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Preliminary Communication

# Noninvasive Prenatal Testing and Incidental Detection of Occult Maternal Malignancies

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**IMPORTANCE** Understanding the relationship between aneuploidy detection on noninvasive prenatal testing (NIPT) and occult maternal malignancies may explain results that are discordant with the fetal karyotype and improve maternal clinical care.

**OBJECTIVE** To evaluate massively parallel sequencing data for patterns of copy-number variations that might prospectively identify occult maternal malignancies.

**DESIGN, SETTING, AND PARTICIPANTS** Case series identified from 125 426 samples submitted between February 15, 2012, and September 30, 2014, from asymptomatic pregnant women who underwent plasma cell-free DNA sequencing for clinical prenatal aneuploidy screening. Analyses were conducted in a clinical laboratory that performs DNA sequencing. Among the clinical samples, abnormal results were detected in 3757 (3%); these were reported to the ordering physician with recommendations for further evaluation.

**EXPOSURES** NIPT for fetal aneuploidy screening (chromosomes 13, 18, 21, X, and Y).

**MAIN OUTCOMES AND MEASURES** Detailed genome-wide bioinformatics analysis was performed on available sequencing data from 8 of 10 women with known cancers. Genome-wide copy-number changes in the original NIPT samples and in subsequent serial samples from individual patients when available are reported. Copy-number changes detected in NIPT sequencing data in the known cancer cases were compared with the types of aneuploidies detected in the overall cohort.

**RESULTS** From a cohort of 125 426 NIPT results, 3757 (3%) were positive for 1 or more aneuploidies involving chromosomes 13, 18, 21, X, or Y. From this set of 3757 samples, 10 cases of maternal cancer were identified. Detailed clinical and sequencing data were obtained in 8. Maternal cancers most frequently occurred with the rare NIPT finding of more than 1 aneuploidy detected (7 known cancers among 39 cases of multiple aneuploidies by NIPT, 18% [95% CI, 7.5%-33.5%]). All 8 cases that underwent further bioinformatics analysis showed unique patterns of nonspecific copy-number gains and losses across multiple chromosomes. In 1 case, blood was sampled after completion of treatment for colorectal cancer and the abnormal pattern was no longer evident.

**CONCLUSIONS AND RELEVANCE** In this preliminary study, a small number of cases of occult malignancy were subsequently diagnosed among pregnant women whose noninvasive prenatal testing results showed discordance with the fetal karyotype. The clinical importance of these findings will require further research.

JAMA. 2015;314(2):162-169. doi:10.1001/jama.2015.7120  
Published online July 13, 2015.

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**N**oninvasive prenatal testing (NIPT) using massively parallel sequencing of cell-free DNA (cfDNA) in maternal plasma has recently changed the clinical paradigm of prenatal screening for the common fetal autosomal aneuploidies (abnormal numbers of whole chromosomes).<sup>1,2</sup> Using this technology, the sensitivities for the detection of fetal trisomies 21 and 18 are, on average, 99% and 96%, respectively, with specificities of 99% to 100%.<sup>3</sup> Many professional societies have recommended that NIPT can be offered to pregnant women at high risk for having a fetus with autosomal aneuploidy, with follow-up diagnostic testing recommended to confirm a positive test result.<sup>1,4-6</sup>

Although NIPT performs well, it is an advanced screen, not a diagnostic test. The reason for this distinction is that the cfDNA in the plasma of pregnant women is a mixture of placental (used as a proxy for the fetus) and maternal DNA. Follow-up studies have shown that some cfDNA results are discordant with the direct fetal karyotype.<sup>7,8</sup> Potential biological explanations for discordance include confined placental mosaicism,<sup>9</sup> co-twin demise,<sup>10</sup> maternal chromosomal mosaicism<sup>11</sup> and DNA copy-number variants,<sup>12</sup> maternal organ transplant from a male donor,<sup>11</sup> and maternal malignancy.<sup>13,14</sup>

The diagnosis of cancer during pregnancy is relatively uncommon, with an incidence of about 1 in 1000 gestations.<sup>15</sup> The most common malignancies observed in pregnant women are breast and cervical cancers, Hodgkin and non-Hodgkin lymphomas, malignant melanoma, leukemia, ovarian cancer, and colorectal cancer.<sup>15</sup>

The purpose of this study was to retrospectively examine DNA sequencing data in a series of pregnant women with abnormal NIPT results involving aneuploidies of chromosomes 13, 18, 21, X, or Y, who were diagnosed with cancer after prenatal testing occurred. In addition, to better understand the frequency with which maternal cancer might provide an explanation for abnormal NIPT results that are discordant with the fetal karyotype, all abnormal test results in the clinical laboratory and available clinical outcomes were reviewed.

## Methods

The current case series was identified from a population of 125 426 pregnant women undergoing plasma cfDNA sequencing in the Illumina clinical laboratory (Redwood City, California) between February 15, 2012, and September 30, 2014. Patients were included if their clinician voluntarily informed the laboratory at any time prior to November 15, 2014, that maternal cancer had been diagnosed after NIPT. As part of a standard laboratory follow-up process, the laboratory contacts the referring clinicians to discuss all positive NIPT results and to recommend a diagnostic procedure to obtain a confirmatory fetal karyotype. When NIPT results and the karyotype are discordant, the medical director (S.B.) and the certified genetic counselors who work with her review possible explanations for the discordant results with the refer-

ring clinician. Maternal cancer had not been reported as a reason for NIPT discordance until publication of a single case report in 2013,<sup>13</sup> so maternal cancer was only included in the differential diagnosis after that time.

Evolving knowledge and experience have resulted in changes in the bioinformatics analytic algorithms used in the clinical laboratory during the time frame of this study. The ability to analyze and visualize whole-genome sequencing results was not technically possible until October 2013. After October 2013, if referring clinicians requested the expanded bioinformatics results, these were communicated directly to the physician. In all cases, the patient's clinician was responsible for determining the follow-up clinical management.

For each patient reported in detail in this article, in addition to the consent obtained for the original, clinically indicated noninvasive prenatal test, a separate individual written consent for medical records review, further genomic analysis, and possible publication of findings was obtained after the abnormal NIPT results were reported to the patient's physician. The Tufts Medical Center institutional review board waived review of this study. Information regarding the patient's pregnancy, cancer diagnosis, and medical history was obtained from her clinicians and medical records, by direct discussion with the first author (D.W.B.), or both. In some of the cases, additional blood samples (including postpartum) were obtained and analyzed. For these samples, the clinical laboratory team performing the sequencing was blinded to the fact that these women were no longer pregnant.

Using whole blood samples, the verifi Prenatal Test (Illumina) screens for the presence of whole chromosome aneuploidy for chromosomes 13, 18, and 21. Testing for sex chromosome aneuploidy by analyzing sequencing counts for chromosomes X and Y is optional.<sup>11</sup> The method uses massively parallel sequencing of cfDNA isolated from maternal plasma.<sup>16-20</sup> To identify on which chromosome the sequenced DNA fragment mapped, a software program known as bowtie<sup>21</sup> was used to align the short (25 base-pair) sequence reads to the 19th reference version of the human genome sequence map (hg19). The data were filtered to remove nonunique alignments and genomic regions associated with high variation. They were then normalized based on the percentage of guanine (G) cytosine (C) representation in the sequence of each chromosome and corrected to remove other assay and sample-specific biases.

Overrepresentation or underrepresentation of the target chromosomes (13, 18, 21, X, and Y) was evaluated by constructing a ratio between the normalized coverage on each chromosome of interest and the sum of normalized coverage on a respective set of reference chromosomes.<sup>16</sup> Typically, there were between 2 and 6 reference chromosomes per target chromosome (eg, 13, 18, 21, X, and Y). Specific reference chromosomes have changed with evolution of the clinical bioinformatics algorithms. Upper and lower normal limits were then applied to the test results to generate an aneuploidy classification status for chromosomes 13, 18, and 21 (aneuploidy detected, aneuploidy suspected, or no

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aneuploidy detected)<sup>17,19</sup> and for sex chromosomes (sex chromosome aneuploidy detected or no sex chromosome aneuploidy detected).<sup>11</sup> If no sex chromosome aneuploidy was detected, a sex chromosome result of XX or XY was provided.<sup>11</sup>

All whole blood samples received within 5 days of sampling with a complete test requisition form authorized by an ordering physician were entered into the laboratory management system. Maternal age, gestational age, and indication for testing (if included) on the test requisition form were recorded. All test results with an aneuploidy “detected” or “suspected” were telephoned to the ordering physician by a certified genetic counselor employed by the clinical laboratory. If a diagnostic procedure for fetal karyotyping was performed, clinicians were requested on 2 separate occasions to inform the laboratory whether the NIPT results were concordant or discordant with the fetal karyotype. Whenever the laboratory was notified of discordant results, pertinent history was obtained from the patients’ physicians and genetic counselors, and possible biological mechanisms for abnormal results were discussed as stated earlier in the Methods. An internal quality assurance process was also followed to evaluate any potential technical explanations for the discordant result.

When detailed bioinformatics analysis of the previously sequenced DNA sample was performed, mapped sections of the human genome were analyzed using circular binary segmentation,<sup>22</sup> in order to identify copy-number variants (CNVs). Copy-number variants are genomic regions associated with significant deviation from the expected 2 copies across a contiguous span of the human genome. For a diploid genome, normalized coverage is expected to be 1.0. If there is a gain of a single copy, the expected result is 1.5 (a 50% gain in amplitude). Similarly, for the loss of a single copy, the expected result is 0.5 (a 50% loss in amplitude). Using this scale, a maternal plasma sample from a woman carrying a fetus with trisomy 21 that contains 10% circulating fetal DNA will have a 5% gain in coverage across the length of chromosome 21 ( $0.1 \times 0.50 = 0.05$ ). In this study, identified CNVs were counted as gains or losses if they exceeded either 10 megabase pairs (Mb) in length and 2.5% in deviation from the expected diploid coverage, or 40 Mb in length and 1% in deviation from the expected diploid coverage. These parameters were only used for visual interpretation of the data and were not intended to identify cancer signatures.

To evaluate the frequency of reported maternal malignancies in relation to the overall frequency of aneuploidy positive results, all clinical laboratory reports, as well as all tests that were cancelled due to abnormal underlying chromosomal patterns generated within the study time frame, were reviewed and the findings were grouped into 1 of 5 categories: single trisomy, single monosomy, single sex chromosome aneuploidy, single sex chromosome aneuploidy plus single trisomy, or multiple aneuploidies.

Statistical analysis of the reported proportions was performed using Clopper-Pearson exact binomial 2-sided confidence intervals at the 95% level (using R version 3.1.2).

## Results

### Review of Clinical Cases

From a cohort of 125 426 NIPT tests, 3757 (3.0%) were positive for 1 or more aneuploidies involving chromosome 13, 18, 21, X, or Y. In 10 of these “aneuploidy-detected” cases, the referring clinician voluntarily reported to the clinical laboratory within weeks to months after the initial discussion regarding the clinical significance of the positive NIPT results that the patient had been diagnosed with a malignancy. The 10 cancer cases were clinically diverse and included 3 cases of B-cell lymphoma and 1 case each of T-cell leukemia; Hodgkin lymphoma; unspecified adenocarcinoma; leiomyosarcoma; and neuroendocrine, colorectal, and anal carcinomas. In 2 cases (leiomyosarcoma and unspecified adenocarcinoma), the referring physicians reported that the women were critically ill, and they declined to approach them for consent to participate in this study.

Table 1 shows demographic factors, NIPT results, fetal status, and cancer stage for the remaining 8 cases, in which permission was granted for further analysis. At the time of initial NIPT, the mean maternal age was 35 years (range, 23-39 years), and the mean gestational age was 13.9 weeks (range, 10-20 weeks). Cancer was subsequently diagnosed (during pregnancy or postpartum) in these women at a mean of 16 weeks (range, 3-39 weeks) after the initial NIPT. The clinical presentations ranged from early-stage to metastatic disease. In 3 patients (cases 4, 5, and 8), the discordant NIPT results prompted a further medical workup that led to the diagnosis of cancer. The 3 patients with B-cell lymphoma (cases 2, 6, and 7) presented with a palpable mass. In 2 cases of maternal malignancy (cases 1 and 3), the patients presented with advanced symptoms: pain due to bone metastases and colon obstruction, respectively.

In 7 of the 8 cases, diagnostic fetal karyotyping was performed and showed a euploid result (46,XY or 46,XX). Of 3 preterm deliveries, 1 was at 29 weeks due to maternal preeclampsia, 1 at 35 weeks due to spontaneous labor, and 1 at 32 weeks to facilitate maternal treatment (cases 5, 7, and 8, respectively, Table 1).

### Bioinformatics Analysis

Detailed genome-wide analysis of the original sequencing data obtained from cfDNA of the 8 study participants revealed CNVs that affected multiple chromosomes and spanned between 4% and 44% (median, 29%) of the genome (Figure 1). Cases with trisomies detected by NIPT could be explained by whole or large partial copy-number gains on the test chromosomes or losses on any of the reference chromosomes. Conversely, cases with monosomies detected could be explained by either losses on the target chromosomes or gains on the reference chromosomes. For 2 cases (3 and 5), in which replicate testing of the same initial blood sample was performed, the CNV detection results were highly consistent, resulting in identical NIPT calls and 91% to 99% identical gain or loss profiles across the entire genome (Figure 1, lines 3B and 3B' and 5A and 5A').

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Table 1. Clinical Details on the 8 Cases of Maternal Cancer That Underwent Genome-wide Analysis

	Case 1 <sup>a</sup>	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Maternal demographics								
Age, y	37	36	33	36	23	37	39	39
GA, wk	13	12	13	20	20	12	11	10
Aneuploidy detection by NIPT								
Chromosome 21	Not detected	Not detected	Not detected	Monosomy	Trisomy	Not detected	Not detected	Trisomy
Chromosome 18	Monosomy	Monosomy	Not detected	Monosomy	Monosomy	Trisomy	Monosomy	Trisomy
Chromosome 13	Trisomy	Not detected	Trisomy	Monosomy	Trisomy	Not detected	Not detected	Trisomy
Sex chromosomes	Not done	Not done	Not done	Not done	XY	XX	XXY	Monosomy X
No. of NIPT aneuploidies	2	1	1	3	3	1	2	4
Fetal/newborn status								
Fetal karyotype	46,XY	Not done	46,XY	46,XY	46,XY	46,XX	46,XY	46,XX
Pregnancy outcome	Term male	Term female	Term male	Term male	Preterm male, preeclampsia, 29 wk	Term female	Preterm male, 35 wk	Preterm female, 32 wk
Cancer characteristics								
Cancer type	Neuro-endocrine (unknown origin)	Non-Hodgkin (B-cell) lymphoma	Colorectal	Hodgkin lymphoma	Acute T-cell lymphoblastic leukemia	Non-Hodgkin (B-cell) lymphoma	Non-Hodgkin (B-cell) lymphoma	Anal
Stage at diagnosis	IV, metastatic	IVB	IIIC	IIA	NA	IV	II	IIIB
Time elapsed from NIPT to diagnosis	28 wk	13 wk	39 wk	3 wk to MRI, 29 wk to biopsy	3 wk	≈20 wk	≈10 wk	8 wk
Timing of cancer diagnosis	Postnatal	Prenatal	Postnatal	Postnatal	Prenatal	Prenatal	Prenatal	Prenatal
Postnatal DNA sequencing results	Not done	Not done	Trisomy 13, monosomy 18	Monosomy 13, monosomy 18, monosomy 21, monosomy X	Not done	Not done	Not done	Not done

Abbreviations: GA, gestational age at time of NIPT blood draw as obtained from test request form; MRI, magnetic resonance imaging; NA, not applicable; NIPT, noninvasive prenatal testing.

<sup>a</sup> Case previously published.<sup>13</sup>

Additional whole blood samples were collected from 3 of the participants at time points subsequent to the initial NIPT: for case 1, 6 weeks after initial NIPT (but still pregnant); for case 3, 5 months after delivery, immediately prior to surgical resection of the colorectal tumor, and 14 months after delivery following completion of radiation and chemotherapy; and for case 4, 8 months after delivery. Detected CNVs were highly consistent prior to treatment in samples obtained up to 11 months apart. Areas of CNV detection overlapped by 76%, 79%, and 93%, respectively, for cases 1, 3, and 4; the differences were mostly due to increased amplitude of signal with time and additional detectable gains/losses in later samples (Figure 1).

Blood samples were obtained at 3 different clinically significant time points for case 3. The sequencing data for chromosome 13 (a test chromosome) and 8 (a reference chromosome for chromosome 18) are shown in Figure 2. An increase in normalized coverage for chromosome 13 is evident in the pretreatment samples, consistent with the original NIPT result of trisomy 13 detected. Confined placental mosaicism was ruled out using microarrays in another laboratory. The magnitude of the CNV in the maternal blood above baseline increased over time (from 1%-3% deviation from the expected diploid genome) in the postpartum presurgical resection sample (Figure 1 and Figure 2). Similarly, chromosome 8 (a ref-

erence chromosome for chromosome 18) displayed partial amplifications and losses in the original NIPT sample. In the second sample, the chromosome 8 signal gained sufficient amplitude to affect the calculations for chromosome 18, causing a monosomy 18-detected test result. The patient's third sample, obtained after completion of all treatment, showed no abnormal deviations from baseline.

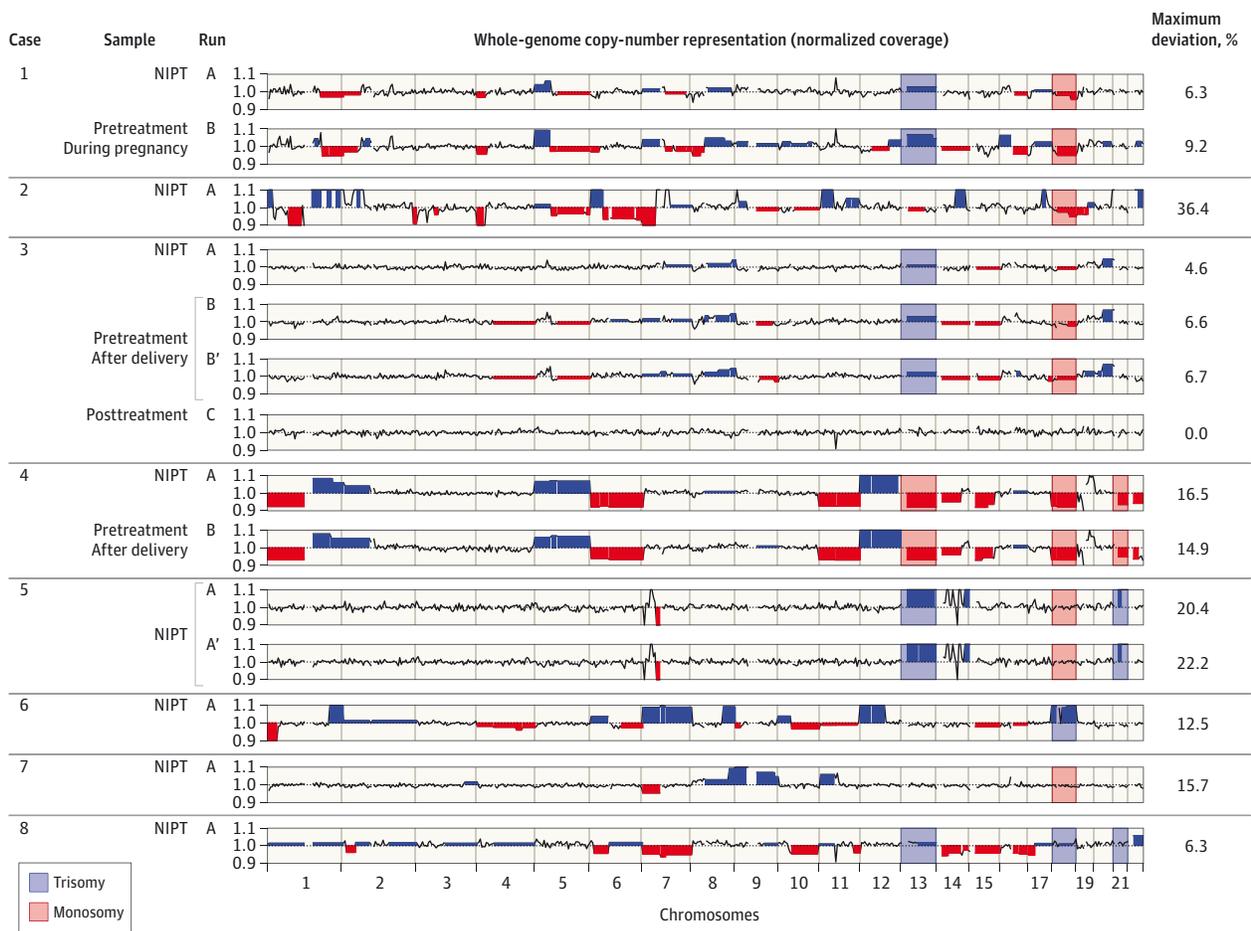
### Aneuploidy Patterns in Maternal Cancer

Follow-up of the 3757 abnormal NIPT results was incomplete. Seven of the 10 cases of maternal cancer reported to the clinical laboratory had multiple aneuploidies (Table 2). Of the 39 cases of multiple aneuploidy, 7 cases (18% [95% CI, 7.5%-33.5%]) were in women with an occult cancer. Of the 39 cases of multiple aneuploidies detected, 4 were concordant or partially concordant, meaning that at least 1 of the aneuploidies detected by NIPT was confirmed by fetal diagnostic testing. Sixteen of the remaining 35 NIPT results were confirmed to be discordant with results from follow-up invasive diagnostic testing. In the other 19 cases, the outcome was unknown because of fetal loss without karyotype information or a lack of clinical information from the referring physician.

Although the patient follow-up was incomplete, we estimate that the risk of maternal cancer in the small subset of preg-

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Figure 1. Whole-Genome View of Copy-Number Changes in 8 Cases of Maternal Cancer



Whole-genome view of copy-number gains and losses in plasma samples from women with known cancer. Smoothed normalized coverage (in black) is plotted along the genomic coordinates (x-axis), sorted by chromosome number and genomic location within the chromosomes. The data for all samples are shown as normalized coverage on the same scale, on the left side of the y-axis (0.9-1.1). The scale chosen for this figure is less than 0.5-1.5 because of fractional representation of the tumor DNA in the mixed sample. For some samples, the amplitude of the copy-number variants exceeded the scale; the maximum

deviation from expected diploid representation is shown as a percentage on the right side of the y-axis. Copy-number gains or losses relative to the diploid reference genome are shown as blue or red, respectively. If a trisomy was reported, the relevant chromosome is shown by a light blue bar. If a monosomy was reported, the relevant chromosome is shown by a light red bar. Cases 3 and 5 include replicates of the same blood sample, identified by an apostrophe ('). Cases 1, 3, and 4 had longitudinal samples obtained (see text for details).

nant women with abnormal discordant NIPT results due to multiple aneuploidies detected and a normal fetal karyotype is as follows. If all 19 cases of multiple aneuploidies in which follow-up information was unavailable were concordant with the fetal karyotype, the risk of maternal cancer as an explanation for the discordant results would be 7 of 16 cases (44%). If, however, all of the 19 cases were discordant with the fetal karyotype (eg, the fetal karyotype is normal), then the risk would be 7 of 35 cases (20%).

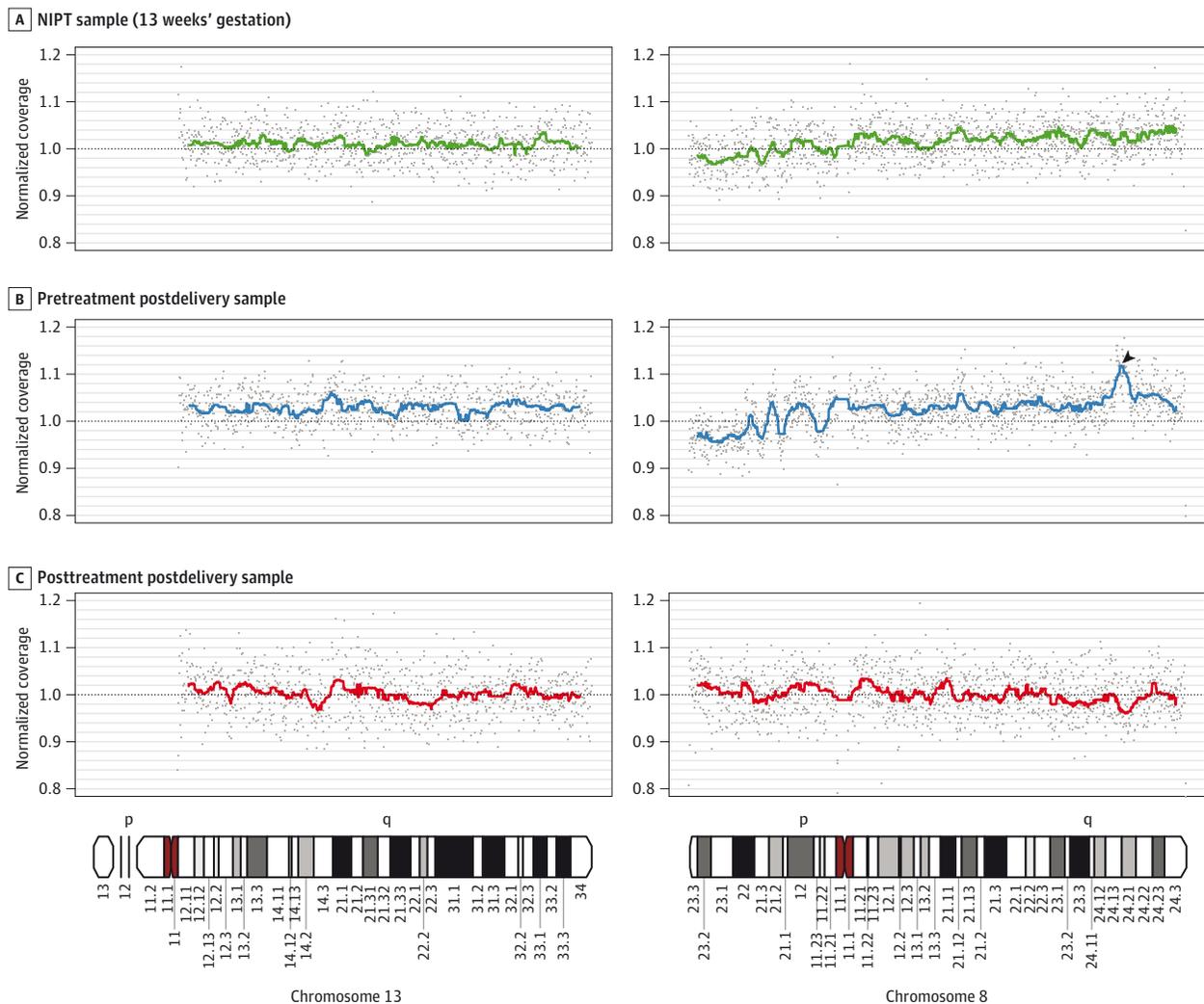
Discussion

In this case series of 125 426 NIPT results, 3757 were positive for 1 or more aneuploidies involving chromosomes 13, 18, 21, X, or Y. Some of the abnormal NIPT results were discor-

dant with the diagnostic fetal karyotypes obtained by amniocentesis or chorionic villus sampling. Here we have shown that occult maternal malignancies may provide a biological explanation for some discordant NIPT results. This is presumably due to the cfDNA that is released into maternal circulation from apoptotic malignant cells. The types of cancers diagnosed were among those most frequently reported in women of childbearing age,<sup>15</sup> although there were more hematologic malignancies than would be expected and no cases of malignant melanoma or cervical cancer. The expected cancer rate in pregnant women is about 0.1%.<sup>15</sup> This series of cancer cases, reported voluntarily, represents 0.008% (10/125 426) of the laboratory case volume, a cancer frequency that is 10-fold lower than what might be expected. However, this patient series is inherently incomplete; maternal cancers diagnosed after delivery

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Figure 2. Longitudinal Evolution of Chromosomal Profiles for Maternal Cancer Case 3



Individual chromosome views of data shown in Figure 1. Chromosomal coverage profiles in samples from case 3 taken at different intervals of time. The gray dots show the normalized coverage, and the solid colored lines show smoothed profiles (obtained from the median values across 31 genomic 100-kilobase bins). The upper panel is from the sample taken during pregnancy at 13 weeks of gestation. The middle panel is after delivery, immediately before surgical resection of an obstructing colorectal tumor. The lower panel is after delivery, following completion of chemotherapy and radiation. The x-axis shows the physical location of the increased counts as mapped against an ideogram for

chromosomes 13 or 8 (SNPchip package, R version 3.1.2; resolution = 1 megabase pair). Chromosome 8 is included because it served as one of the reference chromosomes and contributed to the monosomy 18 classification in the postdelivery sample (see Table 1). The y-axis shows the percentage of signal above or below baseline corresponding to a diploid genome ( $y = 1.0$ ). As an example, in the middle-right panel (chromosome 8 after delivery), the data at the highest peak (indicated by the arrow) show that there is approximately 12% excess representation of this part of the genome compared with the reference. NIPT indicates noninvasive prenatal testing.

might not routinely be reported to the NIPT laboratory. Even cancers diagnosed during pregnancy would not necessarily trigger notification of the laboratory, especially if no aneuploidies had been detected by NIPT. The lower rate may also reflect that the chromosomal aneuploidies and the amount of apoptotic tumor cfDNA released into the maternal circulation could be below the detection limit at the time NIPT is performed. A recent study using sequencing to analyze plasma cfDNA in patients with known cancers<sup>23</sup> found evidence of abnormal cfDNA patterns in more than 80% of metastatic solid tumor cases and 50% of localized cancers. The rates of detection varied widely by tumor type.

Genome-wide bioinformatics analysis for the 8 reported cases revealed extensive copy-number changes involving several chromosomes and ranged from numerous focal amplifications or deletions to multiple whole chromosomes. These types of changes are more likely to be visible using a whole genome rather than a targeted sequencing approach. In addition, the visualized changes were reproducible in replicates obtained from the same blood sample, and the overall pattern of chromosomal changes was stable in samples taken many months apart.

Autosomal monosomies, and especially multiple-aneuploidy test results, are rarely identified in NIPT samples.

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**Table 2. Association of Maternal Cancers With Different Types of Aneuploidies Detected at Noninvasive Prenatal Testing**

Type of Aneuploidy Detected by NIPT	Total No. of Samples	No. of Known Maternal Cancers (%) [95% CI]
Single trisomy <sup>a</sup>	2650	2 (0.08) [0-0.27]
Single SCA <sup>b</sup>	950	0 (0) [0-0.39]
Single trisomy + SCA	30	0 (0) [0-11.5]
Single monosomy	88	1 (1.14) [0-6.1]
Multiple aneuploidy <sup>c</sup>	39	7 (17.9) [7.5-33.5]
Total abnormal NIPT	3757	10 (0.26) [0.12-0.48]

Abbreviations: NIPT, noninvasive prenatal testing; SCA, sex chromosome abnormality.

<sup>a</sup> Single trisomy refers specifically to trisomy of chromosomes 13, 18, or 21.

<sup>b</sup> Single SCA refers to the presence of 1 of the sex chromosome aneuploidies: Turner syndrome (monosomy X), Klinefelter syndrome (XXY), XYY syndrome, or trisomy X (XXX).

<sup>c</sup> The multiple aneuploidy category includes every other combination of autosomal and/or sex chromosome aneuploidy except single trisomy and SCA as noted in the Table.

For this reason, NIPT results demonstrating a single autosomal monosomy or multiple aneuploidies may warrant a more detailed analysis of the whole genome using an advanced bioinformatics review process to determine if a pattern suggestive of malignancy is present.

To date, there have been 3 individual reports of pregnant women with abnormal NIPT results and chromosomally normal fetuses in which the discordant results were explained by the presence of maternal malignancies (metastatic small cell neuroendocrine carcinoma of vaginal origin,<sup>13</sup> lymphomas,<sup>14,24</sup> and ovarian carcinoma<sup>24</sup>) in which tumor DNA was presumably shed into the maternal circulation and detected at the time of noninvasive prenatal testing. The data presented here underscore the necessity of performing a diagnostic procedure to determine the true fetal karyotype whenever NIPT results reveal chromosomal abnormalities.

Many genetic counselors and obstetricians are concerned that an NIPT result of multiple aneuploidies or autosomal monosomy may be suggestive of maternal cancer. When there is discordance between the fetal karyotype and NIPT result, occult maternal malignancy, although very uncommon, may be an explanation for the findings. Based on the results of the study, we estimate there is between a 20% and 44% risk of maternal cancer if multiple aneuploidies are detected. However, until further studies are done to assess the clinical implications of discordant NIPT and fetal karyotype results, it is not clear what, if any, follow-up clinical evaluation is appropriate.<sup>24</sup>

All 8 women in this case series were asymptomatic at the time of their NIPT test. In 3 cases, the NIPT results prompted the diagnosis of malignancy. Whether earlier detection of disease would have made a difference in the course of their illnesses cannot be determined. Cases 1 and 3 presented with advanced symptoms, and their clinicians stated that for them, earlier diagnosis would have had a positive effect on their care.

Limitations of this study include its small size and retrospective design, incomplete clinical follow-up information, potential for bias of ascertainment in the way that the cancer diagnoses were reported back to the clinical laboratory, and the evolving nature of the technical parameters, especially in the bioinformatics analyses over 2.5 years.

## Conclusions

In this preliminary study, a small number of occult malignancies were subsequently diagnosed among pregnant women whose noninvasive prenatal testing results showed discordance with the fetal karyotype. The clinical importance of these findings will require further research to determine appropriate follow-up for the mother and her infant.

### ARTICLE INFORMATION

**Published Online:** July 13, 2015.  
doi:10.1001/jama.2015.7120.

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**Author Contributions:** Dr Bianchi had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Bianchi, Chudova, Sehnert, Murray, Halks-Miller.

**Acquisition, analysis, or interpretation of data:** All authors.

**Drafting of the manuscript:** Bianchi, Chudova, Sehnert, Murray, Goldberg, Halks-Miller.

**Critical revision of the manuscript for important intellectual content:** All authors.

**Statistical analysis:** Chudova, Sehnert, Bhatt.

**Administrative, technical, or material support:**

Bianchi, Murray, Prosen, Garber, Wilkins-Haug, Vora, Warsof, Goldberg, Ziainia, Halks-Miller.  
**Study supervision:** Bianchi, Chudova, Sehnert, Bhatt, Halks-Miller.

**Conflict of Interest Disclosures:** All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Bianchi reported being a member of the Reproductive and Genetic Health Expert Advisory Panel of Illumina, for which she receives an honorarium, and having received sponsored

research funding from Illumina that is administered through Tufts Medical Center. Drs Chudova, Sehnert, Bhatt, and Halks-Miller reported being full-time employees of Illumina. Ms Murray reported having served on the speakers' bureau for Myriad Genetics. Dr Prosen reported being a member of the Illumina speakers' bureau. Dr Garber reported having received sponsored research funding from Myriad Genetics and Novartis and serving as a consultant for Pfizer and Sequenom. Dr Wilkins-Haug reported having received sponsored research support from Ariosa and Sequenom. No other disclosures were reported.

**Funding/Support:** Funding for the study was provided by Illumina. Sponsored research funding from Illumina that is administered through Tufts Medical Center paid for the time that Dr Bianchi spent working with the full-time Illumina employees to design the study, analyze the data, and prepare the manuscript.

**Role of the Funder/Sponsor:** Illumina reviewed and approved submission of the manuscript for publication (as per their internal policies), but they did not make any changes to the manuscript. Illumina had no role in the design and conduct of

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the study; collection, management, analysis, and interpretation of the data; or preparation of the manuscript.

**Additional Contributions:** We thank the case patients whose data are reported herein for giving permission to publish their information. They were gracious and generous with their time and follow-up information with the hope of helping others. We also thank Shannon X. Jeddi, CGC, for her help in obtaining medical record information. She received no financial compensation. We also thank Richard Rava, PhD, for his scientific advice; he was not compensated for his contribution besides salary.

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