Original Article

The role of non-invasive prenatal testing and ultrasound in prenatal screening of fetal chromosomal abnormalities in singleton: a retrospective study

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Background: Non-invasive prenatal testing (NIPT) has good screening performance for common chromosomes, but it may have false positive (FP) and false negative (FN) results for various reasons. For abnormal NIPT results, the combination of fetal ultrasound phenotypes will provide more fetal information for prenatal diagnosis. The aim of this study was to combine NIPT and ultrasound phenotypes to analyze their complementary roles in prenatal screening of fetal chromosome abnormalities.

Methods: From January 2018 to December 2021, 12,803 pregnant women with singleton who successfully underwent NIPT/expanded NIPT (NIPT-Plus) at Xiangya Hospital of Central South University, of which 111 cases were positive results and one case was FN result. We retrospectively collected the clinical features, ultrasonographic findings, prenatal diagnosis, and pregnancy outcomes of these 112 pregnant women and analyzed the ultrasonic manifestations of different chromosomal abnormalities in detail.

Results: The positive predictive values (PPVs) of NIPT/NIPT-Plus for trisomy (T)21, T18, sex chromosome abnormality (SCA), microdeletion/microduplication syndrome (MMS), T13, and rare autosomal trisomy (RAT) were 100.0%, 85.7%, 57.1%, 44.4%, 40.0%, and 7.7%, respectively. The total termination rates of pregnancy for T21, T18, T13, SCA, pathogenic MMS, and RAT were 93.5%, 100.0%, 100.0%, 66.7%, 100.0%, and 100.0%, respectively. From the karyotypes of SCA live-born fetuses, 47,XYY and 47,XXX were more likely to be selected for continued pregnancy. The ultrasound phenotypes of T21 were diverse, including normal, soft marker, and structural malformation. Both T18 and T13 had structural malformations as the main phenotypes. Most ultrasound phenotypes of FP T21, T18, and T13 were normal but occasionally manifested as fetal growth restriction (FGR). The ultrasound phenotypes of SCA, MMS, and RAT were relatively mild and manifested as normal, soft marker, FGR, or polyhydramnios, and the ultrasound phenotypes were similar between FP and true positive (TP) cases.

Conclusions: Ultrasound phenotypes are helpful in identifying FP NIPT/NIPT-Plus results, especially for T18 and T13. Given its mild ultrasound phenotypes, NIPT-Plus has important clinical significance in reducing the missed diagnosis of SCA, MMS, and RAT, but its screening performance needs to be further improved.

Keywords: Non-invasive prenatal testing (NIPT); ultrasound; pregnancy outcomes; aneuploidy; microdeletion/microduplication syndrome (MMS)

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Introduction

Trisomy (T)21, T18, T13, and sex chromosome abnormality (SCA) are the most common chromosomal abnormalities, accounting for 30% of all live births with chromosome abnormalities (1). The development of non-invasive prenatal testing (NIPT) has expanded the detection range from common aneuploidy to SCA, microdeletion/microduplication syndrome (MMS), and even monogenic disorders (2-4). Clinically, NIPT has significant differences in detection rate and positive predictive value (PPV) of different chromosome abnormalities (5,6). A previous study has demonstrated the high sensitivity and specificity of NIPT for screening T21, T18, and T13 (7), but the current guidelines do not recommend its routine application in prenatal screening of SCA and MMS (8,9). Some studies have shown that the combined PPVs of expanded NIPT (NIPT-Plus) for SCA, MMS, and rare autosomal trisomy (RAT) is about 31.97–46.70%, 28.99–49.02%, and 4.88–28.60%, respectively (5,6,10,11). However, methods of increasing the detection rate and decreasing the false positive rate (FPR) of SCA, MMS, and RAT are still in the exploratory stage.

Highlight box

Key findings

- Given its mild ultrasound phenotypes, NIPT-Plus has important clinical significance in reducing the missed diagnosis of SCA, MMS, and RAT. However, its screening performance needs to be further improved.

What is known and what is new?

- NIPT has high sensitivity and specificity for common aneuploidy screening in clinical application.
- The ultrasound phenotypes of fetuses with chromosomal abnormalities are diverse. For fetuses with abnormal NIPT/NIPT-Plus results, the ultrasound phenotypes are helpful in identifying FP results, especially for T18 and T13.

What is the implication, and what should change now?

- The improvement of NIPT-Plus screening performance and standard ultrasound screening are very important for improving the detection rate and reducing the FPR of fetal chromosomal abnormalities.

Ultrasound can dynamically evaluate fetal growth and development, anatomical structure and fetal appendages. Confined placental mosaicism, maternal factors (including low levels of maternal mosaicism or tumors, copy number variants) or low fetal cell-free DNA (cfDNA) concentrations may lead to false negative (FN) or false positive (FP) results of NIPT, and in these cases, ultrasound screening may detect true fetal chromosomal abnormalities (12,13). Conversely, fetal chromosomal abnormalities are not always accompanied by abnormal ultrasound phenotypes, but can be detected by NIPT. Therefore, NIPT and ultrasound complement each other in prenatal screening to provide more diagnostic information for fetal chromosome abnormalities (14). In the cases with abnormal NIPT results, fetal ultrasound phenotypes, although not a substitute for invasive prenatal diagnosis, can indirectly predict the fetal chromosome results in some cases. Therefore, fetal ultrasound phenotypes can provide more supporting information for obstetricians and genetic counselors to recommend invasive prenatal diagnosis and assess fetal prognosis. Currently, most studies have focused on the assessment of the screening performance of NIPT for fetal chromosomal abnormalities, and few studies have analyzed in detail the abnormal ultrasound phenotypes of fetuses with true- and false-positive NIPT results. In the present study, we aimed to analyze the screening performance of NIPT for fetal chromosomal abnormalities and to analyze the differences in ultrasound phenotypes of fetuses with abnormal NIPT results, in order to focus on the complementary role of NIPT and ultrasound in the prenatal screening of fetal chromosome abnormalities. We present the following article in accordance with the STROBE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6343/rc).

Methods

Patient population

From January 2018 to December 2021, this retrospective study enrolled 12803 pregnant women with singleton who successfully underwent NIPT or NIPT-Plus at Xiangya Hospital of Central South University. The NIPT/NIPT-

Plus inclusion criteria were as follows: (I) singleton pregnancy; (II) blood samples were collected from 12–26^{+6} gestational weeks. The exclusion criteria were as follows: (I) multiple pregnancies, including vanishing twins; (II) one or both couples had a definite chromosomal abnormality; (III) pregnant women with malignant tumors or immune system diseases; (IV) the pregnant women had received transplantation, allogeneic blood transfusion or stem cell therapy within 1 year. All patients underwent pretest counseling and were informed of the content and limitations of the test. Pregnant women choose NIPT or NIPT-Plus according to their preferences within the indications.

Sample preparation and sequencing

A total of 8–10 mL maternal peripheral blood was collected using an EDTA anticoagulant tube, and plasma was separated within 8 hours after collection. After cfDNA was extracted from plasma, the DNA library was constructed and quantified according to the manufacturer's instructions of JingXin Fetal Chromosome Aneuploidy (T21, T18, and T13) Testing Kits (Boao Bio-Tech Co., Ltd., Beijing, China). Semiconductor sequencing technology was then used for sequencing on the BioelectronSeq 4000 Platform (Thermo Fisher, Waltham, MA, USA). After the sequencing results were processed by bioinformatics, Z-score was used to identify fetal chromosome aneuploidy or microdeletions/microduplications.

Ultrasound screening

Ultrasound screening was performed by specialized obstetric sonographers, and the corresponding practice were followed to screen for fetal abnormalities (15). Ultrasound follow-up was performed throughout the pregnancy or until the termination of pregnancy. Fetal ultrasonographic findings included soft marker, structural malformation, fetal growth restriction (FGR), and polyhydramnios. FGR was defined as an estimated fetal weight less than the 10th percentile for gestational age (16). Polyhydramnios was defined as the deepest vertical pocket ≥8 cm or amniotic fluid index ≥25 cm. Ultrasonographic findings were divided into seven groups: group (G)1: soft marker; G2: structural malformation; G3: soft marker and structural malformation; G4: soft marker and FGR; G5: soft marker, structural malformation and FGR; G6: FGR; and G7: others.

Prenatal diagnosis

Prenatal diagnosis was recommended when the results of NIPT/NIPT-Plus were high-risk or low-risk but ultrasonographic findings suggested fetal abnormalities. Amniocentesis was the preferred sampling method for prenatal diagnosis. Genetic molecular diagnosis selected karyotype analysis and copy number variation (CNV) analysis. If the fetal gestational week was greater than 26 weeks and the karyotype analysis failed, only CNV analysis was performed. Chromosomal microarray analysis (CMA) or CNV-sequencing (CNV-seq) were performed using CytoScan 750K array (Affymetrix, Santa Clara, CA, USA) or BioelectronSeq 4000 Platform (Thermo Fisher, Waltham, MA, USA).

Follow-up of pregnancy outcomes

We followed up the pregnancy outcomes of pregnant women with NIPT/NIPT-Plus positive or FN results. All newborns were examined by pediatricians, and those suspected of chromosomal abnormalities underwent further examination and diagnosis. Parents were followed up by telephone from the prenatal diagnostic center and home visits were conducted in the community to assess fetal growth and development. Positive and FN results of NIPT/NIPT-Plus were covered by insurance, and only one case of FN NIPT result had been reported during the study period. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All patients who participated in this study signed an informed consent form, and this study was approved by the Medical Ethics Committee of Xiangya Hospital (No. 202006429).

Statistical analysis

SPSS26 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Quantitative data was expressed as n and qualitative data was expressed as the percentage. Screening performance was calculated according to the evaluation indicators of screening tests. The chi-square test or Fisher's exact probability test was used to compare the rates between different groups. P=0.05 was used as the test

Table 1 Comparison between the results of NIPT/NIPT-Plus and prenatal diagnosis

NIPT/NIPT-Plus	TP	FP	FN	RPD	DR (%)	PPV (%)	PDR (%)
T21	30[a]	0	1	0	96.8	100.0	100.0
T18	6	1	0	0	100.0	85.7	100.0
T13	2	3	0	0	100.0	40.0	100.0
SCA	24[b]	18	0	4	100.0	57.1	91.3
MMS	4[c]	5[d]	0	0	100.0	44.4	100.0
RAT	1[e]	12[f]	0	1[g]	100.0	7.7	92.9

[a], including four cases mosaicism, and the proportions of mosaicism were 90%, 40%, 13%, and 5%, respectively. [b], chromosomal results: 11 cases of 47,XXY, including one case mosaicism of 47,XXY(8)/46,XY(54); seven cases of 47,XXX, including one case mosaicism of 47,XXX(68)/45,X(27)/46,XX(10); two cases of 47,XYY; three cases of 45,X, including one case mosaicism of 45,X(37)/46XX(63); one case of Xq21.31q22.1 duplication (8.64 Mb, likely pCNV). [c], chromosomal results: one case of Cri du Chat syndrome (pCNV); one case of 7q36.1q36.3 deletion (11.02 Mb, pCNV); one case of 10q26.13q26.3 deletion (10.85 Mb, pCNV); one case of 3p12.2p11.1 deletion (7.41 Mb, VUS). [d], NIPT results: one case of 1p36 deletion syndrome; one case of Angelman/Prader-Willi syndrome; one case of Cri du Chat syndrome; one case of 19.05Mb deletion on chromosome 4; one case of 37.29 Mb deletion on chromosome 11. [e], chromosomal result: arr(16)*3[0.4]. [f], NIPT results: each one case of T6, T9, T11, T15, and T22; each two cases of T8 and T20; three cases of T3. [g], one case of T8. NIPT, non-invasive prenatal testing; NIPT-Plus, expanded NIPT; TP, true positive; FP, false positive; FN, false negative; RPD, refusal of prenatal diagnosis; DR, detection rate; PPV, positive predictive value; PDR, prenatal diagnostic rate; T, trisomy; SCA, sex chromosome abnormality; MMS, microdeletion/microduplication syndrome; RAT, rare autosomal trisomy; pCNV, pathogenic copy number variation; VUS, variants of uncertain significance.

level, and P<0.05 was considered statistically significant.

Results

Comparison between the results of NIPT/NIPT-Plus and prenatal diagnosis

Among 12,803 pregnant women with singleton who underwent NIPT/NIPT-Plus, 111 cases of positive results and one case of FN result were detected. We retrospectively collected the clinical features, ultrasonographic findings, prenatal diagnosis, and pregnancy outcomes of the 112 singletons. Taking the results of prenatal diagnosis as the gold standard, the screening performance of NIPT/NIPT-Plus for T21, T18, T13, SCA, MMS, and RAT are shown in *Table 1*. NIPT detected 24 cases of T21-true positive (TP), 5 cases of T18-TP, 2 cases of T13-TP, 3 cases of T13-FP, and 3 cases of SCA-TP. NIPT-Plus detected 6 cases of T21-TP, 1 case of T21-FN, 1 case of T18-TP, 1 case of T18-FP, 21 cases of SCA-TP, 18 cases of SCA-FP, 4 cases of SCA-refusal of prenatal diagnosis (RPD), 4 cases of MMS-TP, 5 cases of MMS-FP, 1 case of RAT-TP, 12 cases of RAT-FP, and 1 case of RAT-RPD.

In terms of the proportion of fetal chromosomal abnormalities, T21, SCA, T18, MMS, T13, and RAT accounted for 45.6% (31/68), 35.3% (24/68), 8.8% (6/68), 5.9% (4/68), 2.9% (2/68), and 1.5% (1/68), respectively. NIPT/NIPT-Plus exhibited a high detection rate for common aneuploidy, SCA, and MMS; however, there was a marked difference in the PPVs. In terms of PPV values, T21/T18 ranked first, SCA/MMS/T13 ranked middle, and RAT ranked last. The PPV for RAT was the lowest at 7.7%, increasing the risk of unnecessary prenatal diagnosis. The prenatal diagnosis rates of SCA and RAT were 91.3% and 92.9%, respectively, and those of the other groups were all 100%. There was no significant difference in prenatal diagnosis rate among these groups.

Follow-up of pregnancy outcomes and ultrasonographic findings

The follow-up results of pregnancy outcomes and the ultrasonographic findings of fetuses are shown in *Table 2*. For fetuses with T21, the total pregnancy termination rate was 93.5% (29/31). Only two cases of T21 fetuses chose to continue pregnancy: one case of a T21 fetus had a low proportion of 5% mosaicism and the ultrasound phenotype was normal. The pregnant woman in this case chose to continue the pregnancy after being informed of possible phenotypes by genetic counseling. The other case of T21 fetus with ultrasound abnormalities

Table 2 Follow-up of pregnancy outcomes and ultrasonographic findings of the fetuses using NIPT/NIPT-Plus

NIPT/ NIPT-Plus	Total	USG findings								Outcomes		
		N	G1	G2	G3	G4	G5	G6	G7	TOP	LB	OP
T21-TP	30	13	11	1	4	–	–	–	1[a]	28	2[b]	–
T21-FN	1	–	–	–	1	–	–	–	–	1	–	–
T18-TP	6	1	–	–	4	–	1	–	–	6	–	–
T18-FP	1	–	–	–	–	–	–	1	–	–	1	–
T13-TP	2	–	–	2	–	–	–	–	–	2	–	–
T13-FP	3	3	–	–	–	–	–	–	–	–	3	–
SCA-TP	24	18	5	–	–	–	–	–	1[c]	16	8[d]	–
SCA-FP	18	15	1	–	–	–	–	1	1[c]	–	17	1
SCA-RPD	4	2	1	–	–	–	–	1	–	–	3	1
MMS-TP	4	2	1	–	–	1	–	–	–	3	–	1[e]
MMS-FP	5	3	2	–	–	–	–	–	–	–	5	–
RAT-TP	1	–	–	–	–	1	–	–	–	1	–	–
RAT-FP	12	7	2	–	–	–	–	2	1[f]	1[h]	9	2
RAT-RPD	1	–	–	–	–	1	–	–	–	–	1	–
Total	112	64	23	3	9	3	1	5	4	58	49	5

[a], stillbirth at 19 gestational weeks. [b], 1 case of live birth was T21 with a low proportion of 5% mosaicism; the other case was a fetus with T21 combined with cardiac malformation, and the newborn died 1 day after birth. [c], polyhydramnios. [d], chromosomal results: 4 cases of 47,XXX, 2 cases of 47,XYY, 1 case of 47,XXY(8)/46,XY(54), and 1 case of Xq21.31q22.1 duplication (8.64 Mb, likely pCNV). [e], chromosomal result: 3p12.2p11.1 deletion (7.41 Mb, VUS). [f], widened septum pellucida. [h], termination of pregnancy due to severe preeclampsia and fetal intrauterine hypoxia rather than chromosomal abnormality. NIPT, non-invasive prenatal testing; NIPT-Plus, expanded NIPT; USG, ultrasonography; N, normal; G, group; TOP, termination of pregnancy; LB, live birth; OP, on-going pregnancy; T, trisomy; TP, true positive; FN, false negative; FP, false positive; RPD, refusal of prenatal diagnosis; SCA, sex chromosome abnormality; MMS, microdeletion/ microduplication syndrome; RAT, rare autosomal trisomy; pCNV, pathogenic copy number variation; VUS, variants of uncertain significance.

(complete atrioventricular septal defect, aortic overriding, and nasal bone dysplasia) was recommended to terminate the pregnancy but the pregnant woman chose to continue. After birth, the newborn had a wide eye distance, low and flat nose bridge, eye external oblique, broken palmprint in the right hand, and incomplete sucking and foraging reflexes, and died 1 day after birth.

For the T18 and T13 TP cases, the termination rates of pregnancy were both 100%. For the SCA TP cases, the total termination rate of pregnancy was 66.7% (16/24). The termination rates of 45,X, 47,XXY, 47,XXX, and 47,XYY were 100.0% (3/3), 90.9% (10/11), 42.9% (3/7), and 0% (0/2), respectively. From the karyotypes of live-born fetuses, it could be seen that the fetuses with 47,XYY and 47,XXX were more likely to be selected for continued pregnancy. Among the eight cases of live-born fetuses, five had no abnormal ultrasound phenotype, and the remaining three had the following ultrasound phenotypes: nuchal translucency (NT) 2.7 mm (>95th); short femur length and choroid plexus cyst (CPC); and polyhydramnios.

For the MMS TP cases, one case of continued pregnancy was 3p12.2p11.1 microdeletion [variants of uncertain significance (VUS)] with normal ultrasound phenotype, and the remaining three cases of pathogenic CNV (pCNV) chose to terminate the pregnancy. The termination rate of pregnancy with pathogenic MMS was 100%. For the RAT TP case, only one case of mosaic T16 chose to terminate the pregnancy, and its ultrasound phenotype was FGR, persistent left superior vena cava with dilated coronary sinus, and single umbilical artery.

The details of fetal ultrasonographic findings

Among the 112 cases of pregnant women, a total of 48 cases had fetal ultrasound abnormalities, as shown in *Table 3*. The ultrasound phenotypes of T21 TP fetuses were diverse, including normal, soft marker, soft marker combined with structural malformation, FGR, etc., but ultrasonic normal and soft marker accounted for 43.3% and 36.7%, respectively. The top three ultrasonic soft markers were NT, CPC, and nasal bone aplasia (NBA). The ultrasound phenotypes of T18 and T13 TP fetuses were mainly structural malformations, and the T18 fetuses were often combined with soft markers. Most of the FP cases of T21, T18, and T13 were normal on ultrasound,

Table 3 The details of fetal ultrasonographic findings

Patient	MA (years)	Classification	NIPT/NIPT-Plus	PT	Groups	GW	USG findings	PD results	Outcomes
1	31	T21-TP	T21	NP	G1	18	VM, ASP	T21	TOP
2	35	T21-TP	T21	NP	G1	17	HI	T21	TOP
3	27	T21-TP	T21	ART	G1	16	HI	mos47,+21	TOP
4	38	T21-TP	T21	NP	G1	12	NT, NBA	T21	TOP
5	36	T21-TP	T21	NP	G1	17	CPC	T21	TOP
6	26	T21-TP	T21	NP	G1	17	CPC	T21	TOP
7	29	T21-TP	T21	NP	G1	13–18	NT, NBA	T21	TOP
8	34	T21-TP	T21	NP	G1	13	NT	T21	TOP
9	28	T21-TP	T21	NP	G1	12	NT	T21	TOP
10	40	T21-TP	T21	NP	G1	18	PE	T21	TOP
11	34	T21-TP	T21	NP	G1	12–18	NT, CPC, RPE	T21	TOP
12	28	T21-TP	T21	NP	G2	19	TOF	mos47,+21	TOP
13	36	T21-TP	T21	NP	G3	19–23	AVSD, AOR, NBH	T21	LB
14	30	T21-TP	T21	NP	G3	17	ASD, DBS, CPC	T21	TOP
15	39	T21-TP	T21	NP	G3	17	ARSA, GD	T21	TOP
16	41	T21-TP	T21	ART	G3	12–17	NT, NBA, VSD	T21	TOP
17	34	T21-TP	T21	NP	G7	19	SB	T21	TOP
18	30	T21-FN	LR	ART	G3	22	NF, VSD	T21	TOP
19	43	T18-TP	T18	NP	G3	12–17	VSD, CPC, SUA	T18	TOP
20	31	T18-TP	T18	NP	G3	12–17	NT, CH, CPC, HK	T18	TOP
21	31	T18-TP	T18	NP	G3	19	DORV, PAS, ASD, VSD, NBH	T18	TOP
22	34	T18-TP	T18	NP	G3	19	MVA, VSD, DORV, SH, CPC	T18	TOP
23	35	T18-TP	T18	NP	G5	13–17	NT, NBA, FGR, ASP, VSD, CPC, MG	T18	TOP
24	34	T18-FP	T18	NP	G6	31	FGR	N	LB
25	27	T13-TP	T13	NP	G2	17	HPE, CLP, AS, VSD	T13	TOP
26	37	T13-TP	T13	NP	G2	18	CVA, HV, LVD, VSD	T13	TOP

Table 3 (*continued*)

Table 3 (*continued*)

Patient	MA (years)	Classification	NIPT/NIPT-Plus	PT	Groups	GW	USG findings	PD results	Outcomes
27	27	SCA-TP	SCA	NP	G1	12	NT	47,XXX	LB
28	22	SCA-TP	SCA	NP	G1	12	NT	47,XXX	TOP
29	32	SCA-TP	SCA	NP	G1	13	NT, CH, NIHF	45,X	TOP
30	43	SCA-TP	SCA	NP	G1	32	SFL, CPC	47,XXX	LB
31	30	SCA-TP	SCA	NP	G1	21	EICF	47,XXY	TOP
32	34	SCA-TP	SCA	NP	G7	32	PH	47,XXX	LB
33	32	SCA-FP	SCA	NP	G1	17–32	CPC, SFL	N	LB
34	25	SCA-FP	SCA	NP	G6	36	FGR	N	LB
35	35	SCA-FP	SCA	NP	G7	36	PH	N	LB
36	30	SCA-RPD	SCA	NP	G1	23	HI	–	LB
37	25	SCA-RPD	SCA	NP	G6	34	FGR, SFL	–	LB
38	28	MMS-TP	Cri du Chat syndrome	NP	G1	13	NT	Cri du Chat syndrome	TOP
39	32	MMS-TP	7q deletion syndrome (7q32→qter)	NP	G4	18	FGR, SFL	7q36.1q36.3 deletion (11.02Mb): pCNV	TOP
40	33	MMS-FP	1p36 deletion syndrome	NP	G1	18	CPC	N	LB
41	40	MMS-FP	Chromosome 4 deletion (19.05 Mb)	NP	G1	23	CPC	N	OP
42	25	RAT-TP	T16	NP	G4	19	FGR, PLSVC, SUA	mos47,+16	TOP
43	27	RAT-FP	T11	NP	G1	23	SFL, ARA	N	LB
44	29	RAT-FP	T6	NP	G1	19	CPC	N	LB
45	37	RAT-FP	T8	ART	G6	33	FGR	N	LB
46	36	RAT-FP	T8	ART	G6	23	FGR	N	TOP
47	36	RAT-FP	T3	NP	G7	29	WSP	N	LB
48	36	RAT-RPD	T8	ART	G4	23	FGR, HI	–	LB

MA, maternal age; NIPT, non-invasive prenatal testing; NIPT-Plus, expanded NIPT; PT, pregnancy type; GW, gestational weeks of USG finding; USG, ultrasonography; PD, prenatal diagnosis; T, trisomy; TP, true positive; NP, natural pregnancy; G, group; VM, ventriculomegaly; ASP, absent septum pellucida; TOP, termination of pregnancy; HI, hyperechogenic intestine; ART, assisted reproductive technology; NT, nuchal translucency; NBA, nasal bone aplasia; CPC, choroid plexus cyst; PE, pelviectasis; RPE, right pleural effusion; TOF, tetralogy of fallot; AVSD, atrio-ventricular septal defects; AOR, aortic overriding; NBH, nasal bone hypoplasia; LB, live birth; ASD, atrial septal defect; DBS, double bubble sign; ARSA, aberrant right subclavian artery; GD, gastric dysplasia; VSD, ventricular septal defects; SB, stillbirth; FN, false negative; NF, increased nuchal fold measurement; LR, low risk; SUA, single umbilical artery; CH, cystic hygroma; HK, horseshoe kidney; DORV, double-outlet right ventricle; PAS, pulmonary artery stenosis; MVA, mitral valve atresia; SH, strawberry head; FGR, fetal growth restriction; MG, micrognathia; FP, false positive; HPE, holoprosencephaly; CLP, cleft lip and palate; AS, aortic stenosis; CVA, cerebellar vermis agenesis; HV, hemivertebra; LVD, left ventricular dysplasia; SCA, sex chromosome abnormality; NIHF, nonimmune hydrops fetalis; SFL, short femur length; EICF, echogenic intracardiac focus; PH, polyhydramnios; RPD, refusal of prenatal diagnosis; pCNV, pathogenic copy number variation; MMS, microdeletion/microduplication syndrome; PLSVC, persistent left superior vena cava; ARA, accessory renal artery; WSP, widened septum pellucida; RAT, rare autosomal trisomy.

sometimes presenting with FGR. SCA TP fetuses were mainly characterized by normal ultrasound (75.0%) and soft markers (20.8%) and occasionally presented with polyhydramnios. The SCA FP ultrasound phenotypes were mostly normal (83.3%), with occasional soft marker, FGR, and polyhydramnios. The SCA TP and FP ultrasound phenotypes were basically similar. The ultrasound phenotypes of MMS and RAT TP cases were normal, soft marker and FGR, and the ultrasound phenotypes of TP and FP cases were also similar.

Discussion

For the incidence of fetal chromosomal abnormalities from the TP results of clinical screening of NIPT/NIPT-Plus in our study, T21 and SCA accounted for the majority, followed by T18 and MMS, which was consistent with the proportion of fetal chromosomal abnormalities in other large studies (5,6,17). For PPV values, T21/T18 ranked first, SCA/MMS/T13 ranked middle, and RAT ranked last. However, whether NIPT-Plus, which includes screening for SCA and MMS, should be included in routine screening remains controversial (18,19). From the perspective of the clinical need to reduce birth defects caused by chromosomal abnormalities, SCA and MMS should be included in prenatal screening to maximize the type and number of chromosomal abnormalities that can be detected. A previous study showed that the detection rate of pCNV in prenatal fetuses is significantly lower than that in postnatal fetuses, suggesting that prenatal NIPT-Plus can help improve the diagnostic yield of chromosomal abnormalities in prenatal fetuses (20). In our study, for TP cases of SCA and MMS, 71.4% (20/28) of the fetuses had a normal ultrasound phenotype, and prenatal screening for these chromosomal abnormalities could only be achieved by NIPT-Plus. However, there are still some issues that should be considered carefully in the clinical practice of NIPT-Plus. Firstly, the screening performance of NIPT-Plus for SCA and MMS is not as good as that for T21 and T18 (21,22). Secondly, the phenotypes of SCA and MMS are milder than those of common aneuploidy, and the limited ultrasound phenotypes of intrauterine fetuses present greater challenges to clinical genetic counseling, as well as more anxiety and burden for couples in pregnancy selection. Most of the additional RATs found by NIPT-Plus have a PPV of lower than 10%, and only a few experiments have been carried out for the prenatal screening of monogenic diseases (23). With the improvement of sequencing level and the reduction of cost, fetal whole genome sequencing is expected to be realized in the future. However, based on its current screening performance, it is recommended that NIPT can be used as routine screening, and NIPT-Plus should be performed with genetic counseling and selected more carefully to reduce unnecessary prenatal diagnosis due to the relatively high FPR of SCA and MMS.

Whether the fetus has chromosomal or ultrasound abnormalities are two necessary conditions for clinical obstetricians to evaluate the fetal prognosis. In terms of pregnancy outcomes, we observed that SCA TP fetuses had the highest acceptance, and most of the other fetal chromosomal abnormalities were selected for termination. Although the phenotypes of SCA vary according to different karyotypes, the fetus with relatively mild phenotypes will likely be selected to continue the pregnancy after genetic counseling. Therefore, we found that pregnant women were more inclined to choose fetuses with 47,XYY and 47,XXX karyotypes, which is consistent with previous studies (24,25). At the same time, prenatal diagnosis of SCA can improve the psychological preparation of pregnant women and their families for accepting an imperfect fetus in advance, and provide positive effects for the behavioral phenotype improvement of newborns (26).

In combination with the ultrasound phenotypes and NIPT results, T18 and T13 fetuses are mostly characterized by structural malformations (27), so normal ultrasound is helpful in identifying FP NIPT results. Even if T18 and T13 are not detected by NIPT, ultrasound indicates fetal malformation, which is a clear indication of prenatal diagnosis, and the rate of missed diagnosis can be significantly reduced. T21 fetuses involve various ultrasound phenotypes, including normal, soft marker, and structural malformation. Given the high accuracy of NIPT for T21 screening, the value of ultrasound screening is limited, but it can be applied to screen fetuses for structural malformations to assess the fetal prognosis. For SCA, MMS, and RAT, ultrasound phenotypes can be normal, soft marker, FGR, and polyhydramnios, which are easily ignored clinically. Moreover, the guidelines indicate that under the condition of low risk of aneuploidy testing, the isolated soft marker does not require a further risk assessment, highlighting the advantage of NIPT-Plus in reducing missed diagnoses (28). However, its screening performance still needs to be improved. According to the types of MMS and RAT, some ultrasound phenotypes could be structural malformations (29,30), but the number of TP cases in our study was small and no serious abnormal

ultrasound phenotypes were observed. In conclusion, in prenatal screening for fetal chromosomal abnormalities, NIPT and ultrasound complement each other, providing clinicians with more fetal information for prenatal diagnosis. Therefore, the improvement of NIPT-Plus screening performance and standard ultrasound screening are very important for improving the detection rate and reducing the FPR of fetal chromosomal abnormalities.

Conclusions

In summary, ultrasound phenotypes are helpful in identifying FP NIPT/NIPT-Plus results, especially for T18 and T13. Given its mild ultrasound phenotypes, NIPT-Plus has important clinical significance in reducing the missed diagnosis of SCA, MMS, and RAT, but its screening performance needs to be further improved.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6343/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6343/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All patients who participated in this study signed an informed consent form, and this study was approved by the Medical Ethics Committee of Xiangya Hospital (No. 202006429).

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