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Brief Report

Presymptomatic Identification of Cancers in Pregnant Women During Noninvasive Prenatal Testing

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IMPORTANCE Noninvasive prenatal testing (NIPT) for fetal aneuploidy by scanning cell-free fetal DNA in maternal plasma is rapidly becoming a major prenatal genetic test. Similar to placental DNA, tumor DNA can be detected in the plasma, and analysis of cell-free tumor DNA can be used to characterize and monitor cancers. We show that plasma DNA profiling allows for presymptomatic detection of tumors in pregnant women undergoing routine NIPT.

OBSERVATIONS During NIPT in over 4000 prospective pregnancies by parallel sequencing of maternal plasma cell-free DNA, 3 aberrant genome representation (GR) profiles were observed that could not be attributed to the maternal or fetal genomic constitution. A maternal cancer was suspected, and those 3 patients were referred for whole-body diffusion-weighted magnetic resonance imaging, which uncovered an ovarian carcinoma, a follicular lymphoma, and a Hodgkin lymphoma, each confirmed by subsequent pathologic and genetic investigations. The copy number variations in the subsequent tumor biopsies were concordant with the NIPT plasma GR profiles.

CONCLUSIONS AND RELEVANCE We show that maternal plasma cell-free DNA sequencing for noninvasive prenatal testing also may enable accurate presymptomatic detection of maternal tumors and treatment during pregnancy.

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Over the past years, noninvasive prenatal testing (NIPT) for fetal aneuploidy detection has become a clinical reality.[1] Most NIPT providers focus on the detection of only the most common aneuploidies, trisomies 21, 18, and 13. However, random genome sequencing not only enables the detection of the viable fetal trisomies but also other chromosomal aneuploidies and even segmental fetal imbalances.[2,3] Applying a similar large parallel sequencing approach to plasma-derived DNA from patients with cancer has recently been shown to detect tumor-associated copy number profiles in selected tumors prone to copy number changes.[4-6] We optimized a large parallel sequencing-based NIPT dataset and analysis, which not only interrogates the common trisomies but also allows the genomewide discrimination of fetal and maternal segmental aneuploidies.[3]

All patients undergoing NIPT consented to release of information for study purposes beyond trisomy 13, 18, and 21; this consent and the study protocol were approved by the University Hospitals, Leuven ethical board. Of the first 4000 prospective NIPT samples, we identified 3 profiles with an aberrant quality score and reproducible genomewide representation (GR) profiles reminiscent of cancer-related copy number variation. All 3 women (and only those 3 women) were referred for whole-body diffusion-weighted magnetic resonance imaging (WB-DWI), which revealed a tumorous mass in all 3 cases (eMethods and eFigure in the Supplement).

Report of Cases

Case 1

Bilateral ovarian carcinoma with diffuse peritoneal spread was detected in a pregnant woman who underwent NIPT, along with retroperitoneal lymphadenopathies and the presence of bilateral pleural fluid, consistent with ovarian cancer, FIGO (International Federation of Gynecology and Obstetrics) stage IV-A (**Figure 1**A). The pathological examination confirmed the presence of a high-grade serous ovarian carcinoma with multiple metastases to the omentum (14-mm), the paracolic peritoneum, and the appendix, as well as implants on the small bowel. In addition, 12 of 30 sampled lymph nodes tested positive for tumor cells.

To confirm that the abnormal GR profile was due to tumor-derived cell-free DNA (cfDNA), fluorescence in situ hybridization (FISH) was performed on tumor biopsy using probes for *IRF4*/6p24 (gained), *TCRB*/7q35 (gained), *JAK2*/9p24 (gained) and *BCL2*/18q21 (lost), which confirmed that the genomic imbalances identified in the cfDNA matched the gains and losses of the corresponding chromosomal regions in carcinoma cells (**Figure 2**).

Case 2

In a second woman who underwent NIPT, multiple supradiaphragmatic and infradiaphragmatic lymphadenopathies were revealed on subsequent WB-DWI, as well as diffusion restriction at the spleen and left tonsil, corresponding to Ann Arbor stage III-SE disease (Figure 1B).

An excision biopsy from the involved left tonsil indicated follicular lymphoma (FL), grade 3a ($CD5^-$, $CD10^+$, $CD20^+$, $BCL2^+$, and $Ki67^+$). Cytogenetic analysis of the biopsy material detected an abnormal karyotype in 13 of 20 analyzed cells, described as follows: 48,XX,i(6)(p10),dup(7)(q11q22),+dup(7)(q11q22),+11,dup(12)(q13q15),dup(13)(q21q34),t(14;18)(q32;q21). FISH testing with probe Vysis LSI *IGH/BCL2* (Abbott Molecular) detected the *IGH/BCL2* rearrangement resulting from the FL-characteristic t(14;18) in 49% of the analyzed interphase cells. Array comparative genomic hybridization (aCGH) analysis on DNA of the tumor biopsy confirmed the imbalances detected by cytogenetics (**Figure 3**), and most of the imbalances previously detected by cfDNA GR profiling strongly suggested that the GR profile was FL derived.

At a Glance

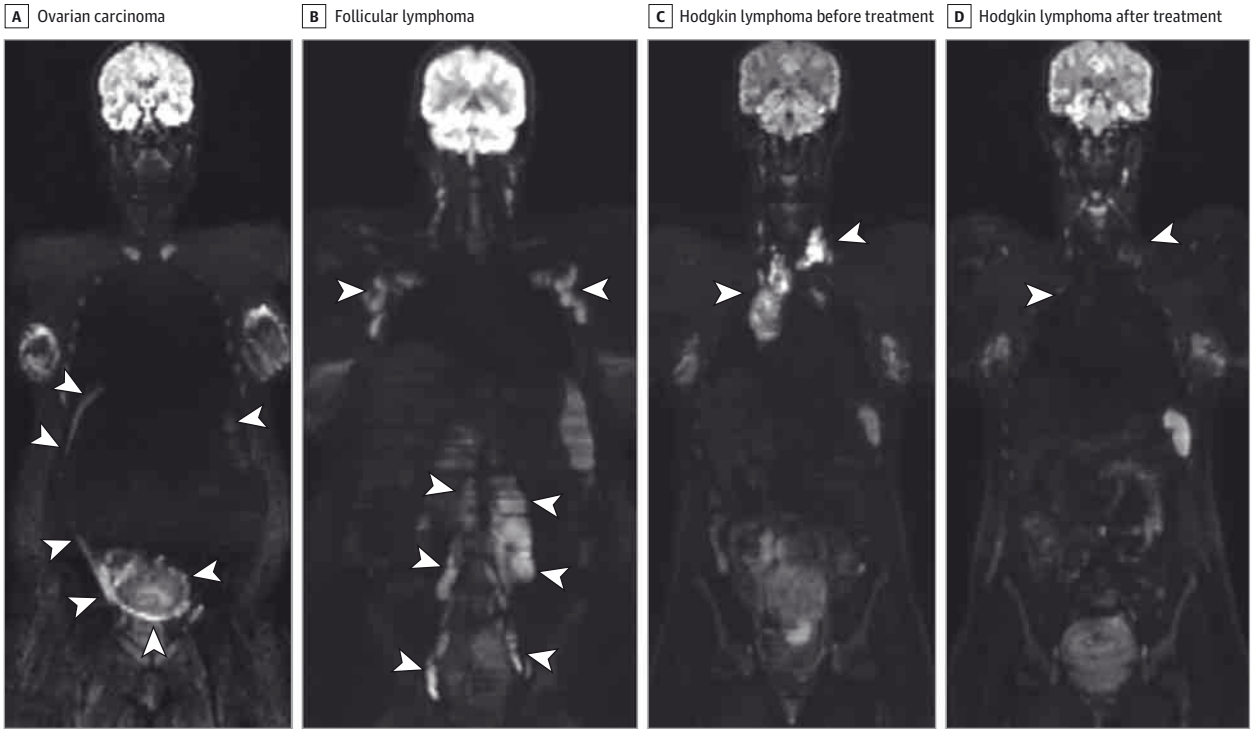
- Noninvasive prenatal testing (NIPT) enables presymptomatic cancer detection in pregnant women.
- Within a series of 4000 routine NIPTs, 3 women were referred for whole-body diffusion-weighted magnetic resonance imaging, and in all 3 cases, a cancer was detected.
- This analysis uncovered the presence of an ovarian carcinoma, a follicular lymphoma, and a Hodgkin lymphoma.
- Cancer detection during pregnancy enables treatment during pregnancy.
- These results suggest that presymptomatic populationwide cancer screening by genomic analysis of plasma DNA may become feasible.

Case 3

In a third woman who had undergone NIPT, subsequent WB-DWI revealed a mass in the anterior mediastinum and multiple lymphadenopathies in the left neck, while excluding involvement of bone marrow, spleen, or visceral organs, corresponding to Ann Arbor stage II disease (Figure 1C).

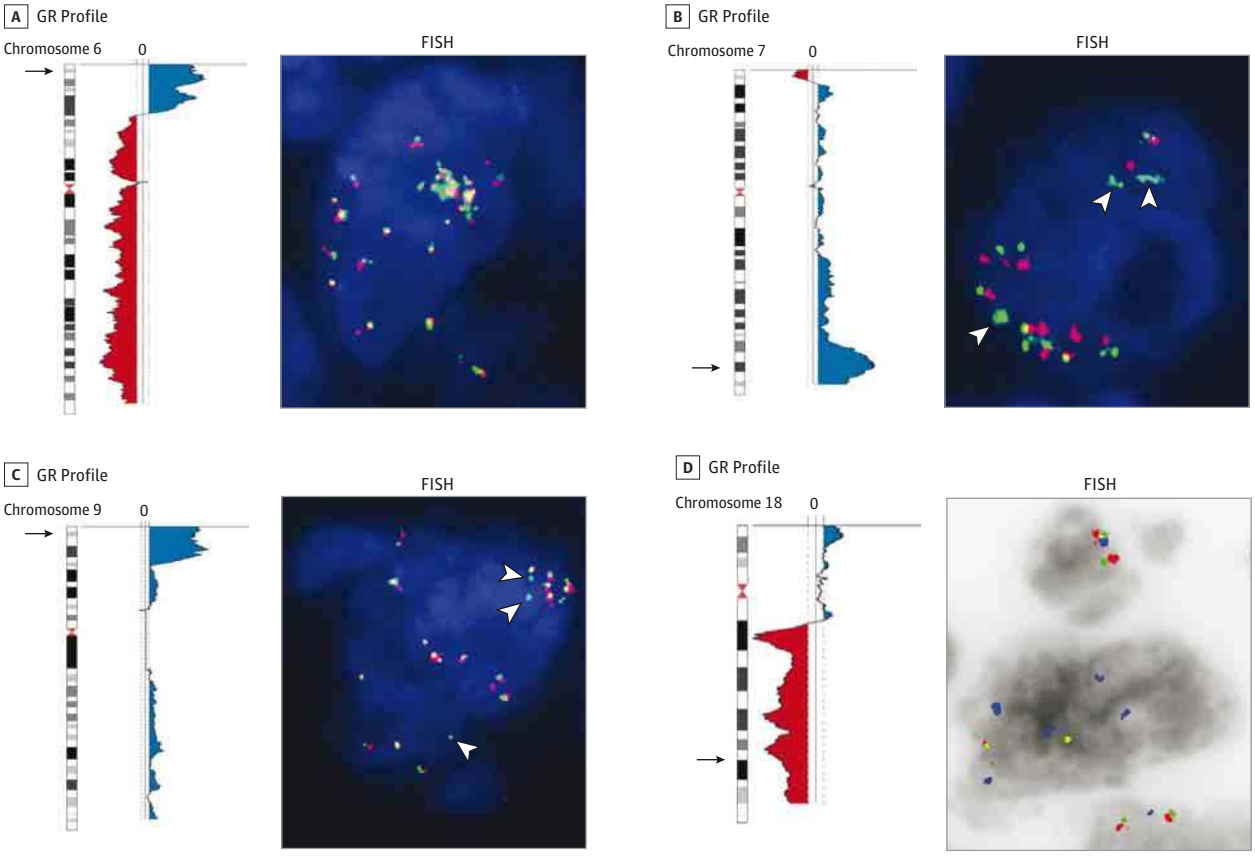
A transthoracic computed tomography–guided punch biopsy of the anterior mediastinal mass was performed. Pathological examination indicated a nodular sclerosis form of Hodg-

Figure 1. Whole-Body Diffusion-Weighted Magnetic Resonance Images



A, Ovarian carcinoma in patient 1. B, Follicular lymphoma in patient 2. C and D, Hodgkin lymphoma before (C) and after (D) treatment in patient 3. The arrowheads in all panels point to the tumor locations. D, Arrowheads point to areas of treatment response (complete remission).

Figure 2. Genome Representation (GR) Profile and Fluorescence In Situ Hybridization (FISH) Images of Ovarian Carcinoma (Patient 1)



A-D, All panels present both chromosome GR profiles (chromosomes 6, 7, 9, and 18; left panel halves) and validation by FISH analyses of large tumor cells in biopsy specimens (right panel halves). In the GR profiles, the positions of the examined genes on the cytoband are indicated by the arrows; the vertical graphs represent the actual GR profiles: dotted lines on either side of the axis, plus or minus 1.5x; red areas, likely deleted regions; and blue areas, likely duplicated or amplified regions. All FISH analyses were performed on 5-µm sections from snap-frozen biopsy tissue. The illustrated FISH probes include the following: A, *IRF4* (6p24) double-color probe (red and green), probe used to proof copy number aberrations, and CEP6 probe (green) (the CEP6 signals are hidden in the amplified *IRF4* area, hence no indicating arrowheads); B, *TCRB* (7q35) double-color probe (red and green), probe used to proof copy number aberrations, and CEP7 probe (green) (arrowheads); C, *JAK2* (9p24) double-color probe (red and green), probe used to proof copy number aberrations, and CEP9 (green) (arrowheads); and D, *BCL2* (18q21) double-color probe (red and green), probe used to proof copy number aberrations, and CEP18 probe (blue). The inverted coloring in panel D is required to visualize the blue probes (spectrum aqua) against the background. A-C, Note numerous and amplified signals of *IRF4*, *TCRB*, and *JAK2*, evidencing gain of these regions in tumor cells detected by GR profiling. D, Two *BCL2* and 5 CEP18 signals confirm loss of the 18q material in carcinoma cells.

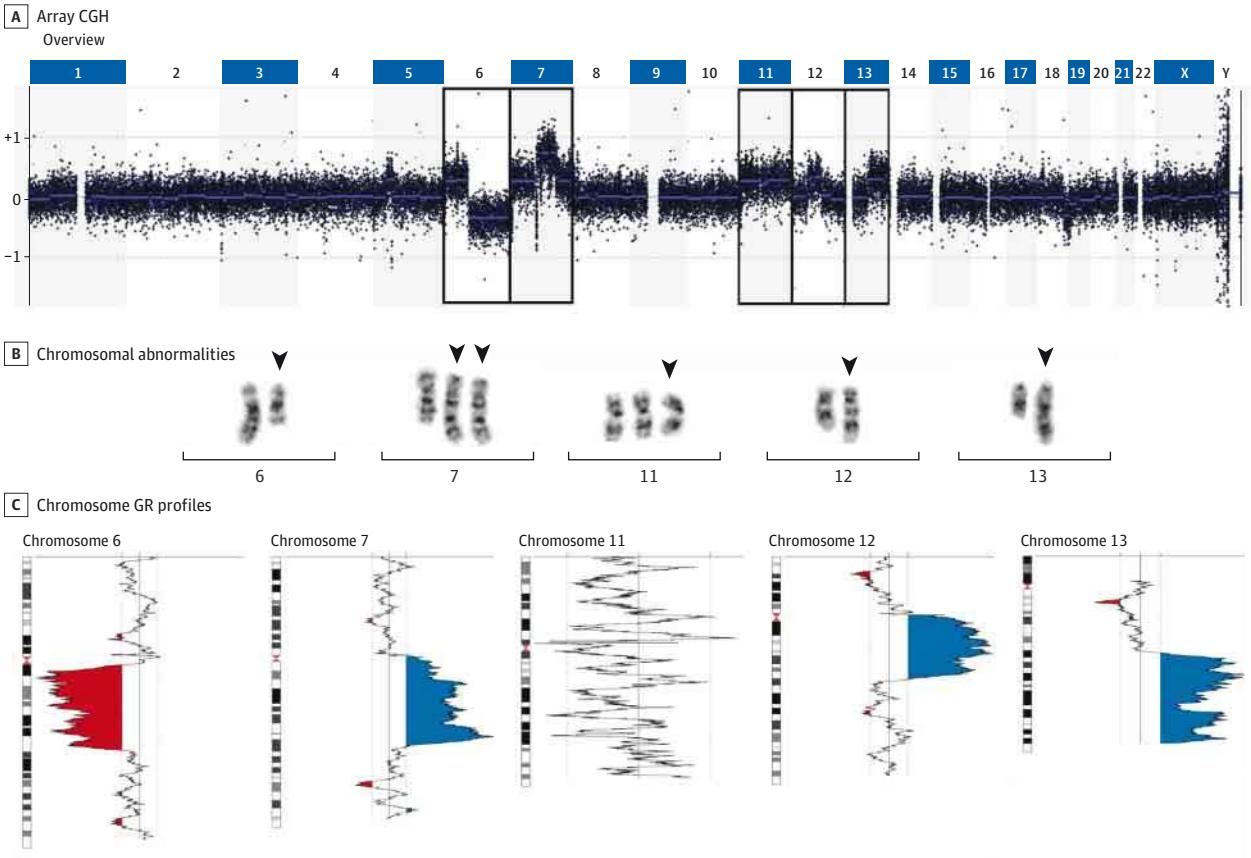
kin lymphoma characterized by the presence of $CD15^+/CD30^+$ neoplastic Hodgkin and Reed-Sternberg (HRS) cells. FISH analysis of the available formalin-fixed, paraffin-embedded tumor biopsy specimens using probes for *MYC*/8q24, *JAK2*/9p24, and *IGH*/14q32 (eTable in the Supplement) confirmed the copy number alterations in the HRS cells, thus indicating that the cfDNA GR profile matched the genomic imbalances in the HRS cells (**Figure 4**).

Interestingly, following blood sampling after the first chemotherapy administration, the aberrant GR profile had "normalized" (eFigure, axis E, in the Supplement), and the profile remained within normal parameters for all successive samplings. This finding led to a pilot study of cfDNA in a series of patients with Hodgkin lymphoma.[7]

Discussion

In 2 previous reports,[8,9] false-positive NIPT results have been attributed to metastatic cancer or uterine fibroids. Our report is the first to our knowledge of aberrant NIPT results prompting investigations that led to the diagnosis of cancer. The identification of 3 cancers in a prospective series of 4000 women is within the range expected from population cancer incidence, which is estimated at 1 per 1000 to 2000 person-years in 20- to 40-year-old women, suggesting that NIPT is a sensitive method to detect tumors characterized by chromosomal imbalances.[10,11] Since all 3 tumorlike cfDNA-derived GR profiles were confirmed by FISH or aCGH

Figure 3. Array Comparative Genomic Hybridization (CGH) Analysis, Chromosomal Abnormalities, and Genome Representation (GR) Profiles of Follicular Lymphoma (Patient 2)



A, In the array CGH analysis, the column labels represent the numbered chromosomes plus X and Y; the y-axis, represents the log2 of the intensity ratios; each graphed point, an array probe; and the boxed areas highlight the chromosomes detailed in panel B. B, Illustrated chromosomal abnormalities (arrowheads) related to the genomic imbalances in the GR profile of follicular lymphoma: [i(6)(p10) (gain of 6p/loss of 6q), dup(7)(q11q22), +dup(7)(q11q22) (gain of 7 and extra gain of 7q11q22), +11 (gain of 11), dup(12)(q13q15) (gain of 12q13q15), and dup(13)(q21q34) (gain of 13q21q34)]. C, The GR profiles of the 5 relevant chromosomes. The partial loss of 6q (but not the entire 6q) and lack of trisomy 7 and 11 in the GR profile of cell-free DNA is likely related to a subclonal/subregional appearance of these aberrations. For an explanation of the graphic conventions used in a GR profile, see the caption of Figure 2. As evidenced by chromosome 11 GR profile, no abnormalities were found.

on biopsy specimens, the test is also specific. A systematic referral to the oncology unit is warranted for those women with cancerlike GR profiles observed during NIPT on repeated sampling.

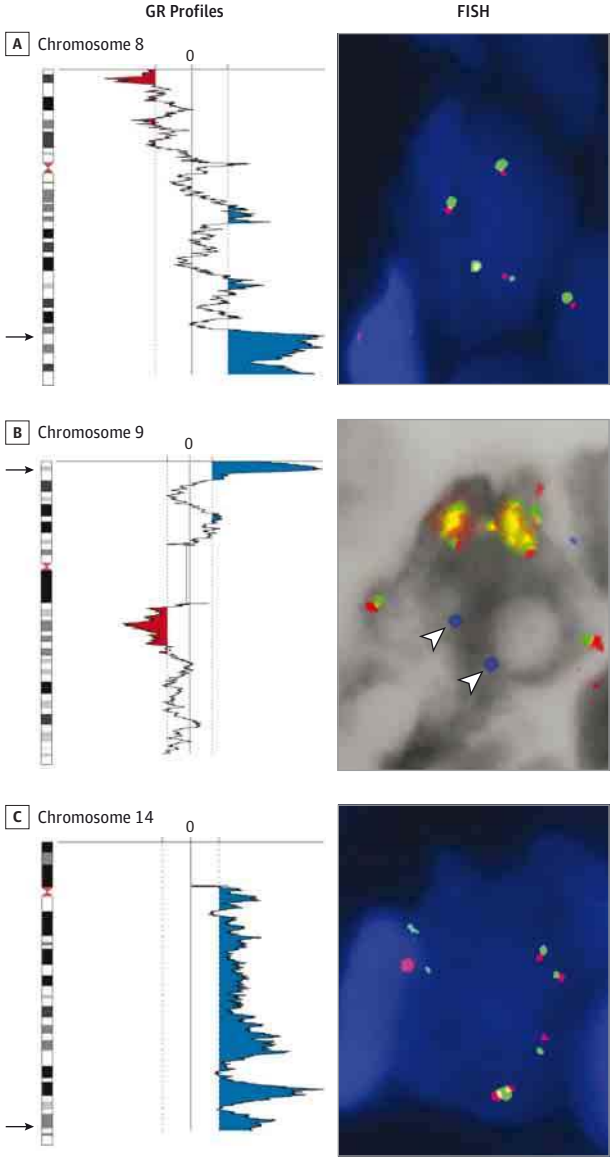
Given the current large scale implementation of NIPT to screen for fetal aneuploidies, it is surprising that there are not more reports of maternal cancers presymptomatically revealed by NIPT.[9] One explanation is that current NIPT analyses focus only on deviations of the viable trisomies 13, 18, and 21. However, our observations suggest that slight adaptations to NIPT analysis enabling the interrogation of (segmental) aneuploidies genomewide could not only avoid false-positive assignment of fetal aneuploidy due to the presence of a maternal cancer but, more importantly, enable identification of the imbalances as cancer-derived anomalies.

Cancer treatment, including chemotherapy, during pregnancy is an option without harming the fetus.[12,13] The prognosis of cancer during pregnancy is similar to the prognosis in nonpregnant women if standard treatment during pregnancy is applied.[14] Since cancer-related symptoms may be masked, especially during pregnancy, we consider the presymptomatic identification of maternal cancer as a potential added value of NIPT. Symptoms such as fatigue, nausea, abdominal pain, and vaginal blood loss can be misinterpreted as physiologic pregnancy-related symptoms.[15]

Of the 3 patients described herein, 2 (patients 1 and 3) underwent successful treatment. Patient 1 had treatment following delivery, and patient 3 underwent treatment during pregnancy without complications and subsequently gave birth to a healthy girl. Patient 2, diagnosed with follicular lymphoma, did not undergo treatment of the slow-growing entity of follicular lymphoma, which may not require treatment for many years.

The limitations of this case series include a small sample size and the detection of only 3 different cancer types, of which 2 were hematological. To address these limitations, we aim to further investigate the potential of NIPT for cancer detection, not only in pregnant women, but also in the general population.

Figure 4. Genome Representation (GR) Profile and Fluorescence In Situ Hybridization (FISH) Images of Hodgkin Lymphoma (Patient 3)



A-C, All panels present both chromosome GR profiles (chromosomes 8, 9, and 14; left panel halves) and validation by FISH analyses of Hodgkin and Reed-Sternberg cells from formalin-fixed paraffin-embedded biopsy tissue (right panels). In the GR profiles, arrows indicate the cytoband positions of the examined genes. For a further explanation of the graphic conventions used in a GR profile, see the caption of Figure 2. The illustrated FISH probes include the following: A, LSI *MYC* (8q24) double-color probe (red and green) and probe used to proof copy number aberrations; B, *JAK2* (9p24) double-color probe, probe used to proof copy number aberrations, and CEP8 (blue, marked with arrowheads); and C, LSI *IGH* (14q32) double-color probe and probe used to proof copy number aberrations. The presence of 4 *MYC* signals (A), 2 CEP8 signals and amplification of *JAK2* (B), and 6 *IGH* signals (C) confirms the gain of 8qter, 9pter, and chromosome 14 detected by GR profiling.

Conclusions

We show that maternal plasma cell-free DNA sequencing for the purpose of NIPT may enable the accurate presymptomatic detection of maternal tumors and treatment during pregnancy. However, the detection of cancer by genomic profiling need not be limited to pregnant women, and additional research on a large scale seems warranted.

ARTICLE INFORMATION

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Author Contributions: Drs Vermeesch had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Amant, Verheecke, Wlodarska, and Dehaspe contributed equally.

Study concept and design: Amant, Verheecke, Dehaspe, Brady, Moerman, Vergote, Putseys, Vandenberghe, Legius, Vermeesch.
Acquisition, analysis, or interpretation of data: Wlodarska, Dehaspe, Brady, Brison, Van Den Bogaert, Dierickx, Vandecaveye, Tousseyn, Vanderstichele, Vergote, Neven, Berteloot, Putseys, Danneels, Vandenberghe, Vermeesch.
Drafting of the manuscript: Amant, Verheecke, Wlodarska, Dehaspe, Brady, Vandecaveye, Vanderstichele, Vergote, Neven, Putseys, Vermeesch.
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Study supervision: Amant, Verheecke, Brady, Vergote, Berteloot, Vandenberghe, Legius, Vermeesch.
Collected biomaterial: Vanderstichele.

Conflict of Interest Disclosures: Dr Vermeesch reports being the founder of and stockholder in Cartagenia, which provides software for clinical analysis of genomics data. The analysis used in this study has been licensed to Cartagenia, for which Dr Vermeesch's laboratory receives license fees. No other conflicts are reported.

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REFERENCES

1. Bianchi DW, Wilkins-Haug L. Integration of noninvasive DNA testing for aneuploidy into prenatal care: what has happened since the rubber met the road? *Clin Chem*. 2014;60(1):78-87.

2. Lau TK, Jiang FM, Stevenson RJ, et al. Secondary findings from non-invasive prenatal testing for common fetal aneuploidies by whole genome sequencing as a clinical service. *Prenat Diagn*. 2013;33(6):602-608.

3. Bayindir B, Dehaspe L, Brison N, et al. Noninvasive prenatal testing using a novel analysis pipeline to screen for all autosomal fetal aneuploidies improves pregnancy management. *Eur J Hum Genet*. 2015.

4. Heitzer E, Ulz P, Belic J, et al. Tumor-associated copy number changes in the circulation of patients with prostate cancer identified through whole-genome sequencing. *Genome Med*. 2013;5(4):30.

5. Chan KC, Jiang P, Zheng YW, et al. Cancer genome scanning in plasma: detection of tumor-associated copy number aberrations, single-nucleotide variants, and tumoral heterogeneity by massively parallel sequencing. *Clin Chem*. 2013;59(1):211-224.

6. 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(224):224ra24.

7. Vandenberghe P, Wlodarska I, Tousseyn T, et al. Non-invasive detection of genomic imbalances in Hodgkin/Reed-Sternberg cells in early and advanced stage Hodgkin lymphoma by sequencing of circulating cell-free DNA: a technical proof-of-principle study. *Lancet Haematol*. 2014;2(2):e55-e65.

8. Osborne CM, Hardisty E, Devers P, et al. Discordant noninvasive prenatal testing results in a patient subsequently diagnosed with metastatic disease. *Prenat Diagn*. 2013;33(6):609-611.

9. McCullough RM, Almasri EA, Guan X, et al. Non-invasive prenatal chromosomal aneuploidy testing—clinical experience: 100,000 clinical samples. *PLoS One*. 2014;9(10):e109173.

10. National Cancer Institute, Surveillance, Epidemiology, and End Results Program (SEER). *SEER Cancer Statistics Fact Sheets: Non-Hodgkin Lymphoma*. Bethesda, MD: National Cancer Institute; 2015.

11. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136(5):E359-E386.

12. Brenner B, Avivi I, Lishner M. Haematological cancers in pregnancy. *Lancet*. 2012;379(9815):580-587.

13. Amant F, Van Calsteren K, Halaska MJ, et al. Long-term cognitive and cardiac outcomes after prenatal exposure to chemotherapy in children aged 18 months or older: an observational study. *Lancet Oncol*. 2012;13(3):256-264.

14. Amant F, von Minckwitz G, Han SN, et al. Prognosis of women with primary breast cancer diagnosed during pregnancy: results from an international collaborative study. *J Clin Oncol*. 2013;31(20):2532-2539.

15. Han SN, Verheecke M, Vandenbroucke T, Gziri MM, Van Calsteren K, Amant F. Management of gynecological cancers during pregnancy. *Curr Oncol Rep*. 2014;16(12):415.