosomal aneuploidies can include complex mosaic results, rearrangements, and copy number variants. In some of our cases, confirmatory testing (eg, clinical postnatal follow-up and the research array) was disparate, and further investigation was not possible. This illustrates a limitation of cfDNA for SCA because complex or mosaic results may not be detected. It is also important to acknowledge that cfDNA screening uses different laboratory methods, limiting the generalizability of these results. In cases with complex or nonreportable results, the data available regarding neonatal outcomes were limited, and future research that includes a large cohort of cfDNA samples with an isolated nonreportable result would be useful to define the magnitude of risk for SCAs associated with this finding. Finally, funding for the SMART study was provided by the commercial laboratory performing the cfDNA screening. Potential bias was minimized using independent study site research coordinators, data reporting to an independent data coordinating site, and confirmatory genetic testing performed by an independent laboratory blind to cfDNA results, which reported directly to the data coordinating site. Moreover, the lead investigators had full independence in decision making.

Overall, this study demonstrates that SNP-based cfDNA screening for the sex chromosome abnormalities is associated with sensitivities of 50% to 87% and specificities of >99.9%. These disorders fulfill the test performance criteria for inclusion in genetic screening,[40-42] including the potential for changes in clinical management in the prenatal and/or postnatal periods. Our study provides useful data for providers to counsel patients regarding inclusion of the sex chromosomes in cfDNA screening.

Data Availability

Data sharing requests should be submitted to the corresponding author (M.E.N.) for consideration. Requests will be considered by the study publication committee, and access may be limited by patient consent considerations. Study protocol and statistical analysis plan will be available upon request. Individual patient data will not be available. Access to deidentified data may be granted after the submission of a written proposal and a signed data sharing agreement. Files will be shared using a secure file transfer protocol. Data will be available immediately after publication and ending 1 year after article publication.

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Ethics Declaration

This study was designed in compliance with an investigational review board–approved protocol (Ethical and Independent Review Services Study ID, 17113; date of certification, August 28, 2017, date of renewal August 20, 2020). Written informed consent was obtained from all study participants, and the SMART study was approved by all site local review boards.

Conflict of Interest

All site principal investigators (Pe'er Dar, Bo Jacobsson, Fergal Malone, Ronald J. Wapner, Ashley S. Roman, Asma Khalil, Revital Faro, Rajeevi Madankumar, Sina Haeri, Robert M. Silver, Nidhi Vohra, Jon Hyett, Cora MacPherson, and Mary E. Norton) received institutional research support from the funding sponsor (Natera). Melissa Egbert, Zachary Demko, and Matt Rabinowitz are employed by the study's funding sponsor (Natera) and hold stock or options to hold stock. Kimberly Martin is a consultant to the funding sponsor (Natera) and holds stock and options to hold stock. Jon Hyett has an ongoing research collaboration that includes financial support for biochemical analytes from Perkin Elmer; has earned honoraria and/or given talks that were not compensated from Natera, Roche, and Canon; and has participated in Asian/Australasian expert consultancies for Natera and Roche. Bo Jacobsson collaborated in the Improving Maternal Pregnancy And Child ouTcomes (IMPACT) study where Roche, Perkin Elmer, and Thermo Fisher provide reagents to Placental Growth Factor (PLGF) analyses. Ronald J. Wapner receives research funding from National Institute of Child Health and Human Development and support from Illumina for research reagents. Mary E. Norton is a consultant to Luna Genetics. All other authors declare no conflicts of interest.

Additional Information

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