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A discordant case in which T21 positive and 47,XXY negative non-invasive prenatal testing result was associated with a 47,XXY mosaic fetus

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Summary

The authors report a discordant case in which T21 positive and 47,XXY negative non-invasive prenatal testing (NIPT) result was associated with a 47,XXY mosaic fetus confirmed by karyotyping. By investigating the genetic cause, the initial evidence for T21 was demonstrated to be induced by the confined placental mosaicism; the initial lack of evidence for 47,XXY could be attributed to the relatively low effective fetal fraction. This case supported that confined placental mosaicism and low effective fetal fraction are major biological risk factors associated with discordant NIPT results, and provided an unusual case for pre- and post-test NIPT counseling.

Key words: False negative; Fetal fraction; Mosaicism; Non-invasive prenatal testing.

Introduction

Non-invasive prenatal testing (NIPT) by next generation sequencing (NGS) has become rapidly integrated into prenatal care worldwide. The clinical data with large sample size demonstrated that the sensitivities and specificities of NIPT are greater than 99.9 % [1]. The investigation of the false positive and negative NITP results is important for improving the technical accuracy and genetic counseling. Confined placental mosaicism [2], fetal mosaicism [3], maternal mosaicism [4], and intrinsic genomic alterations have been demonstrated as major contributors to the false NIPT results. In this study, the authors reported an unusual false NIPT case due to the coexistence of confined placental mosaicism and the fetal mosaicism.

Case Report

A 38-year-old woman with a spontaneously conceived singleton pregnancy underwent NIPT at 13^{+5} weeks' gestation following a published protocol [5]. The results suggested a potential risk of trisomy 21 (Table 1, plasma 1). After genetic counseling, the patient underwent an amniocentesis at 16^{+2} weeks' gestation to confirm the fetal karyotype. In the meantime, a second maternal plasma sample was screened by NIPT, and the results supported the initial findings (Table 1, plasma 2). Interestingly, the fetal karyotype revealed 47,XXY mosaicism (46,XY/47,XXY) by the karyotyping. Ultimately, the patient decided to terminate the pregnancy and the authors received written consent for further sampling to explore the potential causes of the discordant results. Before the induction of labor, maternal plasma was collected at 23 weeks' gestation for a third round of NIPT. Again, the results indicated a high risk for trisomy 21. Notably, while studying plasma containing a high fetal fraction (FF) at this time, the authors also observed some evidence of abnormal sex chromosome ratios, which were not revealed by the previous tests (Table 1, plasma 3). Six placental tissue samples were collected and analyzed by low coverage sequencing following a published protocol [6]. All of the tissue samples were confirmed as 47,XXY mosaicism (mean mosaic ratio, 37%; SD, 16.96%), and two samples showed trisomy 21 mosaicism (Table 2). Simultaneously, maternal mosaicism and intrinsic genomic alterations induced by maternal diseases were excluded by the sequencing of the plasma sample collected 24 hours after placental expulsion (Table 1, plasma 4) and the maternal buffy coat (Table 2). Ultimately, the initial evidence for trisomy 21 was demonstrated to be induced by the confined placental mosaicism; the initial lack of evidence for increased dosages of the sex chromosomes could be attributed to the relatively low effective FF (i.e., the mosaic ratio × FF) [7].

Discussion

The level and nature of the placental mosaicism have been shown significantly influenced the final NIPT result [2, 8]. Although the mosaic ratio was relative higher for 47,XXY than T21 cell line in six placental biopsy samples, the actual output of T21 and 47,XXY DNA from mosaic placenta into the maternal plasma cannot be accurately measured. The authors only can reasonably postulate that the mosaic placenta preferentially releasing greater amounts of T21 than 47,XXY DNA into the maternal plasma where only T21 was detectable by NIPT in the early

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Table 1. — *Tests for maternal plasma and amniotic fluid at different gestational age.*

Samples	Gestational age (week)	[a]T-value for chr 21	[b]T-value for sex chromosomes	FF estimated from Y chromosome (%)	Detection method	Test interpretation
Plasma 1	13^{+5}	2.60	2.23	8.86	NGS	Potential risk for T21
Plasma 2	16^{+2}	3.68	1.82	11.06	NGS	High risk for T21
Amniotic fluid	16^{+2}	-	-	-	Karyotyping	46,XY [32] /47,XXY [18]
Plasma 3	23	9.56	2.86	28.93	NGS	High risk for T21; potential risk for sex chromosome aneuploidy
Plasma 4	23^{+3}	-0.21	1.34	0.76	NGS	Euploidy

[a] According to previous literature [5], t-value > 3 is considered the high risk criteria for fetal trisomy 21. In this clinical practice, t-value > 2.5 is considered the potential risk criteria for fetal trisomy 21. [b] According to previous literature [5], t-value >2.5 was an indicator of sex chromosome aneupoidy.

Table 2. — *Investigating the genetic cause of the discordant result by low coverage sequencing.*

Samples	Gestational age (weekz)	Copy number ratio for chr21	Copy number ratio for chrX	Test interpretation
Buffy coat	23	1.00	1.00	100% XX
Placental tissue 1	23^{+2}	1.00	1.16,	32% XXY; 68% XY
Placental tissue 2	23^{+2}	1.00	1.15,	30% XXY; 70% XY
Placental tissue 3	23^{+2}	1.00	1.35	70% XXY; 30% XY
Placental tissue 4	23^{+2}	1.00	1.19	38% XXY; 62% XY
Placental tissue 5	23^{+2}	1.26	1.11	52% T21; 22% XXY; 26% XY
Placental tissue 6	23^{+2}	1.25	1.15	50% T21; 30%XXY; 20% XY

pregnancy.

In conclusion, the authors reported an unusual false NIPT case and investigated the genetic cause of the discordant result. This study not only supported the previous finding that placental mosaicism combined with confined regional fluctuations in the level of tissue mosaicism appears to be a major biological risk factor associated with discordant NIPT results [2], but also provided an unusual case for pre-test and post-test NIPT counseling.

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