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Case Report

Copy number variation profile in noninvasive prenatal testing (NIPT) can identify co-existing maternal malignancies: Case reports and a literature review

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ABSTRACT

Objective: The coexistence of maternal malignancy and pregnancy has received increasing attention in Noninvasive prenatal testing (NIPT) studies. Malignancy in pregnant women potentially affects the copy number variation (CNV) profile in NIPT results. Only one case of hematologic cancer has been reported in a Hong-Kong pregnant women, and solid tumors have never been reported in pregnant Chinese women.
Case report: The patients with dysgerminoma and cervical cancer showed aberrant chromosomal aneuploidies in NIPT and concordant patterns of genome disruption in tumor tissues. The genomic aberrations in the gastric cancer patient had similar copy number variation pattern of gastric cancer.
Conclusion: The findings in this study and the literature review further validate the effect of maternal malignancy on the copy number variation profile in NIPT data and strengthen the possibility of detecting malignant tumors with NIPT in the future.

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Introduction

Over the past few years, noninvasive prenatal testing (NIPT) has become a common technology for prenatal screening of common fetal aneuploidies, such as trisomy 13, 18 and 21. The high sensitivity and specificity of NIPT have been shown in large-scale studies conducted in different populations [1,2]. However, despite the high accuracy of NIPT, false and failed test results occur in larger numbers due to the rapidly increasing population of patients who are undergoing NIPT. The discordance between cell-free DNA (cfDNA) and fetal karyotype results could be a major concern for NIPT. This discordance can be attributed to various factors, including confined placental mosaics [3], co-twin demise [4], maternal chromosomal mosaics [5] and maternal malignancy [5–7]. Despite the relatively low incidence of maternal malignancy (1:1000 to 1:5000), the incidental discovery of maternal cancer has been reported among pregnant women undergoing routine NIPT [5–8]. However, previous studies were mainly conducted in Western populations. Only one case of hematologic cancer has been reported in an Asian population, and no solid tumors have been reported in this population [8] which is inconsistent with the rapidly increasing number of pregnant women undergoing NIPT in Asia. In this brief report, we present three cases of aberrant chromosomal aneuploidies revealed by NIPT that were diagnosed as solid tumors: ovarian dysgerminoma, cervical cancer and gastric cancer. We also summarize the reported cases of maternal malignancy. The case reports and literature summary support the potential of applying NIPT for the pre-symptomatic detection of maternal malignancies in pregnant women in the future.

Materials and methods

Sample collection, sequencing and bioinformatics analysis

Ten milliliters of maternal peripheral blood were collected in a Cell-Free DNA BCT® blood collection tube (Streck Inc, La Vista, NE, USA) and processed within 4 days of collection. Details of the NIPT method, also called the noninvasive fetal trisomy test (NIFTY), have been published previously [5]. In brief, plasma was separated by sequential centrifugation of the blood sample at 1600 g at 4 °C for 10 min. Cell-free DNA was extracted from plasma and subjected to library construction. The quantity and quality of the library were examined by real-time PCR and size distribution. Then, the library was sequenced, and the generated data were analyzed using bioinformatics algorithms to detect fetal chromosomal aneuploidy, including alterations to chromosomes 1 to 22 and the sex chromosomes and large deletions/duplications, as previously described [9].

Somatic copy number alterations (Affymetrix OncoScan FFPE Express 2.0)

Somatic copy number analysis was performed using OncoScan FFPE Express 2.0 (Affymetrix-Thermo Fisher Scientific, Santa Clara, CA, USA) with 334,183 sequence tag site probes to measure DNA copy number variations (CNVs) [10]. Copy number data were processed and normalized, and quality control metrics were applied according to the manufacturer's instruction. Copy numbers were estimated with NEXUS software, and only samples that passed Affymetrix quality control metrics (median absolute pairwise difference [MAPD] ≤ 0.6) were considered [11].

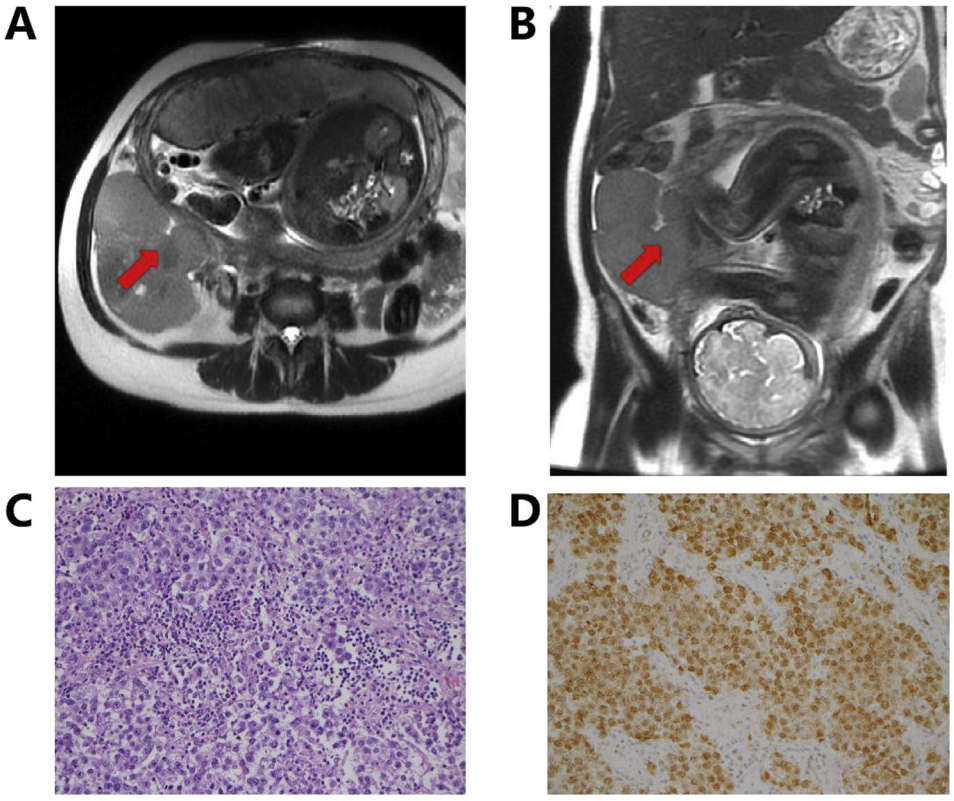

Fig. 1. Case 1 with Dysgerminoma. A and B. A 112 × 109 × 75 mm mass on T2-weighted MRI images at 35 gestational weeks (A: axial plane, B: coronal plane). The red arrow heads indicate the malignant tumors in all panels. C. solid sheets of large tumor cells with prominent nucleoli and clear cytoplasm (H&E staining, ×200). D. the tumor cells were immunoreactive for OCT4 (anti-OCT4, ×200).

Case summaries

Case 1

This 30-year-old patient had a history of bilateral ovarian endometriosis. During her first obstetrics ultrasound, bilateral ovarian cysts (left: 2.8 × 2.0 cm; right: 7.6 × 4.4 cm) were identified with endometriosis according to her prior history. The patient underwent a NIPT at 13 weeks gestation that showed multiple aneuploidies. Amniocentesis and array comparative genomic hybridization (array CGH) of the amniotic fluid showed a normal fetal karyotype (46, XX). A fetal anatomic ultrasound survey also showed normal fetal anatomy. However, the right ovarian cyst with internal flow enlarged gradually, and abdominal MRI revealed that an 112 × 109 × 75 mm lobulated soft tissue mass at the right adnexa at 35 weeks gestation (Fig. 1A,B). Malignancy was highly suspected based on NIPT and imaging results, but the patient decided to wait for fetal maturity. At 36 weeks gestation, the patient underwent a cesarean section and conservative debulking surgery, including right salpingo-oophorectomy, dissection of the right pelvic lymph node, omentectomy and appendectomy. A female infant with an Apgar score of 5/8 and a weight of 2498 g appeared normal without any obvious dysmorphic features. The final pathologic diagnosis was right ovarian dysgerminoma, pT3aN0M0, FIGO IIIa with tumor invasion of the appendix (Fig. 1C,D). We found concordance of the chromosomal alterations between maternal plasma DNA (Fig. 2A) and tumor tissue performed by OncoScan® (Fig. 2B), including gains at 7p, 8q, 12p and 21q. These results suggested that circulating tumor DNA contributed to the aberrant NIPT results. Additional NIPT was conducted after surgery and six courses of chemotherapy; the aberrant CNVs disappeared after treatment was completed (Fig. 2C).

Case 2

This 37-year-old pregnant patient had persistent vaginal bleeding for 5 months during pregnancy, and a cervical mass was found at 27 weeks gestation. The patient underwent amniocentesis that revealed a normal fetal karyotype, and a prior obstetric ultrasound revealed normal fetal growth. The cervical mass was biopsied and diagnosed as squamous cell carcinoma at stage IV. After options were discussed with the patient and her family, it was

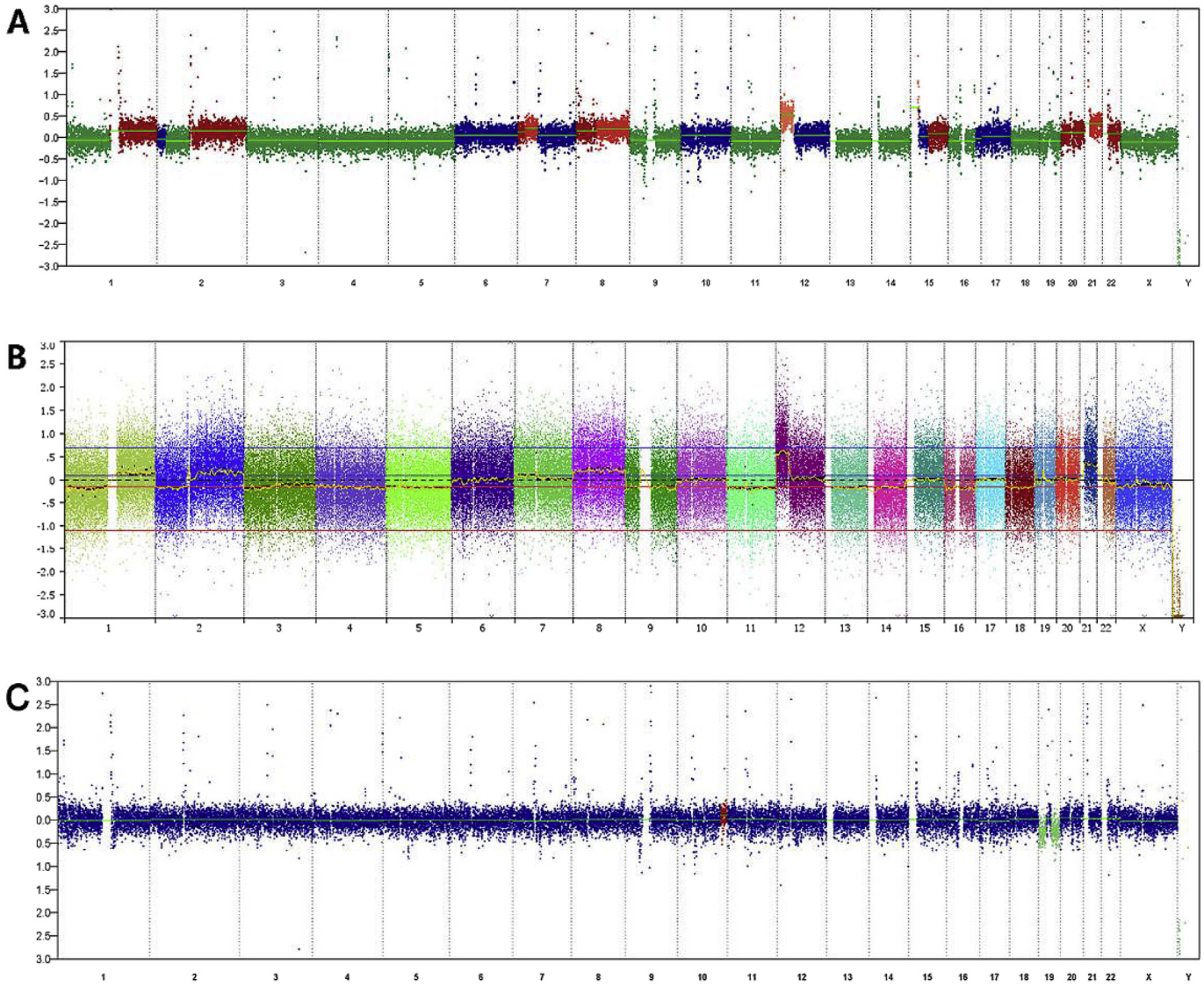

Fig. 2. CNV profile in Case 1 with dysgerminoma A. Whole-genome view of copy-number gains and losses in plasma (A), malignant tumors (B) and plasma samples after treatment (C).

decided that medical treatment was urgently needed for this patient and that the fetus might have a poor prognosis because of prematurity and the state of local medical service. Hysterectomy, bilateral salpingo-oophorectomy and dissection of right internal iliac lymph nodes were conducted at 28 gestational weeks without saving the fetus (Fig. 3A). The final diagnosis was stage IV small cell neuroendocrine cancer of the cervix with invasion of the right ovary (Fig. 3B). Before surgery, the patient underwent NIPT, which showed multiple aneuploidies. A concordant pattern of chromosome alterations was observed between maternal plasma DNA (Fig. 3C) and tumor tissue performed by Oncoscan® (Fig. 3D), including gains at 3q, 9p, 14q, 16p-q, 17q, 18p-q and 20q and losses at 3p, 4p-q, 13q, 16q, 19p and 21q. Unfortunately, this patient died 2 months later during adjuvant chemotherapy.

Case 3

The third patient is a 36-year-old woman who underwent NIPT testing at 16 and 20 weeks gestation. The patient had a history of peptic ulcers and suffered from worsening nausea and vomiting during the pregnancy. The patient underwent gastroscopy and was diagnosed with advanced gastric cancer (Fig. 4A,B). One week after diagnosis, she died of gastric cancer. Due to a lack of cancer samples, no experiments were performed to validate the copy number changes in the gastric cancer tissue. NIPT at 30-fold depth, which equals 3X coverage in whole genome sequencing (WGS), was performed using the NIPT DNA library. Both the original NIPT and the 30-fold NIPT results displayed a severe disruption of genome stability, including gains at 7q, 8p-q, and 9q and losses at 3q and 17p (Fig. 4C,D).

Summary of maternal malignancies

In total, 16 cases of maternal malignancies with abnormal NIPT results have been reported in the literature including our case report since 2011 (Table 1). Eleven cases were diagnosed at stage III or IV, and 3 were diagnosed at stage II, and other 2 cases with unknown stage. Of the cases of maternal cancer, 13 were diagnosed prenatally and 3 postnatally respectively. Some cases showed

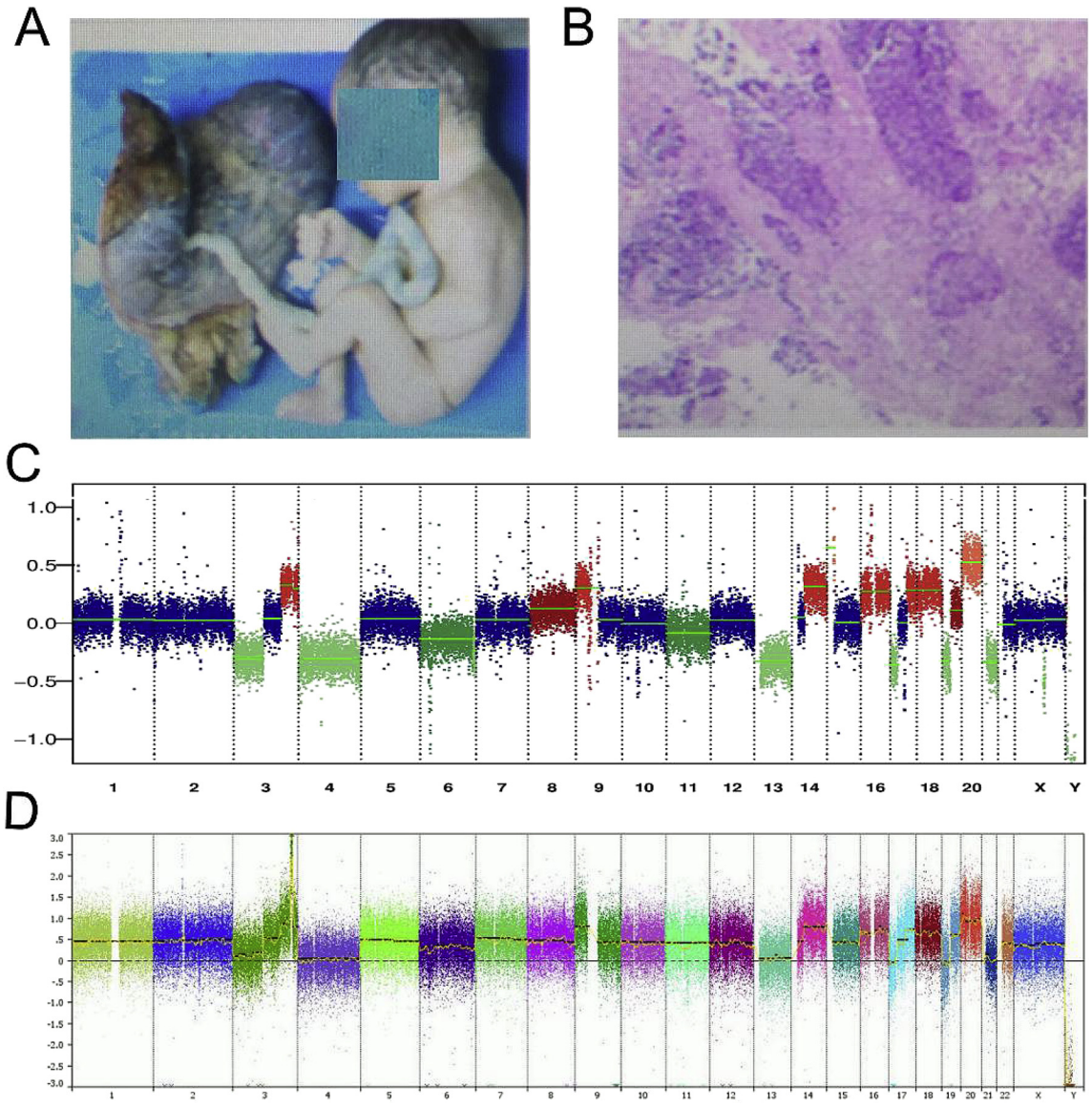

Fig. 3. Case 2 with neuroendocrine tumor of the cervix. A. Gross anatomy of uterine, placenta and fetus. B. Pathology of neuroendocrine tumor of the cervix (H&E staining). C. Whole-genome view of copy number alterations in plasma revealed by NIPT. D Whole-genome view of copy number alterations of malignant tumor tissue.

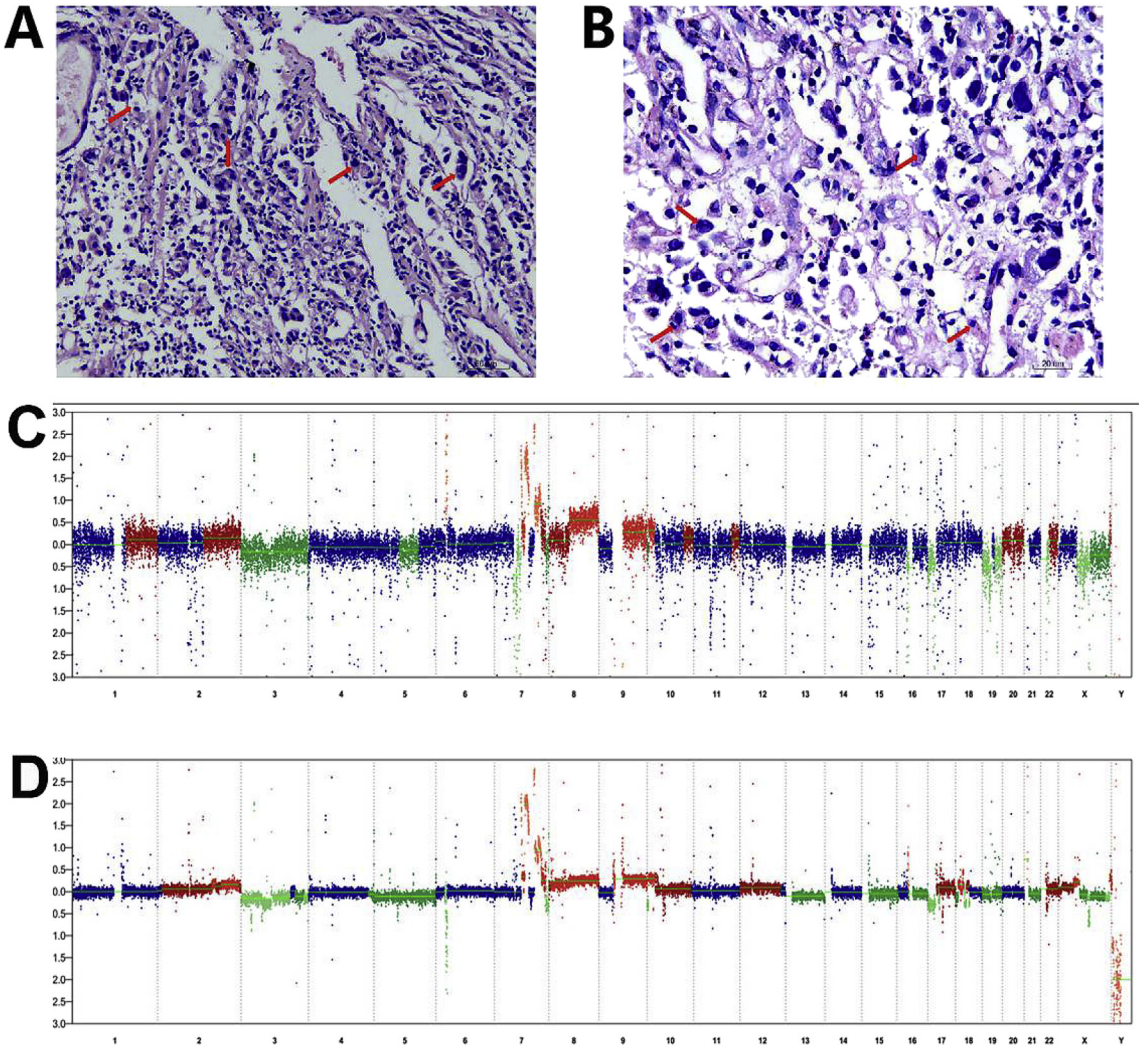

Fig. 4. Case 3 with advanced gastric cancer. A. B. The red arrowhead marks signet ring cells in gastric cancer biopsy samples (A: H&E staining, ×20, B: H&E staining, ×40). C, D. Whole-genome view of copy-number alterations in plasma revealed by NIPT (C) and 30 fold NIPT (D).

multiple chromosomal aneuploidies in NIPT; 8 were confirmed to harbor aberrant CNVs in tumor tissue by fluorescence in situ hybridization (FISH), genome-wide DNA sequencing or OncoScan microarray (Table 1).

Discussion

The incidental discovery of maternal cancer has been reported among pregnant women undergoing routine NIPT in Europe and the US [5–7]. Sun et al. published the only report of recurrent follicular lymphoma during pregnancy in an Asian woman [8] (Table 1). The three cases in our study revealed the association between maternal solid cancer and aberrant NIPT results in an Asian population. According to a previous study, the finding of multiple aneuploidies in a patient is strongly associated with maternal cancer [5]. This conclusion was also confirmed by our results, which is strengthening the potential implications of NIPT in screening for maternal cancer and monitoring women during pregnancy.

The most frequently diagnosed malignancies during gestation are breast cancer, cervical cancer, Hodgkin's disease, malignant melanoma, and leukemia [12,13]. Previous reports of maternal malignancies included a large proportion of hematologic cancers [5,6]. Solid tumors, including neuroendocrine, colorectal, anal, and serous ovarian cancer, ovarian dysgerminoma, neuroendocrine cancer of the cervix and gastric cancer (Table 1), have been detected with aberrant NIPT results, further expanding the range of cancer types detected by NIPT screening.

Fluorescent in situ hybridization (FISH) for selected target loci was mainly used to screen for CNV patterns in tumor samples in the study by Bianchi et al. [5] and Amant et al. [6] (Table 1). In our two cases and the case reported by Sun et al. [8], Single Nucleotide Polymorphism (SNP) array analysis or genome-wide DNA sequencing of tumor samples revealed more comprehensive and comparable whole genomic CNV patterns than FISH because the detected aberrations were not limited to specific chromosomes. Our results also suggest the possibility of detecting circulating tumor DNA from blood using extreme-low depth WGS. However, in a study on non-pregnant women, Cohen et al. reported that NIPT only had a sensitivity of 40.6% and a specificity of 93.8% in detecting early- and late-stage high-grade serous ovarian carcinoma without pregnancy [14]. Given the relatively low sensitivity of NIPT, refined bioinformatics algorithms and genome-wide CNV analysis may be needed to improve the performance of NIPT in detecting occult malignancies.

Table 1
Summary of maternal malignancies with NIPT results.

Reference	NIPT platform	Case no.	Tumor type/stage	NIPT results	Time of cancer detection	Tissue CNV analysis (method)	Tissue results
Solid tumor							
Osborne et al. (2013) [7]	13, 18, 21	Case	Neuroendocrine of vagina origin/IV	Monosomy 18 Trisomy 13	Postnatal	Done (FISH)	Monosomy 18 Trisomy 13
Bianchi et al. (2015) [5]	13, 18, 21 and sex chromosomes	Case 1	Neuroendocrine (unknown origin)/IV, metastatic	Monosomy 18 Trisomy 13	Postnatal	Done (FISH)	Monosomy 18 Trisomy 13
		Case 3	Colorectal cancer/IIIC	Trisomy 13	Prenatal	Not done	N/A
		Case 8	Anal cancer/IIIB	Trisomy 13,18 and 21 Monosomy	Prenatal	Not done	N/A
Amant et al. (2015) [6]	Whole genome	Case 1	High-grade serous ovarian cancer/stage IVA	Gain: 6p, 7q, 9p Loss: 6q, 7p, 18q	Prenatal	Done (FISH)	Gain: 6p, 7q, 9p Loss: 18q
This study	Whole genome	Case 1	Ovarian dysgerminoma/stage IIIC	Gain: 7p, 8q, 12p, 21q	Postnatal	Done (OncoScan)	Gain: 7p, 8q, 12p, 21q
		Case 2	Neuroendocrine of cervix/IV	Gain: 3q, 9p, 14q, 16, 17q, 18, 20q Loss: 3p, 4, 13q, 16q, 19p, 21q	Prenatal	Done (OncoScan)	Gain: 3q, 9p, 14q, 16, 17q, 18, 20q Loss: 3p, 4, 13q, 16q, 19p, 21q
		Case 3	Gastric cancer/IV	Gain: 7q, 8p-q, 9q Loss: 3p, 17p	Prenatal	Not done	N/A
Hematologic cancer							
Bianchi et al. (2015) [5]	13,18, 21 and sex chromosome	Case 2	Non-Hodgkin (B-cell) lymphoma/IVB	Monosomy 18	Prenatal	Not done	N/A
		Case 4	Hodgkin lymphoma/IIA	Monosomy 13, 18 and 21	Postnatal	Not done	N/A
		Case 5	Acute T-cell lymphoblastic leukemia	Trisomy 21 Monosomy 18 Trisomy 13	Prenatal	Not done	N/A
		Case 6	Non-Hodgkin (B-cell) lymphoma/IV	Trisomy 18	Prenatal	Not done	N/A
		Case 7	Non-Hodgkin (B-cell) lymphoma/II	Monosomy 18	Prenatal	Not done	N/A
Amant et al. (2015) [6]	Whole genome	Case 2	Follicular lymphoma/III	Gain: 7q, 12q, 13q Loss: 6q	Prenatal	Done (FISH)	Gain: 7q, 11q, 12q, 13q Loss: 6q
		Case 3	Hodgkin lymphoma/II	Gain: 8q, 9p, 14q Loss: 8p, 9q	Prenatal	Done (FISH)	Gain: 8p, 9p, 14q
Sun et al. (2015) [8]	Whole genome	Pregnant case	Recurrent follicular lymphoma	Gain: 1p, 17, 21q Loss: 6q	Prenatal	Done (genome-wide DNA sequencing)	Gain: 1p, 17, 21q Loss: 6q8

So far, only three abnormal NIPT results have been confirmed to stem from concurrent maternal malignancies, despite the prevalence of NIPT in Asia is much higher. One possible explanation is that current NIPT products focus mainly on trisomy 13, 18, and 21; multiple aneuploidies are frequently correlated with maternal cancer, but these might result in screening failures in NIPT and thus be overlooked. Another possibility is that maternal malignancies are relatively rare and often misdiagnosed as pregnancy symptoms, resulting in a low diagnostic rate. Many patients may have experienced concurrent cancer during pregnancy after NIPT and not reported it to their NIPT provider. Therefore, more attention and medical follow-up are greatly needed for those patients who have multiple aneuploidies by NIPT.

The limitation of this study is that we failed to obtain tumor samples from the gastric cancer patient and thus tumor DNA information. The NIPT result for this gastric patient showed similar CNVs to cases of gastric cancer reported in a prior study, such as aneuploidy of chromosomes 7 and 8 [15]. The other limitation of this study is NIPT cannot specify the exact origin of cfDNA, and therefore, diagnosing the primary tumor remains a difficult and complicated task. Further investigations are needed.

Taken together, our findings combined with those of prior studies highlight the potential value of NIPT for the presymptomatic or co-current detection of cancer in pregnant Chinese women, indicating a possible strategy for early cancer detection.

Conflicts of interest statement

The authors declare no conflict of interest.

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References

[1] Chiu RW, Akolekar R, Zheng YW, Leung TY, Sun H, Chan KC, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. BMJ 2011;342:c7401.
[2] Zhang H, Gao Y, Jiang F, Fu M, Yuan Y, Guo Y, et al. Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146,958 pregnancies. Ultrasound Obstet Gynecol — Offic J Int Soc Ultrasound Obstet Gynecol 2015;45(5):530–8.
[3] Lau TK, Jiang FM, Stevenson RJ, Lo TK, Chan LW, Chan MK, 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):602–8.
[4] Curnow KJ, Wilkins-Haug L, Ryan A, Kirkizlar E, Stosic M, Hall MP, et al. Detection of triploid, molar, and vanishing twin pregnancies by a single-nucleotide polymorphism-based noninvasive prenatal test. Am J Obstet Gynecol 2015;212(1). 79 e71–79.
[5] Bianchi DW, Parsa S, Bhatt S, Halks-Miller M, Kurtzman K, Sehnert AJ, et al. Fetal sex chromosome testing by maternal plasma DNA sequencing: clinical laboratory experience and biology. Obstet Gynecol 2015;125(2):375–82.
[6] Amant F, Verheecke M, Wlodarska I, Dehaspe L, Brady P, Brison N, et al. Presymptomatic identification of cancers in pregnant women during noninvasive prenatal testing. JAMA Oncol 2015;1(6):814–9.
[7] Osborne CM, Hardisty E, Devers P, Kaiser-Rogers K, Hayden MA, Goodnight W, et al. Discordant noninvasive prenatal testing results in a patient subsequently diagnosed with metastatic disease. Prenat Diagn 2013;33(6):609–11.
[8] Sun K, Jiang P, Chan KC, Wong J, Cheng YK, Liang RH, et al. Plasma DNA tissue mapping by genome-wide methylation sequencing for noninvasive prenatal, cancer, and transplantation assessments. Proc Natl Acad Sci USA 2015;112(40):E5503–12.
[9] Chen S, Lau TK, Zhang C, Xu C, Xu Z, Hu P, et al. A method for noninvasive detection of fetal large deletions/duplications by low coverage massively parallel sequencing. Prenat Diagn 2013;33(6):584–90.
[10] Wang Y, Carlton VE, Karlin-Neumann G, Sapolsky R, Zhang L, Moorhead M, et al. High quality copy number and genotype data from FFPE samples using Molecular Inversion Probe (MIP) microarrays. BMC Med Genom 2009;2:8.
[11] Darvishi K. Application of Nexus copy number software for CNV detection and analysis. Curr Protocols Hum Genet 2010 [Chapter 4]:Unit 4.14.1–28.
[12] Zagouri F, Dimitrakakis C, Marinopoulos S, Tsigginou A, Dimopoulos MA. Cancer in pregnancy: disentangling treatment modalities. ESMO Open 2016;1(3), e000016.
[13] Pavlidis NA. Coexistence of pregnancy and malignancy. Oncology 2002;7(4):279–87.
[14] Cohen PA, Flowers N, Tong S, Hannan N, Pertile MD, Hui L. Abnormal plasma DNA profiles in early ovarian cancer using a non-invasive prenatal testing platform: implications for cancer screening. BMC Med 2016;14(1):126.
[15] Cancer Genome Atlas Research N. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014;513(7517):202–9.