

# 1      **Incidental identification of maternal malignancies in two Asian** 2                      **women underwent noninvasive prenatal test.**

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## 46 **Abstract**

47 Noninvasive prenatal test (NIPT) has been widely used as a screening test for trisomy  
 48 13, 18 and 21 worldwide. Recently, coexistence of maternal malignancy and  
 49 pregnancy has drawn increasing attention in NIPT studies. Malignancy in pregnant  
 50 women potentially affected NIPT results, which may cause false positive results or  
 51 failed tests. However, no such case has ever been reported in Asian population. In this  
 52 study, for the first time, we reported a stage III dysgerminoma and advanced gastric  
 53 cancer during pregnancy accidentally identified during NIPT tests. These two women  
 54 showed aberrant chromosome aneuploidies in NIPT results and concordant pattern of  
 55 genome disruption found in tumor samples. The findings in this study further validate  
 56 the effect of maternal malignancy on NIPT results and strengthen the possibility of  
 57 detecting malignant tumors through NIPT in the future.

58

59 Keywords: Noninvasive prenatal test (NIPT), massively parallel sequencing, maternal  
 60 malignancy, multiple aneuploidies

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62

## 63    **Introduction**

64    Over the past few years, noninvasive prenatal testing (NIPT) has become a common  
65    technology screening for the common fetal aneuploidies, including 13, 18 and 21  
66    –trisomy. The high sensitivity and specificity of NIPT has been demonstrated by  
67    large-scale studies conducted in different populations (Chiu et al., 2011; Zhang et al.,  
68    2015). Moreover, NIPT is recommended as a better substitute to traditional Down  
69    syndrome screening.

70    However, despite the high accuracy of NIPT, false or even failed test results still exist  
71    in a large amount due to the rapid increasing population who underwent NIPT. The  
72    discordance between cell-free DNA (cfDNA) and fetal karyotype could be a major  
73    concern of NIPT technic, which could be attributed to various factors including  
74    confined placental mosaics, co-twin demise, maternal chromosomal mosaics and  
75    maternal malignancy (Bianchi et al., 2015).

76    Of them, maternal malignancy has drawn more and more attention, due to its fatal  
77    consequences during pregnancy. Despite the relatively low incident rate of maternal  
78    malignancy (1:1000 to 1: 5000), incidental discovery of maternal cancer have been  
79    reported among pregnant women undergoing routine NIPT screening (Amant,  
80    Verheeecke, et al., 2015; Bianchi et al., 2015; Osborne et al., 2013). However, all of  
81    those studies were conducted in western population. Rare case had been reported in  
82    Asian population, which is inconsistent with the rapidly increasing number of  
83    pregnant women who took NIPT screening. In addition, the majority of maternal  
84    malignancies discovered by NIPT are hematological malignancies, few cases had

85 been reported on other cancer types.

86 In the current report, we presented two cases of aberrant chromosomal aneuploidies  
87 unraveled by NIPT subsequently diagnosed with dysgerminoma and gastric cancer,  
88 respectively. These case reports supported the potential of applying NIPT to  
89 pre-symptomatic detection of malignant tumors in pregnant women in the future.

90

## 91 **Methods and materials**

### 92 **Sample collection, sequencing and bioinformatics analysis**

93 Ten millimeters of maternal peripheral blood were collected in Cell Free DNA BCT ®  
94 blood collection tube and was processed within 4 days of collection. Details of the  
95 NIPT method, also called Non-invasive fetal trisomy test (NIFTY), have been  
96 published previously(Dan et al., 2012). In brief, plasma was separated by sequential  
97 centrifugations of the blood sample at 1600g at 4°C for 10mins. Cell free DNA was  
98 extracted from plasma and subjected to library construction. The quantity and quality  
99 of the library were examined by real time PCR and size distribution. Qualified library  
100 was sequenced and the data generated was analyzed using the bioinformatics  
101 algorithm to detect fetal chromosomal aneuploidy and large deletion/duplication, as  
102 previously described(Chen et al., 2013; Lau et al., 2012).

103

### 104 **Somatic copy-number alterations (Affymetrix OncoScan FFPE Express 2.0)**

105 Somatic Copy number analysis for this tumor was performed using MIP array  
106 technology (Affymetrix OncoScan FFPE Express 2.0) with 334,183 sequence tag site

107 probes to measure DNA copy number variations(Wang et al., 2009). Copy number  
108 data were processed and normalized by Affymetrix as previously described with  
109 passed Affymetrix quality control metrics(Wang et al., 2009). Copy numbers were  
110 estimated with the NEXUS software and only samples that passed Affymetrix quality  
111 control metrics (median absolute pairwise difference [MAPD] value of  $\leq 0.6$ ) were  
112 considered(Darvishi, 2001).

113

## 114 **Cases summary**

### 115 **Case 1**

116 The first patient is a 30-year-old woman, who was diagnosed with bilateral ovarian  
117 endometriosis and received laparoscopic cystectomy and 6-months  
118 gonadotropin-releasing hormone agonist treatment from December 2013 to June 2014.  
119 During her first obstetrics ultrasound, bilateral endometriosis, including left ovarian  
120 cyst (2.8 cm and 2.0 cm) and one right ovarian cyst (7.6 x 4.4 cm) were diagnosed  
121 according to her prior history. The patient received two NIPT tests at 13 and 32  
122 gestational weeks respectively. After the failure of the first test, the second test after  
123 resampling of peripheral blood failed again, which excluded the possibility of sample  
124 degradation during transportation. To rule out fetal aneuploidies, amniocentesis and  
125 array comparative genomic hybridization (array CGH) were conducted on the  
126 amniotic fluid, showing a normal fetal karyotype (46, XX). A fetal anatomic  
127 ultrasound survey also demonstrated normal fetal anatomy and growth with normal  
128 placenta appearing at 19 gestational weeks.

129 However, the right ovarian cyst with internal flow enlarged gradually as gestational  
130 week and fetal MRI revealed that an 8.7x7.9x6.0cm lobulated soft tissue mass at right  
131 adnexa with engorged regional collateral vessels noted with diffusion restriction  
132 (Figure 1 A and B). At 36 weeks gestational age, patients underwent a cesarean  
133 section combined with a conservative debulking surgery, including right  
134 salpingo-oophorectomy, right pelvic lymph node sampling, omentectomy and  
135 appendectomy. A female infant with Apgar score 5/8 and weight of 2498 g appeared  
136 normal without any obvious dysmorphic features. The final pathologic diagnosis was  
137 a right ovarian dysgerminoma, pT3aN0M0, FIGO IIIa with tumor invasion to  
138 appendix (Figure 1C and D). Thereafter, the patient received 6-course of  
139 chemotherapy (bleomycin, etoposide and cisplatin).

140 After knowing concurrent pregnancy and dysgerminoma in this patient, we  
141 re-examined the NIPT results and found multiple aneuploidies in the blood DNA,  
142 which was the main reason of NIPT failure (Figure 2A).

143 To study the resource of genetic variations found in NIPT, MIP array technology  
144 (Affymetrix OncoScan FFPE Express 2.0) with paraffin-embedded tumor tissues was  
145 performed. We found the concordance of chromosome alterations between maternal  
146 plasma DNA and tumor tissue (Figure 2A and B), such as gain of whole short arm of  
147 chromosomal 12 (12p11.1-13.3) and gain of whole chromosomal 21 (p11.2-q22.3).

148 Those results suggested that tumor cfDNA contributed to the aberrant NIPT results.

149 To further validate this result, another cfDNA test was conducted after the surgery and  
150 six courses of chemotherapy. As shown in figure 2C, aberrant copy number variations

151 disappeared after the complete treatment, further supporting the hypothesis that NIPT  
152 could detect tumor cfDNA in maternal plasma. These results strengthened the  
153 potential implement of non-invasive prenatal test in screening and monitoring  
154 maternal cancer during pregnancy.

## 155 **Case 2**

156 The second patient is a 36-year-old woman, who received two NIPT tests at 16 and 20  
157 gestational weeks, respectively. The patient had peptic ulcer history, she suffered from  
158 growing nausea and vomiting during the pregnancy. The patient received a  
159 gastroscopy and was diagnosed with an advanced gastric cancer (Figure 3). After one  
160 week of diagnosis, she died of gastric cancer. Due to lack of cancer samples, no  
161 experiment was performed to validate the copy number changes in gastric cancer  
162 tissues. A NIPT test at 30-fold depth, which equals to 3X WGS, was performed using  
163 the NIPT DNA library. Both original NIPT and the 30-fold NIPT results displayed a  
164 severe disruption of genome stability, further supporting abnormal copy number  
165 variations reported by NIPT test is a strong indicator of maternal malignancies (Figure  
166 4). In line with previous reports (CALCAGNO et al., 2005; Network, 2014), genomic  
167 changes commonly seen in gastric cancer, such as aneuploidy of chromosome 7 and 8,  
168 were observed, providing further evidence that NIPT test is able to detect the tumor  
169 ctDNA in maternal blood.

170

## 171 **Discussion**

172 Incidental discovery of maternal cancer has been repeatedly reported among pregnant

173 women undergoing routine NIPT screening in Europe and US (Amant,  
174 Vandenbroucke, et al., 2015; Bianchi et al., 2015; Osborne et al., 2013). Although  
175 different groups have reported their findings of maternal cancer in pregnant women  
176 undergoing NIPT screening, our report is the first to reveal the association between  
177 maternal cancer and aberrant NIPT results in Asian population. In this study, two  
178 Asian pregnant women showed aberrant NIPT results and subsequently diagnosed  
179 with maternal malignancies, both harboring extensive copy-number changes across  
180 the whole genome. According to previous study(Bianchi et al., 2015), patients with  
181 multiple aneuploidies are strongly associated to maternal cancer, which is also  
182 confirmed by our results.

183 Moreover, further investigation of NIPT result, in combination of SNP array outcome  
184 demonstrated similar copy-number variation patterns in samples from peripheral  
185 blood and tumor tissue, which provides strong evidence for the detection of  
186 circulating cancer DNA from blood using extreme-low depth whole genome  
187 sequencing.

188 The most frequently diagnosed malignancies during gestation are breast cancer,  
189 cervical cancer, Hodgkin's disease, malignant melanoma, and leukemia (Pavlidis,  
190 2002). Previous reported maternal malignancies contain a large proportion of blood  
191 cancer(Amant, Verheেকে, et al., 2015; Bianchi et al., 2015). For the first time, we  
192 showed a stage III dysgerminoma and advanced gastric cancer during pregnancy,  
193 further expanding the range of cancer types detected by NIPT screening.

194 According to previous reports, the incidence rate of cancer

195 complicating pregnancy ranges from 1:1000 to 1: 5000 among pregnancies (Pavlidis  
196 NA,2002) whereas the reported incidence of maternal cancer associated with  
197 abnormal NIPT results is much lower, which is the case in our study. So far, only two  
198 cases of abnormal NIPT results were confirmed with concurrent maternal  
199 malignancies, despite the large number of NIPT tests conducted in Asia. One possible  
200 explanation is that present NIPT products focus mainly on the abnormal trisomy-13,  
201 18, and 21, multiple aneuploidies were shown to be frequently correlated with  
202 maternal cancer whereas in NIPT screening, it often causes screening failure thus may  
203 be overlooked. Another possibility is that maternal malignancy is a relatively rare  
204 disease often misdiagnosed by other pregnant symptoms and results in a low  
205 diagnostic rate. The complicated conditions during pregnancy makes it even more  
206 difficult to perform medical follow-up for patients. Many patients may have  
207 concurrent cancer during pregnancy after their NIPT tests and do not report to their  
208 NIPT providers. Therefore, more attention and medical follow-up are greatly needed  
209 for those patients who have multiple aneuploidies in NIPT test. However, how to  
210 conduct reasonable clinical follow-up and provide proper medical interventions  
211 becomes an important question that should be answered by NIPT provider and  
212 medical staff in the future.

213 Of these two patients described herein, one patient gave birth to a healthy girl and  
214 subsequently underwent successful resection of tumor tissue, followed by  
215 chemotherapy. The other patient died one week after diagnosis with gastric cancer.  
216 The limitation of this study is that we failed to obtain enough tissue samples for the

217 second patient to discover tumor DNA information.

218 Taken together, our findings underlined potential value of NIPT for pre-symptomatic

219 cancer screening in pregnant women, indicating a possible direction of early cancer

220 screening strategy.

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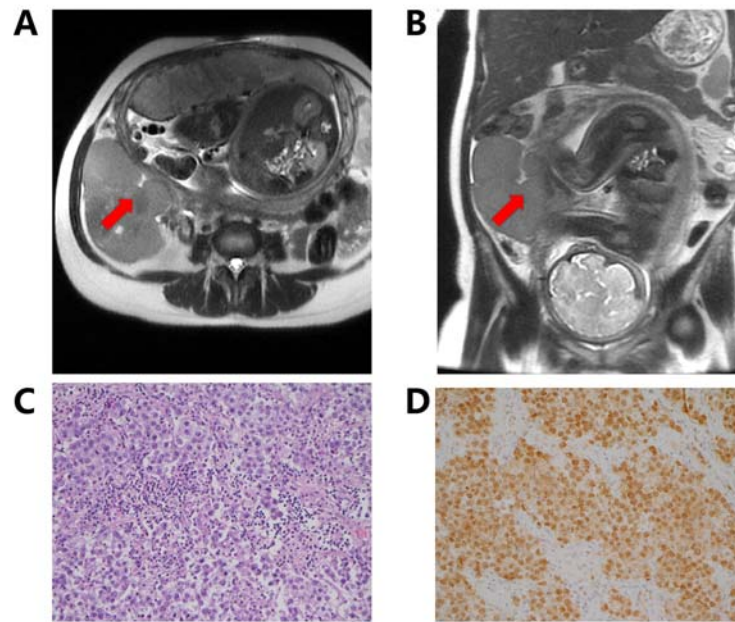
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## 279    **Figures and legends**

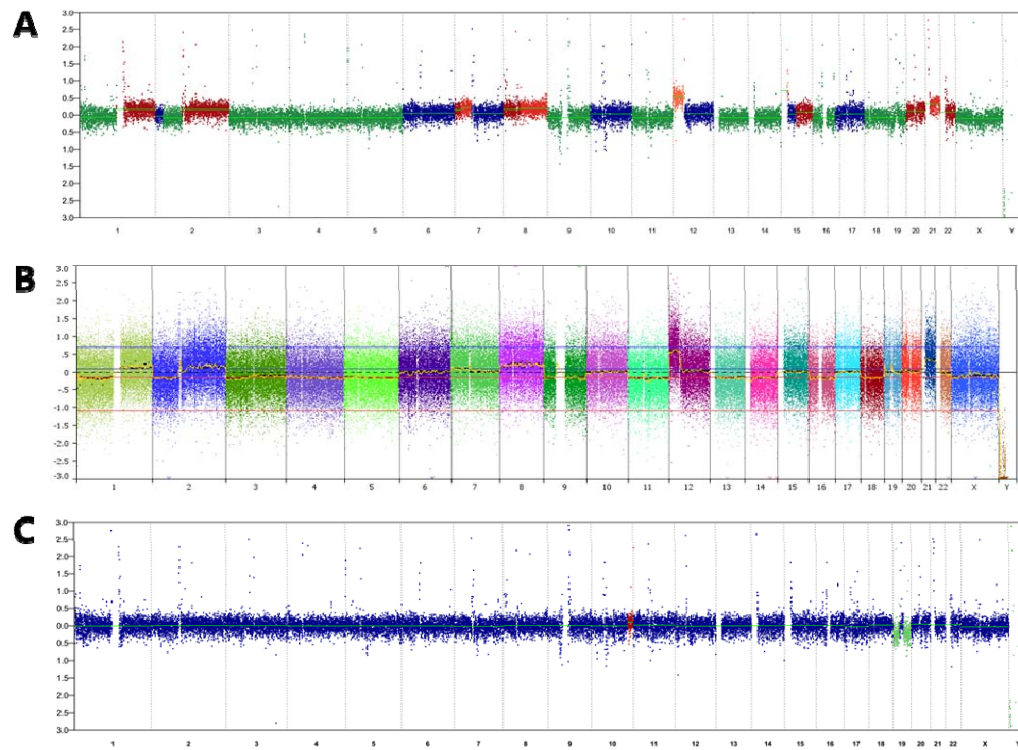


280    Figure1. Dysgerminoma in a 31-year-old pregnant female patient. A 112x109x75 mm  
 281    mass on T2-weighted MRI images at 35 gestational weeks (A: axial plane, B: coronal  
 282    plane). The red arrow heads indicate the malignant tumors in all panels. (C) solid  
 283    sheets of large tumor cells with prominent nucleoli and clear cytoplasm (H&E  
 284    staining, x 200). (D) the tumor cells were immuno-reactive for OCT4 (anti-OCT4,  
 285    x200).

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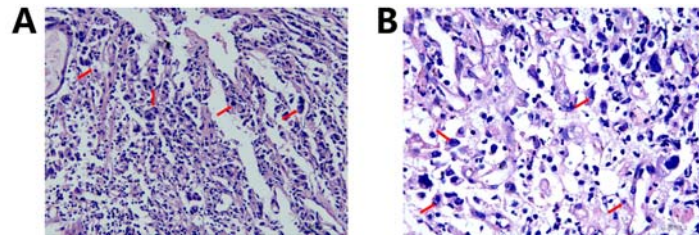


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290 Figure2. Whole-genome view of copy-number gains and losses in plasma (A),

291 malignant tumors (B) and plasma samples after treatment (C).

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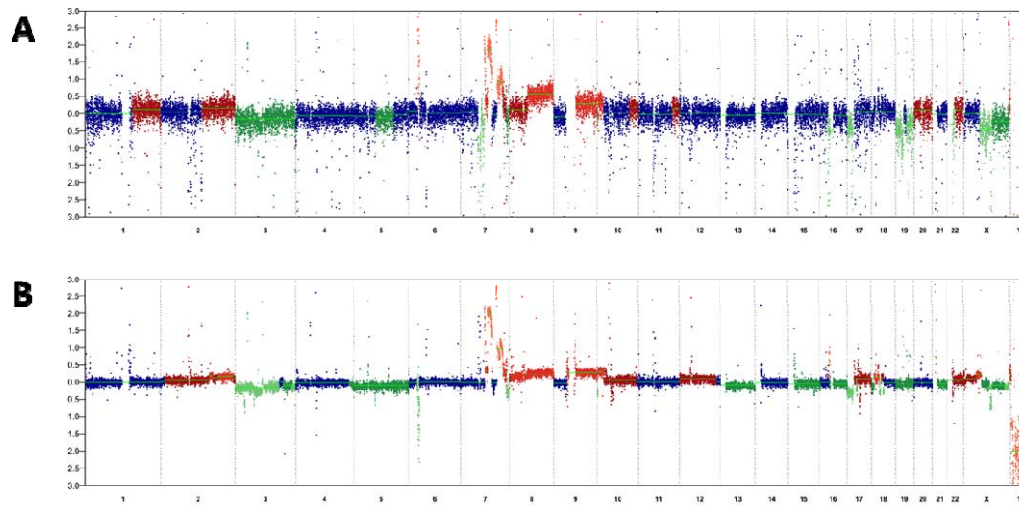
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294 Figure3. A gastric cancer in patient 2. The red arrowhead marks signet ring cells in

295 gastric cancer biopsy samples (A: H&E staining, x 20, B: H&E staining, x 40).

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299 Figure4. Whole-genome view of copy-number alterations in plasma revealed by NIPT

300 (A) and 30-fold NIPT (B).

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