

Incidental Detection of Maternal Neoplasia in Noninvasive Prenatal Testing

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BACKGROUND: Noninvasive prenatal testing (NIPT) uses cell-free DNA (cfDNA) as an analyte to detect copy-number alterations in the fetal genome. Because maternal and fetal cfDNA contributions are comingled, changes in the maternal genome can manifest as abnormal NIPT results. Circulating tumor DNA (ctDNA) present in cases of maternal neoplasia has the potential to distort the NIPT readout to a degree that prevents interpretation, resulting in a nonreportable test result for fetal aneuploidy.

METHODS: NIPT cases that showed a distortion from normal euploid genomic representation were communicated to the caregiving physician as nonreportable for fetal aneuploidy. Follow-up information was subsequently collected for these cases. More than 450 000 pregnant patients who submitted samples for clinical laboratory testing >3 years are summarized. Additionally, in-depth analysis was performed for >79 000 research-consented samples.

RESULTS: In total, 55 nonreportable NIPT cases with altered genomic profiles were cataloged. Of these, 43 had additional information available to enable follow-up. A maternal neoplasm was confirmed in 40 of these cases: 18 malignant, 20 benign uterine fibroids, and 2 with radiological confirmation but without pathological classification.

CONCLUSIONS: In a population of pregnant women who submitted a blood sample for cfDNA testing, an abnormal genomic profile not consistent with fetal abnormalities was detected in about 10 out of 100 000 cases. A subset of these observations (18 of 43; 41.9%) was attributed to maternal malignant neoplasms. These observational results suggest the need for a controlled trial to

evaluate the potential of using cfDNA as an early biomarker of cancer.

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Circulating cell-free DNA (cfDNA)⁵ originates predominantly from apoptotic white blood cells and typically reflects the euploid chromosome state of its donor (1). In certain instances, a genetically different cell population hosted within the same person may also contribute to the pool of total cfDNA. Two well-described examples include cell-free fetal DNA originating from placental cells during pregnancy (2) and circulating tumor DNA (ctDNA) released from tumor cells into the bloodstream (3–6). Therefore, in a pregnant woman harboring a tumor, there is a possibility that both cell-free fetal DNA and ctDNA would be contributing to total cfDNA. Although rare, malignancies during pregnancies have been reported (7–9). Incidental detection of maternal neoplasms during noninvasive prenatal testing (NIPT) will be discussed.

Materials and Methods

SAMPLES

Maternal blood samples (approximately 10 mL) were collected in Streck BCT tubes primarily from patients with high-risk pregnancies and were submitted to CLIA-certified, College of American Pathologists accredited clinical laboratories (San Diego, CA and Morrisville, NC) for DNA-sequencing-based screening for fetal aneuploidy by use of a validated laboratory-developed test (LDT). In this LDT, cfDNA is purified from maternal plasma and subjected to next-generation sequencing to determine the presence of aneuploidy, manifested as an overrepresentation of chromosome-specific sequences relative to a reference euploid sample. A high-risk preg-

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⁵ Nonstandard abbreviations: cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; NIPT, noninvasive prenatal testing; LDT, laboratory-developed test; CNAs, copy-number alterations.

nancy is defined as one with advanced maternal age, abnormal ultrasonography findings, abnormal serum screen result, or personal/familial history.

NEXT-GENERATION SEQUENCING

Anticoagulated blood samples were subjected to plasma fractionation, DNA extraction, library preparation, and next-generation sequencing as previously described (10–13). When needed for clinical decision-making, or for in-depth retrospective analysis (research-consented samples only), genome-wide chromosomal representations were evaluated.

RESULTS REPORTING

Sequencing data were used to calculate Z -scores, which are estimates of normalized chromosomal representation compared with a euploid genome. Trisomy of chromosome 21, 18, or 13 (3 of the analytes offered in the LDT) was identified when the Z -score for the respective chromosome was increased. When multiple chromosomal and/or subchromosomal abnormalities were observed (i.e., a sample showing abnormal Z -scores for both chromosomes 21 and 18), the test outcome was “nonreportable” for fetal aneuploidy, and a possibility of ctDNA confounding the NIPT result was hypothesized. It is important to emphasize that the laboratory does not report a finding of neoplasia to the physician because the LDT is not clinically or analytically validated for cancer detection. In such cases, the laboratory director reviewing the case assumes the role of a clinical consultant and discusses the findings with the caregiving physician, who identifies the most appropriate management for the patient.

DATA ANALYSIS: CLINICAL COHORT

NIPT measures fetal aneuploidy by determining the over- or underrepresentation of each chromosome of interest in maternal plasma. The chromosome Z -score measures the differential representation, in standard deviation units, as compared to a plasma sample from a woman carrying a euploid fetus. The algorithm calculating the Z -score assumes that the chromosome complement of the pregnant woman is 46, XX. In our laboratory, a Z -score of 3 or higher is considered significant if the sample passes quality metrics. The NIPT used in this patient cohort reported on chromosomes 21 (Down syndrome), 18 (Edward syndrome), 13 (Patau syndrome), 16, and 22; sex chromosomes (X and Y); and a set of subchromosomal copy-number alterations (14). When a true fetal trisomy for a specific chromosome is observed, the Z -score for that chromosome is above the threshold, but the Z -scores for other analytes are within normal limits. For example, a sample positive for trisomy 13 might have a Z -score of 8 for chromosome 13, but the Z -scores for chromosome 21 and 18 would be between -3.0 and 2.99 . When multiple Z -scores are significantly

increased or decreased, additional genome-wide data review is considered before issuing a result. Since multiple such abnormalities do not describe any specific syndrome in the fetus (the main indication of NIPT), a nonreportable result is issued, and the findings are communicated by the laboratory director to the ordering physician through peer-to-peer communication in a HIPAA compliant fashion; maternal neoplasia is mentioned during this communication as one potential explanation for the unusual findings.

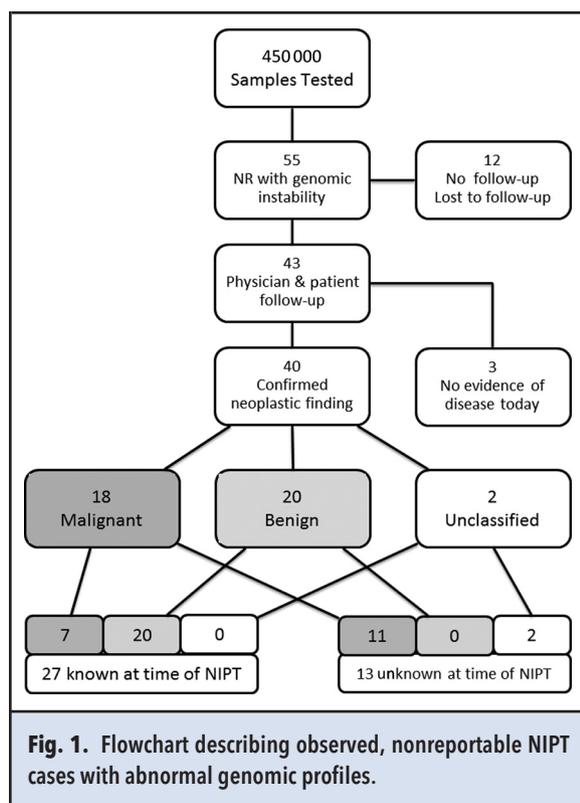
DATA ANALYSIS: RESEARCH COHORT

Because it is reasonable to suggest that not all neoplasms will have a copy-number alteration (CNA) affecting chromosomes 13, 18, or 21, we developed a novel algorithm to identify additional neoplasms with CNAs located elsewhere in the genome. To do this, we first trained our algorithm on the basis of 31 of the known cases of neoplasia discussed herein. The sequence characteristics of these samples were used within a principal component-like cluster analyses that identifies outlier behavior conditional on the overall CNA features. Specifically, principal components describe the severity of CNAs from high-dimensional data into low-dimensional space. We subsequently used the features identified in the training cohort to identify samples that had a genomic CNA profile consistent with a malignancy and identified those as being likely derived from a neoplasm.

Results

During >3 years of noninvasive testing for fetal aneuploidies, we identified 55 samples with Z -scores increased or decreased across multiple genomic regions (Fig. 1). These findings prompted additional genome-wide data review, which typically revealed widespread and profound CNAs. In these cases, no result for fetal aneuploidy could be issued, but a clinical consultation was warranted. The discussion between the laboratory director and the physician focused on the technical description of the sequencing-based observations, and touched on possible explanations for the unusual findings: analytical challenges associated with the sample and its processing and biological reasons including, but not limited to, systemic lupus erythematosus (15) and neoplastic conditions, either benign or malignant. No diagnosis was offered.

As a result of these consultations, clinical follow-up was obtained for most patients. In 43 of the 55 cases, additional testing was performed; for the remaining 12 cases, no further clinical outcome was received, as patients were either not available for follow-up or no information was available (Table 1; see Table 1 in the Data Supplement that accompanies the online version of this article at <http://www.clinchem.org/content/vol64/issue2>). In total, 40 of the 43 cases that underwent additional



testing had a confirmed neoplastic finding (93%); in the remaining 3 cases, no evidence of disease was found at the time this report was compiled. In 1 of the 40 cases that underwent additional diagnostic testing, approximately 6 months passed between the abnormal cfDNA findings and a pathological diagnosis of stage I Hodgkin lymphoma (see Table 1 in the online Data Supplement; patient 1). The group of 40 diagnostically confirmed neoplastic events were stratified as follows: 18 malignant cases and 20 benign cases. For 2 cases, a mass was observed by radiologic examination and deemed to be neoplastic in origin; however, no tissue diagnosis was available (Table 1).

Within this set of 40 maternal neoplasms, gains and losses of genetic material were found on most chromosomes. This cohort size, however, is inadequate to explore the relationship between tumor types and specific CNA profiles. In this sample set with significantly aberrant *Z*-scores, the confirmed neoplastic conditions originated from or affected the reproductive system, lymph nodes, blood, breast, colon, and various soft tissues (see Table 1 in the online Data Supplement).

KNOWN NEOPLASIA

For 27 of the 40 samples (all 20 leiomyoma and 7 malignant cases), an existing neoplastic condition was known to the ordering physician (but not to the clinical labora-

tory) at the time of testing, and the CNAs could be readily associated with these clinical diagnoses (Fig. 1). For example, after clinical consultation about a sample with genome-wide CNAs, we became aware of a case of invasive ductal carcinoma of breast. Compared with a sample with a euploid NIPT result (Fig. 2A) with normalized genome-wide trace superimposed on the baseline, and a sample with trisomy 13 result showing an increase of only chromosome 13 trace (Fig. 2B), the breast carcinoma sample showed striking genome-wide changes involving multiple chromosomes (Fig. 2C). Similar genome-wide CNA profiles are shown for cases of follicular lymphoma and esophageal carcinoma (Fig. 2, D and E).

UNKNOWN NEOPLASIA

In the remaining 13 out of 40 samples (11 malignant and 2 unclassified cases), no neoplasm had been diagnosed at the time of NIPT (Fig. 1). In this cohort, additional exploratory clinical testing was performed by the caregiving physician, which led to identification of neoplastic conditions and subsequent primary diagnoses. For example, in 1 case, communication of the NIPT (nonreportable) result with simultaneous clinical consultation concerning the genomic observations contributed to timely decision-making and clinical management of a patient (see Table 1 in the online Data Supplement; patient 14). This patient had suffered from several months of vague abdominal pain and constipation with no primary diagnosis. On learning of the observed global genomic abnormalities on NIPT performed at 10 weeks of gestation (Fig. 3A), she and her physician decided to proceed with an MRI examination that identified a 7 cm sigmoid colon mass pathologically consistent with well to moderately differentiated adenocarcinoma. The patient underwent surgical resection of the mass at 15 weeks of gestation. A postsurgical blood sample drawn at 27 weeks of gestation was subjected to the same genomic profiling analysis at the request of the patient and her physician. This showed the complete disappearance of all the chromosomal aneuploidies detected in the presurgical blood specimen (Fig. 3B) and allowed an NIPT result of the euploid male fetus to be reported. Subsequently, the patient delivered a healthy male infant. In similar fashion, other patients with nonreportable NIPT result were subsequently diagnosed with Hodgkin lymphoma, pleomorphic leiomyosarcoma of the uterus, right fallopian tube mass, non-Hodgkin lymphoma of the thyroid gland, epithelioid angiosarcoma of the retroperitoneum, and small-cell carcinoma of the anterior vaginal wall (see Fig. 1, A–F in the online Data Supplement). Given that these were incidental findings and that patients were subsequently referred to other physicians or oncologists, we were unable to obtain detailed clinical history and outcome on most cases. Our clinical correlations were highly dependent on telephonic conversations with the obstetri-

Table 1. Individual number of cases by category.		
Category	Clinical diagnosis	No. of cases
Malignant neoplasms		
	Hodgkin lymphoma	4
	Follicular lymphoma	1
	Non-Hodgkin lymphoma	1
	Cutaneous T-cell lymphoma	1
	Multiple myeloma	1
	Chronic myeloid leukemia	1
	Breast carcinoma	4
	Colon carcinoma	1
	Epithelioid angiosarcoma of retroperitoneum	1
	Uterine leiomyosarcoma	1
	Small-cell carcinoma of anterior vaginal wall	1
	Esophageal carcinoma	1
Benign neoplasms		
	Uterine leiomyoma	20
Unknown		
	Fallopian tube mass	1
	Ovarian teratoma (dermoid cyst)	1
Lost to follow-up		12
No history of neoplastic condition		3
Total		55

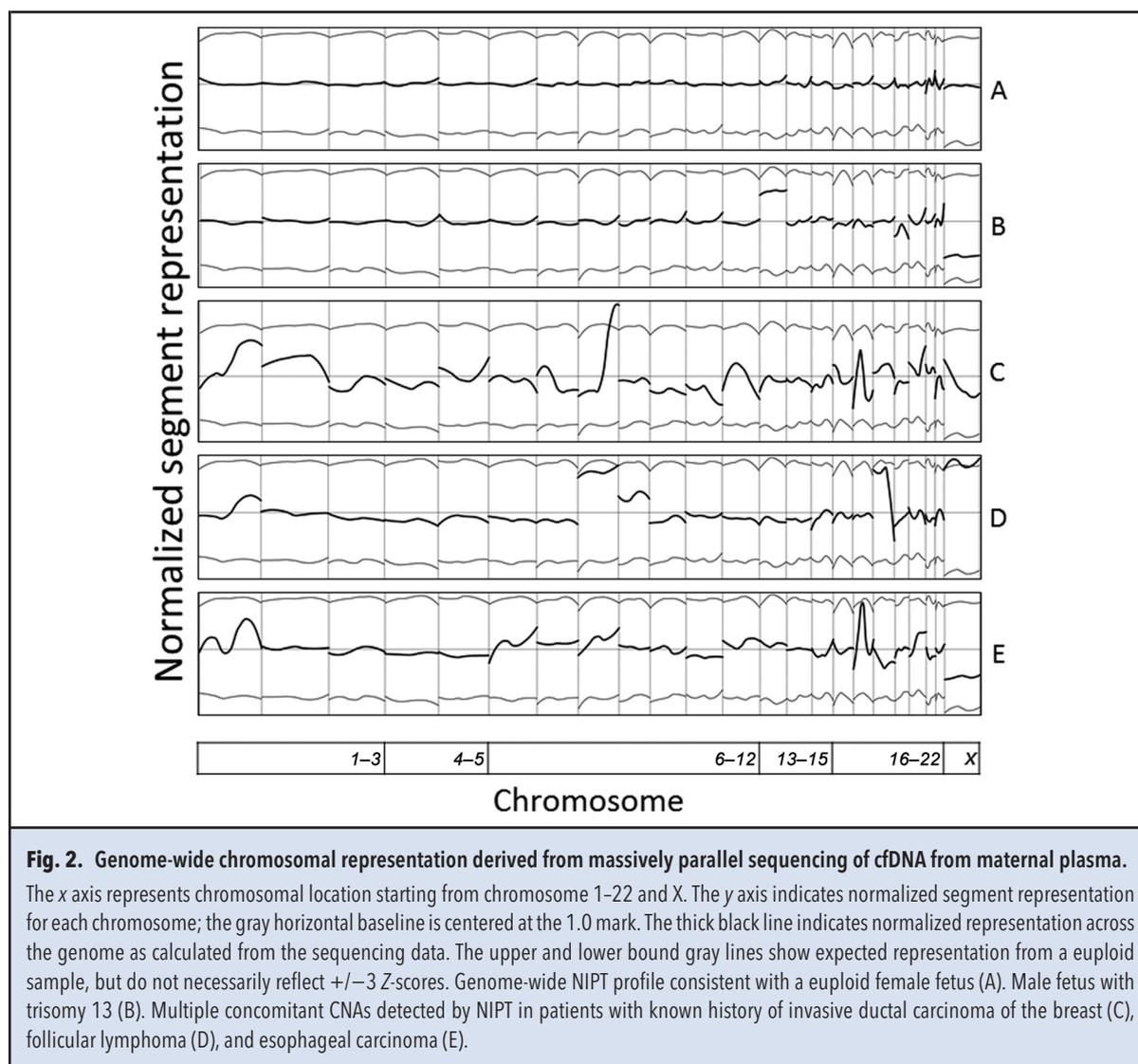
cian or maternal fetal medicine physician who ordered NIPT. Because these samples were analyzed as part of routine clinical care and not as a part of a formal study protocol, we were unable to obtain detailed outcome data for most of these patients.

LEIOMYOMAS

Uterine leiomyomas comprised a large proportion (20 of 40; 50%) of this cohort (Table 1). Although considered benign in nature, leiomyomas are monoclonal in origin and are suspected to undergo malignant transformation in rare instances. CNAs have been described for leiomyomas and cover a broad range of chromosome regions, including deletions of 1p, 1q, 3q, 7q, 11q, 13, 15q, 17p, 22q, and 14q (16–19). In one previously published case of uterine leiomyoma, microarray analysis of DNA from a tissue biopsy from the leiomyomectomy specimen was available and was found to mimic the CNAs observed in cfDNA extracted from the maternal plasma specimen. Similar to the colon adenocarcinoma case described above, postpartum evaluation of circulating cfDNA after surgical removal of the leiomyoma demonstrated no significant genomic deviations, consistent with the hypothesis that the aberrant CNAs were due to the uterine leiomyoma (20).

RESEARCH COHORT

In the aforementioned samples, an in-depth data review of the entire genome was performed when Z -scores for 2 or more core chromosomes (21, 18, and 13) were abnormal. It is reasonable to assume that CNAs associated with neoplastic changes will also involve chromosomes other than 21, 18, and 13 (21, 22); however, the Z -scores of chromosomes 21, 18, and 13 can be altered even when they are not directly affected. When a Z -score is calculated, the totality of all chromosomes serve as a reference; large chromosomal aberrations can sufficiently alter this reference and therefore increase or depress other Z -scores. An in silico simulation revealed that a typical q-arm deletion at a 25% fraction of circulating DNA is sufficient to increase the Z -scores of chromosomes 21, 13, and 18 above the relevant threshold. In addition, we developed a novel, systematic genome-wide approach for CNA detection to account for cases that did not directly or indirectly affect the 3 measured core chromosomes (21, 18, and 13). We used 31 samples with confirmed neoplasia to derive sequencing characteristics that are sensitive and specific predictors for CNAs. We applied this algorithm to a cohort of 79 407 samples that had been consented for additional research beyond the clinical NIPT reporting; this cohort included 7 of the confirmed neoplasia sam-



ples. The algorithm detected 10 samples, including all 7 of the confirmed neoplastic samples that exhibited high degrees of genomic instability as evidenced by multiple CNAs. For the additional samples, unfortunately no clinical validation data were available, making the results for these samples somewhat speculative. Based on these findings, the estimated incidence for pregnancy-associated neoplasia is 25–29 per 100 000. This is consistent with previous studies evaluating the incidence rates of all cancers diagnosed during pregnancy, 16–25 per 100 000 (7, 23–26).

Discussion

NIPT has been developed to identify specific chromosomal CNAs in a cfDNA mixture comprised of a minority of fetal and a majority of maternal DNA. It is not

surprising that the same concepts apply when a different source, such as neoplastic tissue, contributes ctDNA to euploid maternal background cfDNA. The incidental identification of an association between aberrant NIPT findings and neoplasia has been previously reported (8, 9, 27). Of particular interest, the largest previously published cohort comprised 10 cases of occult maternal malignancies among 125 426 NIPT samples (9). The current report represents the largest case series published to date, with 40 confirmed neoplastic cases in a cohort of >450 000 pregnancies undergoing routine clinical NIPT, a rate consistent with previously published literature.

Special attention should be paid to the findings in cases of uterine leiomyomas, the most common example of neoplasms observed in this study (20 out of 40 cases). Considering the high prevalence of uterine leiomyomas in the first trimester of pregnancy (approximately 10%)

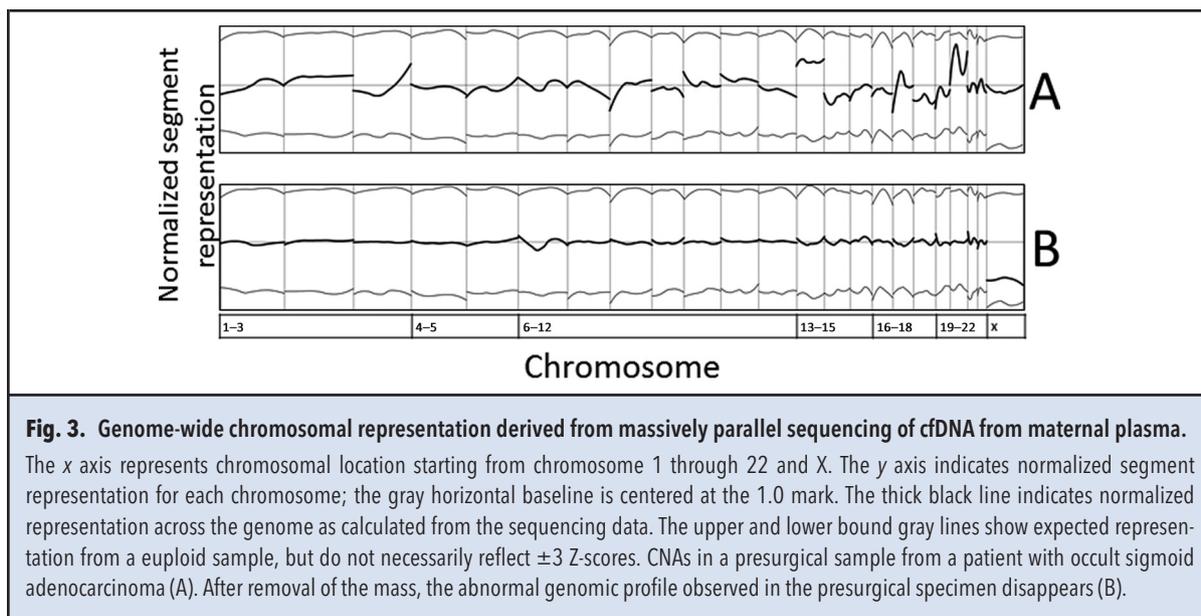


Fig. 3. Genome-wide chromosomal representation derived from massively parallel sequencing of cfDNA from maternal plasma.

The x axis represents chromosomal location starting from chromosome 1 through 22 and X. The y axis indicates normalized segment representation for each chromosome; the gray horizontal baseline is centered at the 1.0 mark. The thick black line indicates normalized representation across the genome as calculated from the sequencing data. The upper and lower bound gray lines show expected representation from a euploid sample, but do not necessarily reflect ± 3 Z-scores. CNAs in a presurgical sample from a patient with occult sigmoid adenocarcinoma (A). After removal of the mass, the abnormal genomic profile observed in the presurgical specimen disappears (B).

and the previously reported genomic instability for a subset of them, it is not surprising that they contributed to the set of detected maternal neoplastic conditions (28). Overall, however, aberrant genomic profiles associated with leiomyomas were detected at a relatively low prevalence in our cohort. This might be explained by the observation that many cytogenetic abnormalities in uterine fibroids are balanced translocations, which do not lead to altered Z-scores (29). Furthermore, it is unclear how much DNA is shed from these benign neoplasms into the circulation, and how many of these may be in the process of undergoing malignant transformation. Finally, differentiation of benign and malignant leiomyomas by use of the detected CNA profiles has not been evaluated as part of this study and would benefit from being directly studied as part of prospective clinical trials.

Much larger systematic evaluations are needed to determine which tumor types and stages lend themselves best to assessment using cfDNA analysis. While lack of this information limits our ability to make accurate predictions about the detectability of tumors or the corresponding expected sensitivity of a clinical laboratory test, we can now estimate that a test of this nature will have a high specificity. The analysis of a research cohort of >79 000 samples indicated a detection rate that is slightly reduced relative to previously published incidence rates. Especially in light of recent data that indicate no adverse pediatric outcome after chemotherapy treatment during pregnancy, a high performing screening test could be very compelling (30).

While it is too early to suggest cfDNA screening should be used as a routine public health tool, further research is warranted to investigate which groups of pa-

tients may derive benefit from it. In this report, the studied population was pregnant women. Because we are not aware of any biological mechanism that exists in pregnancy that alters the release of tumor DNA, it can be reasonably assumed that these findings can be extended to other populations. For a screening test to be effective, the detection of the condition must result in an opportunity to change the outcome. In this report, the detection of neoplasia preceded or coincided with the onset of symptoms in 13 of the 40 cases. While large-scale studies will be required to ultimately determine if cfDNA testing can be an effective general screening tool for asymptomatic individuals, more targeted studies in high-risk populations could be a good preliminary investigative step. In Hong Kong, 1 study investigated populations of chronic hepatitis patients with remarkable success in predicting liver tumors (31). The same group also reported detection of CNAs in plasma DNA of patients with breast cancer, lung cancer, nasopharyngeal cancer, smooth muscle sarcoma, and neuroendocrine tumors (32). A similar approach was used in detecting genomic imbalances in Reed–Sternberg cells in Hodgkin lymphoma (33). Other high-risk candidate populations could include longtime smokers, workers exposed to benzidine, and patients with hereditary cancer syndromes.

Detection of genomic abnormalities suggestive of a neoplastic condition following NIPT currently triggers more questions than answers, with important implications for the informed consent process for women contemplating NIPT (34). The data in this report, however, suggest a significant longer-term opportunity for disease detection and monitoring through cfDNA analysis. In our experience to date, we found the putative detection

rate in the study population to be comparable to previous reports of cancer incidence during pregnancy. Furthermore, the relative rarity of the described findings is consistent with a predicted high specificity. Taken together, these data warrant further investigation in large clinical trials, particularly in populations at high risk for cancer. Routine analysis of cfDNA holds considerable potential to shape the way early diagnosis of cancer is made in the future.

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