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Letter to the Editor

False-negative trisomy 18 non-invasive prenatal test result due to 48,XXX,+18 placental mosaicism

Analysis of cell-free DNA (cfDNA) in maternal plasma by massively parallel sequencing (MPS) has been used recently as a non-invasive prenatal testing (NIPT) approach to detect fetal chromosome aneuploidy with high sensitivity and specificity[1,2]. Here we report a rare placental condition of 48,XXX, trisomy 18 mosaic that led to a partially inaccurate NIPT result.

The 43-year-old patient (gravida 5 para 2) with uncomplicated spontaneous pregnancy had a Down syndrome risk of 1/70 (first-trimester combined test results: pregnancy-associated plasma protein-A, 0.49 multiples of the median (MoM); free beta human chorionic gonadotropin, 1.58 MoM; and nuchal translucency, 0.66 MoM). The patient underwent NIPT at 13 + 2 weeks' gestation; 5 mL maternal peripheral blood was collected for cfDNA extraction, library construction and sequencing using the Illumina Hiseq 2000 platform[3].

The NIPT result showed low risk of fetal trisomy 13, 18 and 21, but high risk of XXX. The fetal cfDNA fraction was estimated by T-score of the X chromosome to be 7.4%. Ultrasound examination at 16 weeks' gestation showed bilateral clubfeet, bilateral choroid plexus cysts and a single umbilical artery (Figure S1). The patient received counseling and agreed to undergo amniocentesis. Both rapid diagnosis of common aneuploidies by quantitative fluorescent polymerase chain reaction (QF-PCR) and G-banding of cultured amniocytes proved the fetal karyotype to be 48,XXX,+18 (Figures 1 and S1), suggesting a trisomy 18 condition that had not

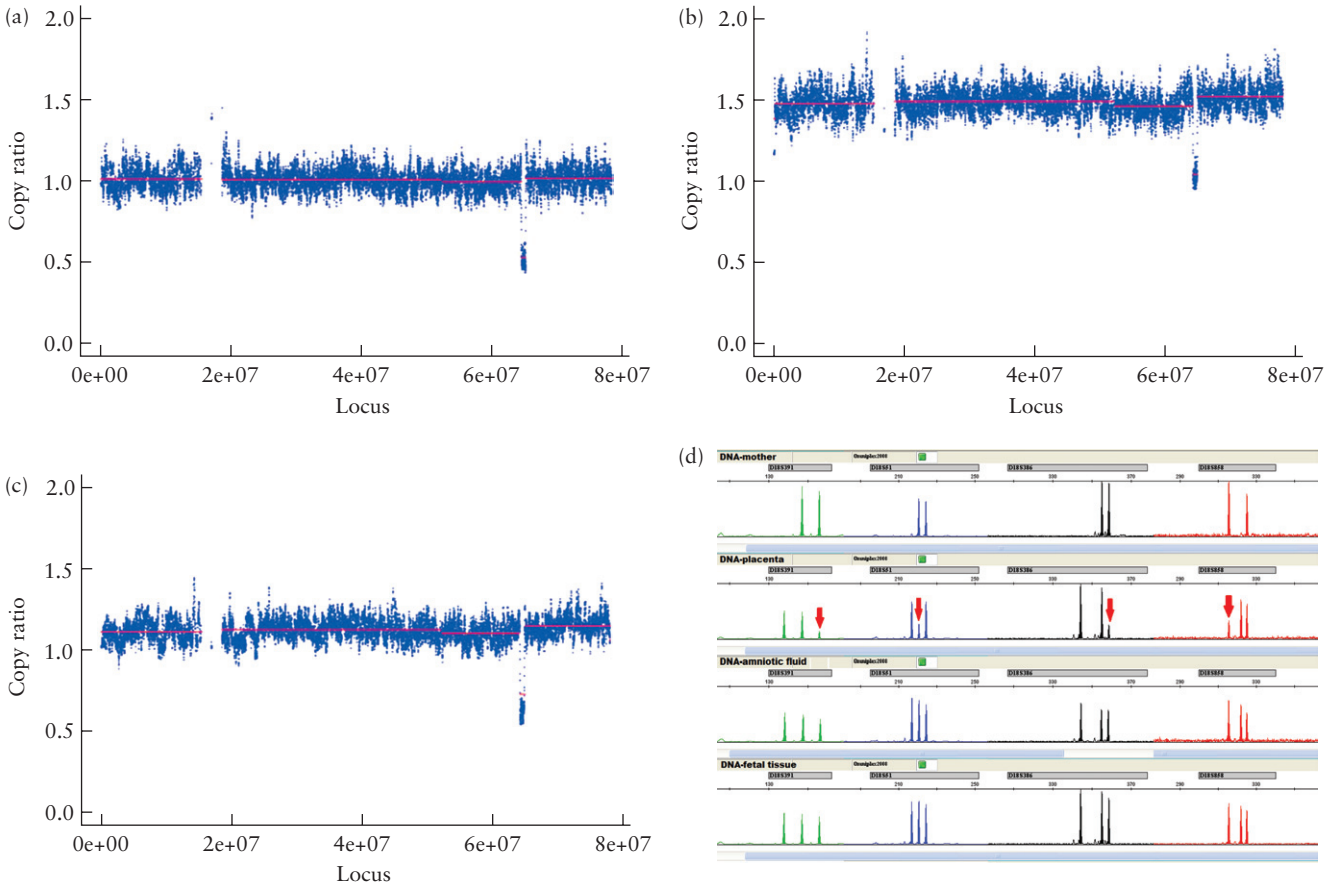

Figure 1 (a,b,c) Massively parallel sequencing results obtained with: (a) DNA from maternal peripheral white blood cells, showing a copy ratio of chromosome 18 of 1.0, suggestive of the euploid status of the maternal background; (b) DNA from fetal tissue, showing a copy ratio of chromosome 18 of 1.5, suggestive of fetal trisomy 18; (c) DNA from the placenta, showing a copy ratio between 1.0 and 1.5, indicative of a low level of mosaicism of trisomy 18 (copy ratio plotted in blue and segmentation indicated by red lines). (d) Quantitative fluorescent polymerase chain reaction results obtained with DNA from maternal blood, placenta, amniotic fluid and postmortem fetal tissue using different short tandem repeat markers from chromosome 18. Presented are results for markers D18S391, D18S51, D18S386 and D18S858. A low level of mosaicism in the placenta is indicated by reduced marker signals (arrows).

been revealed by NIPT. After post-test counseling, the couple decided on pregnancy termination and agreed to further analyses.

Karyotyping and QF-PCR of the maternal peripheral blood confirmed the maternal genetic background as being euploid. A fluorescence *in-situ* hybridization assay on placental tissues with chromosome 18- and X-specific probes revealed a total of seven different clones concerning chromosomes 18 and X with a majority (73/103) of disomic nuclei, showing the existence of placental mosaicism (20–30%) (Figure S1). This was also confirmed by QF-PCR and MPS results of placental samples which revealed XXX and various levels of trisomy 18 mosaicism (Figure 1). In contrast, G-banding, QF-PCR and MPS results of the postmortem fetal tissue all demonstrated complete XXX and trisomy 18, showing a genetic discordance between the fetal and placental tissue.

In the case reported here, the discrepant result between NIPT, first-trimester combined screening and fetal karyotyping was due to genetic discordance between the placenta and fetus. With the estimated fetal fraction of 7.4% and euploid maternal background, the influence of an insufficient fetal fraction and an abnormal maternal background could be excluded. Confined mosaic placenta has been reported to cause a small but non-negligible number of false NIPT results[4,5]. In this case we discovered that the level of mosaicism is also important since the 7.4% fetal cfDNA fraction and the 30% trisomy 18 mosaicism resulted in a reduced (< 2.2%) effective fetal fraction for trisomy 18 detection, which is under the detection threshold of our NIPT method (3.5%)[3].

In conclusion, both the discrepancy between placental and fetal karyotypes and the level of mosaicism can compromise the NIPT result. Although a superior screening test, NIPT results should be interpreted in the context of other information (clinical and family) and comprehensive genetic counseling is essential.

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Disclosure

Y.G., F.J. and W.W. are employees of BGI-Shenzhen.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figure S1 (a,b) Abnormal ultrasound findings at 16 weeks' gestation showing a single umbilical artery (a) and clubfoot (b), suggesting high risk of chromosomal abnormality. (c) Placental fluorescence *in-situ* hybridization showing a combination of XXX and trisomy 18 mosaicism. (d) G-banding of cultured amniocytes showing fetal karyotype as 48,XXX,+18.