A Retrospective Single-center Analysis of Prenatal Diagnosis and Follow-up of 626 Chinese Patients with Positive Non-invasive Prenatal Testing Results

Xiufen Bu
Changsha Hospital for Maternal & Child Health Care Affiliated to Hunan Normal University
Shihao Zhou
Changsha Hospital for Maternal & Child Health Care Affiliated to Hunan Normal University
Xu Li
Yiyang Medical College
Shihong Li
Changsha Hospital for Maternal & Child Health Care Affiliated to Hunan Normal University
Hongyu Li
Changsha Hospital for Maternal & Child Health Care Affiliated to Hunan Normal University
Siyi Ding
Changsha Hospital for Maternal & Child Health Care Affiliated to Hunan Normal University
Jun He
Changsha Hospital for Maternal & Child Health Care Affiliated to Hunan Normal University
Siyuan Linpeng (linpengsiyuan@hotmail.com)
Changsha Hospital for Maternal & Child Health Care Affiliated to Hunan Normal University

Research Article

Keywords: non-invasive prenatal screening (NIPT), invasive prenatal diagnosis (IPD), karyotyping, chromosomal microarray analysis (CMA)

Posted Date: May 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1641089/v1

Abstract

Background: This study explores the diagnostic efficiency of different prenatal diagnostic approaches for women with positive non-invasive prenatal testing (NIPT) results by analyzing their clinical information and pregnancy outcome.

Methods: We collected data on 626 NIPT-positive pregnant women from January 2017 to June 2021 and arranged subsequent prenatal diagnostic operations for them after genetic counseling, along with long-term intensive follow-up. A total of 567 women accepted invasive prenatal diagnosis (IPD) (90.57%), and 262 cases were confirmed as true positives of NIPT.

Results: The positive predictive value for trisomy 21 (T21), trisomy 18 (T18), trisomy 13 (T13), sex chromosome aneuploidies (SCAs), rare autosomal trisomies (RATs), and microdeletion and microduplication syndromes (MMS) were 81.13%, 37.93%, 18.42%, 48.83%, 18.37%, and 41.67%, respectively. Discordant results between NIPT and IPD were seen in 53 cases, with the discordance rate being 9.35%. Additionally, there were 43 cases with discordant results between karyotyping and chromosomal microarray analysis (CMA)/copy number variation sequencing.

Conclusions: Additional reporting of RATs and MMS with routine NIPT that only detects T21/T18/T13 and SCAs can yield diagnosis more accurately. However, NIPT cannot be used as a substitute for IPD owing to its high false positive rate and discordances with other diagnostic methods. We recommend CMA combined with karyotyping as the preferred method for accurate diagnosis of NIPT-positive women.

1. Introduction

Approximately 900,000 new cases of birth defects, including congenital structural, functional, and/or biochemical-molecular defects, are recorded each year in China, with a prevalence rate of approximately 56.0 per 1000 live births [1]. Approximately 80% of the cases with birth defects have unknown causes; however, there is strong evidence that genetic conditions contribute to their etiologies [2, 3]. Chromosomal abnormalities such as trisomy 21 (T21), trisomy 18 (T18), trisomy 13 (T13), and sex chromosome aneuploidies (SCAs) are the main causes of birth defects [4]. In addition, multiple lines of evidence indicate that copy number variants (CNVs) in submicroscopic chromosomal structures can also play an important role in the etiology of some cases of birth defects [5, 6]. These defective infants with chromosomal or genetic abnormalities are often accompanied by varying degrees of intellectual disabilities, multiple malformation syndrome, growth retardation, and multiple dysfunction syndrome [7], which cause a great economic burden for families and society, thus highlighting the importance of prenatal genetic screening and diagnosis.

Non-invasive prenatal testing (NIPT), introduced into clinical practice in 2011, has gained repute in recent years as a screening test for genetic abnormalities during pregnancy. NIPT identifies genetic abnormalities from the analysis of maternal blood during pregnancy by employing next-generation sequencing (NGS) techniques to detect highly fragmented circulating cell-free fetal DNA (ccfDNA), which is derived from placental tissues and has rapid post-delivery clearance profiles [8, 9]. Therefore, the risks associated with conventional invasive techniques are avoided, which makes it more acceptable to pregnant women as a preferred diagnostic method compared to the conventional methods. In the last ten years, numerous studies have focused on the clinical applicability of NIPT mostly in the detection of common autosomal trisomies (T21, T18, and T13) and SCAs, and extended the applicability to rare autosomal trisomies (RATs) and microdeletion and microduplication syndromes (MMS) gradually with the rapid development of NGS technologies such as whole genome sequencing. NIPT involves the direct examination of DNA derived from the placenta, which has the same origin as the fetus, and has been shown to have much higher specificity and sensitivity than that of traditional serum analyte screening, which requires taking into account additional biochemical indicators as well as maternal age, race, and weight [10]. However, on the NIPT-based identification of enhanced risk, invasive prenatal diagnostic approaches such as amniocentesis (AC), chorionic villi sampling (CVS), and/or percutaneous umbilical cord blood sampling (PUBS) are recommended to identify a false positive finding [11, 12].

In this study, we present the clinical data of 626 NIPT-positive cases detected based on whole genome sequencing of patients in a tertiary medical center in China from January 2017 to June 2021. The confirmatory invasive test results and the detailed follow-up information were summarized to assess the performance of NIPT for the detection of common autosomal trisomies, SCAs, RATs, and MMS and to analyze the clinical outcomes following high-risk results. In addition, we analyzed and compared the invasive test results along with those of karyotyping and chromosomal microarray analysis (CMA)/copy number variation sequencing (CNV-seq) in order to evaluate the accuracy, efficacy, and incremental yield of CMA/CNV-seq as compared with that of karyotyping for routine prenatal diagnosis.

2. Materials And Methods

2.1. Subjects

From January 2017 to June 2021, we selected 626 NIPT-positive pregnant women who had been consulted at the Prenatal Diagnosis Center of Changsha Hospital for Maternal and Child Health Care before testing. The average age of the pregnant women was 31.0 ± 5.7 years. Among the study participants, 122 women were of advanced maternal age (≥ 35 years), accounting for 19.49% of the study population. Maternal blood was collected at gestational ages between approximately 12 ± 5 weeks and 28 ± 6 weeks.

The DNA library was constructed using a fetal chromosome aneuploidy (T21/T18/T13) detection kit (BGI, Wuhan, China) and high-throughput sequencing (0.5X) was performed using the BGISEQ-500 platform (BGI, Wuhan, China). We mainly analyzed T21-, T18-, and T13-positive cases, along with an additional analysis of positivity for SCAs, RATs, and MMS. Our procedure involved providing genetic counseling and recommending invasive prenatal diagnosis (IPD) for pregnant women at high risk based on NIPT results. Excluding 59 women who refused prenatal diagnosis, a total of 567 women chose to undergo prenatal diagnostic operations, which included 565 opting for AC and two for PUBS. Among the pregnant women who chose to proceed with the diagnostic procedures,

512 cases were diagnosed in our hospital. Pregnant women chose suitable prenatal diagnosis techniques according to indications and economic conditions, as shown below (Table 1).

Table 1
Selection of prenatal diagnostic techniques for 512 pregnant women with positive NIPT results based on diagnosis at our center

Diagnostic techniques	Case number (*n*)	Percentage (%)
Only Karyotyping	200	39.06
Karyotyping +CMA	287	56.05
Karyotyping +CNV-seq	21	4.10
Only CMA	2	0.39
Only CNV-seq	2	0.39

CMA: chromosomal microarray analysis; CNV-seq: copy number variation sequencing.

2.2. Sample Processing

The amount of amniotic fluid or umbilical cord blood to be extracted was determined according to the prenatal diagnosis method selected by the pregnant women: for G-banded karyotyping, 16 mL of amniotic fluid (2 tubes) or 2 mL of umbilical cord blood (subjected to heparin anticoagulation) were required; for CMA and CNV-seq, 8 mL of amniotic fluid (1 tube) or 2 mL of umbilical cord blood (EDTA anticoagulation) were required. Immediately after amniocentesis, the samples were centrifuged for 10 min at 1500 rpm/min to collect amniocytes separately.

2.3 G-banded Karyotyping

Amniocytes were transferred to an amniotic cell culture medium (Biosan, Zhejiang, China) and cord blood cells to a T cell culture medium (Biosan, Zhejiang, China) on an ultra-clean workbench for in vitro cell culture. When a specified number of cells were in the metaphase of active division, colchicine was added to inhibit mitosis. After the cells were digested by trypsin to isolate amniocytes, treated with hypotonic solution, fixed, and subjected to G-banded karyotyping, the karyotype was captured by a automatic scanner (Leica, Inc., Wetzlar, Germany). We then manually counted 30 or more integrity cleavage phases and analyzed five or more to be described according to the principles stated in *An International System for Human Cytogenetic Nomenclature, ISCN2020* [13].

2.4 Chromosomal Microarray Analysis (CMA)

Genomic DNA (250 ng) of amniocytes or umbilical cord blood cells was extracted by QIAamp® DNA Mini Kit (Qiagen GmbH, Hilden, Germany), following which, it was digested, ligated, PCR amplified, purified, fragmented, labeled, and hybridized to the Affymetrix CytoScan 750K array. The raw data were analyzed by Chromosome Analysis Suite (ChAS) 4.2 (Affymetrix, Santa Clara, CA, USA). The interpretation and reporting of constitutional CNVs was performed according to the standards and guidelines released by the American College of Medical Genetics [14]. We described the clinical significance of CNVs under a five-tiered system: pathogenic, likely pathogenic, variants of uncertain significance, likely benign, and benign. In accordance with the aforementioned standards, we did not report microdeletions less than 500 kb, microduplications less than 1 Mb, and some CNVs with low penetrance [15, 16]. Regions of homozygosity (ROH) with a size of more than 10 Mb were reported.

2.5 Copy Number Variation Sequencing (CNV-seq)

Genomic DNA was extracted from amniocytes by using Qiagen DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany). Genomic DNA (50 ng) was prepared as a template to construct a sequencing library and sequenced using a NextSeq CN500 System (Illumina, Inc., CA, USA). The sequencing results were subjected to bioinformatics analysis and annotated by the chromosome aneuploidy and gene microdeletion analysis software (Berry, Inc., Beijing, China). The whole experiment process was commissioned by Beijing Berry Company (Berry, Inc., Beijing, China). The clinical evaluation of results showing CNVs (> 100 kb) is based on the aforementioned guidelines.

2.6 Follow-up

The follow-up procedure for pregnant women with positive NIPT test results comprised the following four telephone-based interactions: 1. Informing pregnant women of the NIPT results and suggesting them to seek genetic counseling; 2. Inquiring about the occurrence of pregnancy loss after amniocentesis; 3. Informing pregnant women of the IPD results and advising them to seek genetic counseling; 4. Recording pregnancy outcomes and the development of newborns through online follow-up in addition to telephone interaction.

3. Results

3.1. Positive Predictive Value of NIPT

Among the 626 positive cases recorded at Changsha Hospital for Maternal and Child Health Care from January 2017 to June 2021, 59 patients refused prenatal diagnosis, while 567 patients underwent IPD, with a diagnostic rate of 90.58%, which included 115 confirmed cases of common autosomal trisomies, 104 of SCAs, 18 of RATs, and 25 of MMS (Table 2). Moreover, the positive predictive value (PPV) for T21, T18, T13, SCAs, RATs, and MMS was 81.13% (86/106), 37.93% (22/58), 18.42% (7/38), 48.83% (104/213), 18.37% (18/98), and 41.67% (25/60), respectively. In addition, among the different types of SCAs, 47, XXY had the highest PPV (40/49, 81.63%), followed by 47, XYY (22/30, 73.34%), 47, XXX (23/44, 52.27%) and 45, X (19/90, 21.11%).

Table 2
Performance of NIPT for detecting trisomies and MMS in 626 positive samples

Types of abnormalities		NIPT	Prenatal diagnosis (*n*)		Diagnostic	With diagnosis results		PPV
		(*n*)	Accepted (*n*)	Refused (*n*)	Rate (%)	Accordance (*n*)	Discordance (*n*)	
Common autosomal trisomies	T21	110	106	4	96.36	86	20	81.13%
	T18	59	58	1	98.31	22	36	37.93%
	T13	40	38	2	95.00	7	31	18.42%
SCAs	45, X	95	90	5	94.74	19	71	21.11%
	47, XXY	54	49	5	90.74	40	9	81.63%
	47, XXX	51	44	7	86.27	23	21	52.27%
	47, XYY	38	30	8	78.95	22	8	73.34%
RATs		114	98	16	85.96	18	80	18.37%
MMS		71	60	11	84.51	25	35	41.67%
Total		632*	573*	59	90.57	262	311	45.28%

NIPT: non-invasive prenatal screening; SCAs: sex chromosome aneuploidies; RATs: rare autosomal trisomies; MMS: microdeletion and microduplication syndromes; PPV: positive predictive value; *: Six cases suggested abnormalities on two chromosomes, so 6 more than the total of 626 and 567.

3.2. Discordance Between NIPT and Positive IPD Results

Among 567 NIPT-positive samples, 53 cases were found to be discordant with the positive IPD results. We divided these cases into the following four categories according to the number of chromosomes considered for the evaluation based on NIPT and IPD (Table 3): 1. Multiple-to-one: NIPT results suggested abnormalities of multiple chromosomes, whereas IPD identified abnormality only on one of those chromosomes; 2. One-to-one: NIPT results suggested abnormality of one chromosome; IPD results also suggested abnormality of the same chromosome but were discordant with the NIPT result in terms of the location/type of the chromosomal aberration. This included mosaicism in 21 cases, partial deletion/duplication in eight cases, from monosomy to trisomy in three case, and Robertsonian translocation resulting in trisomy 21 in one case; 3. One-to-multiple: NIPT results suggested abnormality of one chromosome, whereas IPD results revealed multiple chromosomal abnormalities that included the target chromosome; 4. One-to-another one: NIPT results suggested abnormality of one chromosome, whereas IPD identified the abnormality on another chromosome. In types “one-to-multiple ” and “one-to-another one ”, IPD reported several additional findings involving other chromosomes compared to those of NIPT, which included trisomy/partial trisomy, microdeletions/microduplications, balanced/unbalanced structural rearrangements. Details were shown in the supplementary table (Supplementary Table S1).

Table 3
Cases showing discordance between NIPT and positive IPD results

NO.	Categories	NIPT results	Diagnosis results		Cases	Total
			Primary classification	Secondary classification	(n)	(n)
1	Multiple-to-one	Abnormality of multiple chromosomes	Abnormality only on one of those chromosomes	Trisomy (*n* = 3)	3	53
2	One-to-one	Abnormality of one chromosome	Abnormality of the same chromosome	Mosaicism (*n* = 21)	33	
				Partial deletion or duplication (*n* = 8)		
				From monosomy to trisomy (*n* = 3)		
				Robertsonian translocation resulting in trisomy (*n* = 1)		
3	One-to-multiple	Abnormality of one chromosome	Multiple chromosomal abnormalities that included the target chromosome	Trisomy of two or more (*n* = 2)	11	
				Trisomy + sSMC (*n* = 1)		
				Trisomy + balanced structural rearrangement (*n* = 2)		
				Unbalanced structural rearrangement (*n* = 6)		
4	One-to-another one	Abnormality of one chromosome	Abnormality on another chromosome	Trisomy of another (*n* = 1)	6	
				Balanced structural rearrangement (*n* = 4)		
				Microdeletion (*n* = 1)		

NIPT: non-invasive prenatal screening; sSMC: small supernumerary marker chromosomes.

3.3. Discordance Between Results of Karyotyping and CMA/CNV-seq

Among the 512 NIPT-positive cases that were diagnosed at our hospital, 308 pregnant women opted for both karyotyping and CMA/CNV-seq. Discordant results between karyotyping and CMA/CNV-seq were found in 43 cases, accounting for 13.96% of the study population (Table 4). This excluded chromosome polymorphisms such as inv(9)(p12q13), inv(1)(p13q21), and inv(Y)(p11.2q11.2), Seven discordant cases were associated with mosaicism, which included four cases of sex chromosome mosaicism and three cases of autosomal mosaicism. Among these, six cases were successfully detected by karyotyping but not by CMV/CNV-seq, and for Case 304, while a normal karyotype was observed, the CMV result was arr(2)x3[0.52] hmz. Case 108 showed positive results with both karyotyping and CMA, with the CMA identifying the source of the small supernumerary marker chromosomes (sSMC) detected by karyotyping. Reciprocal translocation and inversion were detected in case 140 and case 437, respectively by karyotyping. These balanced chromosome rearrangements were not identified by CMA. Moreover, 10 cases with MMS and six cases with ROH were detected by CMA in 193 samples with normal karyotype, thus having an improved diagnostic rate of 5.18% and 3.11%, respectively, in comparison to that of karyotyping. In addition, chromosome breakpoints in 17 cases with unbalanced rearrangements were located relatively accurately by CMA/CNV-seq (Supplementary Table S2).

Table 4
Cases showing discordance between karyotyping and CMA/CNV-seq results

N0.	Case number	Maternal Age (Years Old)	Gestational Age (Weeks *)	NIPT results	Karyotype	CMA/CNV-seq results	Size (Mb)	Ultrasound findings	P o
1	Case 309	30	16^{+5}	XO	45, X[6]/46, XX[75]	N	/	N	B
2	Case 312	27	18^{+1}	XO	45, X[41]/47, XXX[20]	N	/	Single umbilical artery	T
3	Case 353	29	19^{+1}	XO	45, X[6]/46, XX[84]	N	/	N	T
4	Case 493	28	16^{+3}	XO	47, XXX[18]/46, XX[37]	N	/	N	B
5	Case 122	37	20^{+}	T13, T20	47, XN, + 20[28]/46, XN[22]	N	/	N	T
6	Case 386	25	14^{+3}	T4	47, XX, + 4[19]/46, XX[71]	N	/	N	T
7	Case 304	31	17^{+3}	T2	N	arr(2)x3[0.52] hmz	/	FGR, Oligohydramnios	T
8	Case 108	28	18^{+}	T16	47, XN, +mar[14]/46, XY[18]	arr[GRCh37] 16p11.2q22.1(33766659_67589639)x3[0.52]	33.8	/	T
9	Case 140	48	20^{+}	T16	46, XY, t(4;9)(q12;q22)[9]/ 46, XY[31]	N	/	N	B
10	Case 437	33	20^{+1}	XXX	46, XX, inv(6)(p21q13) mat	N	/	N	B
11	Case 109	28	17^{+}	MMS	N	arr[GRCh37] 5p15.33 (113576_2835831)x1	2.7	N	T
12	Case 121	36	22^{+}	MMS	N	arr[GRCh37] 3q23q25.31(141158071_155492129)x3	14.3	N	T
13	Case 123	24	27^{+}	MMS	N	arr[GRCh37] 2q24.1q31.1(158448403_174291185)x1 dn	15.8	NT was 3.3 mm at 12 gestational age	T
14	Case 172	33	19^{+}	MMS	N	arr[GRCh37] 16p13.11p12.3(15319277_18172468)x1	2.8	N	B
15	Case 242	30	17^{+3}	T16	N	arr[GRCh37] 16p13.11p12.3(15325072_18242713)x3 mat	2.9	N	B
16	Case 347	31	17^{+3}	T15	N	arr[GRCh37] 1p36.33 (849466_1996635)x1 dn	1.15	Fetal tetralogy of Fallot, PLSVC, Thoracic vertebral abnormality	T
17	Case 64	28	26^{+}	T21	N	arr[GRCh37] 13q33.3q34(107382604_115107733)x1	7.7	FGR	T
18	Case 376	28	18^{+4}	MMS	N	arr[GRCh37] 15q13.1q13.3(28635057_32444261)x1 mat	3.81	N	B
19	Case 164	27	20^{+}	T15	N	arr[GRCh37] 15q11.2q13.1(23281885_28526905)x4	5.2	N	T
20	Case 500	33	19^{+4}	MMS	N	arr[GRCh37] 22q13.33(50207711_51197766)x1	0.99	Normal indicators at 12 weeks	T
21	Case 86	38	18^{+}	MMS	N	arr[GRCh37] 5p15.33p15.1(113576_16203210)x2 hmz	16.0	Missed follow-up	

N0.	Case number	Maternal Age (Years Old)	Gestational Age (Weeks *)	NIPT results	Karyotype	CMA/CNV-seq results	Size (Mb)	Ultrasound findings	P o
22	Case 146	36	19^{+}	MMS	N	arr[GRCh37] 2q31.1q37.3 (174605494_242773583)x2 hmz	68.1	FGR, Placental thickening, Oligohydramnios	T
23	Case 156	31	18^{+}	T16	N	arr[GRCh37] 16p13.3p12.3 (94807_17705580)x2 hmz, 16q22.3q24.3(73772289_90146366)x2 hmz	17.6, 16.3	N	B
24	Case 240	32	20^{+}	T13	N	arr[GRCh37] 18p11.23q12.2(7131233_34755544)x2 hmz	27.6	N	B
25	Case 477	27	16^{+5}	MMS	N	arr[GRCh37] 18q21.32q23(56947979_77997606) hmz	21.05	N	B
26	Case 552	30	16^{+4}	CNV	N	arr[GRCh37] 18p11.32p11.21(136305_11807701)x2 hmz	11.67	N	B

NIPT: non-invasive prenatal screening; MMS: microdeletion and microduplication syndromes; XO: 45, X high risk; XXX: 47, XXX high risk; N: Normal; /: No; CMA: chromosomal microarray analysis; CNV-seq: copy number variation sequencing; PLSVC: persistent left superior vena cava; NT: nuchal translucecy; TOP: termination of pregnancy; *: Weeks$^{+\text{ days}}$.

3.4. Analysis of Pregnancy Follow-up

Among the 567 pregnant women who underwent IPD, 263 were confirmed as true positive cases. Tracking the pregnancy outcomes of 261 pregnant women among these led to the following observations: mothers of all fetuses diagnosed with T21, T13, T18, RATs, Klinefelter syndrome, and Turner syndrome had been induced labor, excluding one case of T21 (Supplementary Table S1, Case 439) and two cases of haploid chromosome X with a low rate of mosaicism and normal ultrasound findings throughout pregnancy (Supplementary Table S1, Case 309 and Case 512); five cases diagnosed as having fetuses with Triple X syndrome and 8 cases diagnosed as having fetuses with 47, XYY syndrome had been induced labor, with a birth rate of 77.27% (17/22) and 63.64% (14/22), respectively; Among the MMS detected by NIPT, the clinical significance of most cases was unknown, and due to the presence of pathogenic CNVs, only 54.17% (13/24) cases were induced labor. Additionally, among 304 cases confirmed to be false positives, tracking of the pregnancy outcomes of 295 pregnant women showed the following: two patients underwent spontaneous abortion; six patients terminated pregnancy due to other genetic abnormalities; two patients had abortion due to abnormal ultrasound findings; two patients terminated pregnancies for unknown reasons, and the remaining 274 mothers had infants that were born healthy.

Among the 59 pregnant women who refused prenatal diagnosis, the pregnancy outcomes of 42 pregnant women were tracked: eight patients had abortion due to multiple malformations found by ultrasound, and 34 women underwent delivery. Among the cases leading to live birth, a confirmed occurrence of T21 was found in an infant from a twin pregnancy, and the remaining 33, which included one case of T13, reported healthy birth that was confirmed by long-term follow-up.

4. Discussion

From 2012 onwards, NIPT for fetal aneuploidies was broadly implemented for detecting common autosomal trisomies and SCAs owing to the advantages associated with it such as non-invasiveness, zero risk for the unborn baby, capability to acquire diagnostic hints as early as the 10th week of gestation onwards, immediate results within as early as two weeks, as well as high sensitivity (99.3% for T21, 97.4% for T18, and 97.4% for T13) and specificity (pooled specificity was 99.9% for all three trisomies) [17, 18]. However, this approach identifies only 75 to 85% of clinically relevant aneuploidies [19]. Therefore, additional screening based on identifying RATs and MMS is necessary. Here, we assessed a series of 626 NIPT-positive cases with low genomic coverage and detected a broad range of aneuploidy classes, namely the common autosomal trisomies, SCAs, RATs, and MMS. The PPV of T21 (81.23%) observed using our platform in the present study was within the range of values reported in published literature (between 80% and 90%) [20]. The PPVs of T18 and T13, presented as the main positive results, were 37.93% and 18.42%, respectively, which were slightly lower than those reported by previous studies on the same platform [21]. The PPVs of SCAs, RATs, and MMS, presented as the additional positive results, were 48.83%, 18.37%, and 41.68%, respectively, which were slightly higher than those reported by previous studies on the same platform [22]. PPV obtained via NIPT, excluding that of T21, is known to have large variation associated with prior risk factors, such as maternal age, and the individual trisomies [23, 24]. NIPT results are affected by an insufficient or absent fetal fraction, fetoplacental mosaicism, the presence of a vanishing twin, maternal mosaicism, maternal CNVs, and maternal malignancy leading to false positives that are discordant with results obtained by other methods [25, 26]. Moreover, technical factors such as testing procedures, sequencing algorithms and depths, as well as Z-scores may also be important in terms of their effect on NIPT results [20]. This makes the fluctuation of the PPV of NIPT in different study populations a common occurrence. In our research, we found RATs to have a PPV of 18.37%, which was similar to that of T13 presented as the main positive results, and could therefore act as an extension of NIPT screening. For MMS, there was a higher PPV than that of T18 shown as positive results, but most of the CNVs were identified as hereditary and of unknown significance. Disclosure of these results to pregnant women did not provide them any substantial help with pregnancy-related decisions and had a negative psychological impact on them. Therefore, for cases of MMS suggested by NIPT results, it is recommended to only present the diagnoses to pregnant women if the CNVs are in genomic regions that have definite associations with certain syndromes or present them after pathogenicity has been identified.

Discordant results associated with NIPT often occur during screening and diagnosis. Reports of discordant results focused on the causes of false positives and false negatives detected by NIPT highlight the impact of confined placental mosaicism and true fetal mosaicism on NIPT-based analyses [25]. In this study, our analysis of the discordance between the positive results of NIPT and IPD showed 53 discordant cases (which accounted for 9.35% of the total cases). As expected, NIPT was ineffective in identifying balanced structural rearrangements due to the limitations of sequencing depth and fragment read length of NGS. The assessment of the cases in our analysis confirmed the importance of testing by IPD in addition to NIPT, which could not accurately determine the abnormality as being on one or two chromosomes, being a trisomy or monosomy, euploidy or mosaic, or a trisomy or partial abnormality. Among cases of the “one-to-one” type of discordance, we found that NIPT suggested trisomy/monosomy for 21 cases where IPD results indicated mosaicism. This accounted for the largest proportion of discordance that was observed between NIPT and IPD results. NIPT uses ccfDNA fragments that originate from the cytotrophoblast cells of the chorionic villi in the placenta to detect fetal trisomies; however, the karyotype of the cytotrophoblast cells does not always represent the fetal chromosome constitution [27]. Our observations show that in some cases that were diagnosed with very low rates of mosaicism confirmed by multiple detection methods, pregnant women choosing to continue the pregnancy had fetuses that developed well after birth (Supplementary Table S1, Case 439). Therefore, it is advised that pregnant women getting a positive NIPT result should not hastily be driven to a negative attitude, and should actively undergo a follow-up consultation to determine the proportion of fetal mosaicism by means of IPD, and only then make decisions regarding the continuation or termination of pregnancy. Some unbalanced structural rearrangements involving other chromosomes and trisomies that were not identified by NIPT and sSMC detection were discovered by accident in our research. It must be taken into account that NIPT, which is based on second-generation sequencing technologies, is not sensitive to some DNA fragments with a high average content of guanine and cytosine bases. Therefore, NIPT cannot be regarded as a diagnostic tool for conclusive diagnoses, and positive NIPT results must further be assessed by invasive prenatal diagnostic approaches.

G-banded karyotyping, which has limited resolution (5 ~ 10 Mb), is a common diagnostic technique and the gold standard for the diagnosis of chromosomal disorders. It can detect chromosomal aneuploidy or polyploidy, large deletions/duplications in chromosomes, and balanced chromosomal rearrangement. Other commonly used prenatal diagnostic techniques, namely CMA and CNV-seq, can be used to analyze aneuploidy as well as microdeletion and microduplication (≥ 100 kB) [15, 28]. In our study, a total of 43 discordant cases were found in the chromosomal analysis of 308 patients performed by means of both karyotyping and CMA/CNVseq. Four instances of sex chromosome mosaicism were detected by karyotyping, which were not indicated by CMA. For cases of sex chromosome abnormality indicated by NIPT, karyotyping was seen to be more effective than CMA in confirming true positive detection of sex chromosome mosaicism. Additionally, two cases of autosomal mosaicism were detected by karyotyping, and not suggested by CMA; while one case of autosomal mosaicism were detected by CMA, and not suggested by karyotyping.

Karyotyping and CMA each have certain advantages and disadvantages for their use in the detection of autosomal mosaicism. Although karyotyping requires cell culture, it can detect mosaics of different types, including those of a very low proportion. However, multiple factors, such as aberration of the primary amniotic cells themselves and cell aberration resulting from in vitro culturing, may lead to pseudomosaicism, loss or increase of the abnormal cell line resulting in a change in the proportion of mosaic cells, or even to missing detection of autosomal mosaicism [29]. Conversely, CMA can only stably detect mosaicism in cells with larger proportions (> 30%) of it and can detect the genome of the amniotic fluid directly, thus being capable of reflecting the proportion of true mosaicism in the sample. Additionally, CMA has unique advantages in detecting CNVs and ROH, which cannot be detected by karyotyping. Our results show that in comparison with CNVs detected by karyotyping, 10 more cases of pathogenic CNVs were detected by CMA, which thus had an improved diagnostic rate of 5.18% compared to that of karyotyping. In addition, for NIPT positive samples showing normal karyotypes, a total of 3.11% ROH was detected by SNP-based microarrays. The presence of large fragments of ROH in the fetus is associated with the risk of uniparental disomy (UPD), which is the result of the successful rescue of cells from aneuploidy to euploidy after fertilization of germ cells. A UPD diagnosis should be considered when NIPT suggests trisomy, especially on chromosomes 6, 7, 11, 14, 15, and 20 [30]. Thus, it can be seen that a single detection method can easily lead to misdiagnosis. Therefore, the combination of karyotyping with CMA seems to be preferable for obtaining accurate diagnoses of chromosomal abnormalities.

At the later stages of follow-up, most women with fetuses diagnosed with autosomal trisomies had terminated pregnancy, excluding one case of T21 with a low rate of mosaicism. SCAs are the most frequent chromosomal abnormalities encountered in NIPT. In true positive cases, the overall termination of pregnancy rate was 22.7% (5/22) for Triple X syndrome and 36.36% (8/22) for 47, XYY syndrome, which was significantly lower than those of other chromosomal syndromes. The prevalence of Triple X syndrome and 47, XYY among newborns is high, Triple X: 11 per 100,000 females, and 47, XYY: 18 per 100,000 males respectively [31]. Although an increased risk of psychosocial problems or psychiatric disorders (such as autism) during childhood has been associated with the 47, XYY syndrome, long-term, unbiased follow-up studies have concluded that Triple X syndrome and 47, XYY syndrome, do not cause postnatal development disorders, children with these conditions have IQs in the normal range despite physical abnormalities being occasionally observed [32]. The acceptance of fetuses with SCAs tends to be affected by many factors, such as social and cultural background, disease type, genetic counseling methods, and the economic status of the family. In China, an increasing number of people are accepting children with Triple X syndrome and 47, XYY syndrome. Therefore the exclusion of Triple X syndrome and 47, XYY syndrome from the NIPT process is expected in the near future. Moreover, the true or false positive nature of ultrasound findings is also an important factor in determining the decision to continue a pregnancy.

5. Conclusions

NIPT has a high positive rate for detecting common trisomies and SCAs in general testing of pregnant women, and testing for RATs and MMS can be additionally conducted with the informed consent of the pregnant woman for obtaining a more accurate diagnosis. However, NIPT cannot be used as a substitute for amniocentesis and prenatal diagnosis techniques owing to its high rate of false positives and discordances with the diagnoses provided by IPD. CMA combined with karyotyping can be recommended as the most preferred method of prenatal diagnosis for the cases having NIPT results showing high risk in pregnancy.

Abbreviations

NIPT: non-invasive prenatal screening; IPD: invasive prenatal diagnosis; CMA: chromosomal microarray analysis; CNV-seq: copy number variation sequencing; SCAs: sex chromosome aneuploidies; RATs: rare autosomal trisomies; MMS: microdeletion and microduplication syndromes; CNVs: copy number variants; ROH: Regions of homozygosity; NGS: next-generation sequencing; ccfDNA: circulating cell-free fetal DNA; PPV: positive predictive value; sSMC: small supernumerary marker chromosomes; TOP: Termination of pregnancy; AC: amniocentesis; CVS: chorionic villi sampling; PUBS:percutaneous umbilical cord blood sampling;

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Changsha Maternal and Child Health Hospital, China (7 May 2021, 2021004) and written informed consent was obtained from the patient.

Consent for publication

The authors declare that there are no financial or other relationships that might lead to a conflict of interest of the present article. The manuscript is approved by all authors for publication.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

Funding

This research was funded by Program of Health Commission of Hunan Province, grant number 20200365.

Authors' contributions

All authors have materially participated in the study and manuscript preparation. S.D. (Siyi Ding), H.L. (Hongyu Li) and J.H. (Jun He) carried out all the molecular genetic analysis, and participated in the design of the work; S.L. (Shihong Li) and S.L. (Siyuan Linpeng) collected all clininal data and participated in conceiving the work; X.B. (Xiufen Bu), S.Z. (Shihao Zhou) and X.L. (Xu Li) designed the work, drafted and revised the manuscript. All authors have approved the final article.

Acknowledgements

We would like to thank BGI-Shenzhen/Wuhan (China), Biosan-Zhejiang (China), and Berry-Beijing (China) for their technical contributions to this study.

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Figures

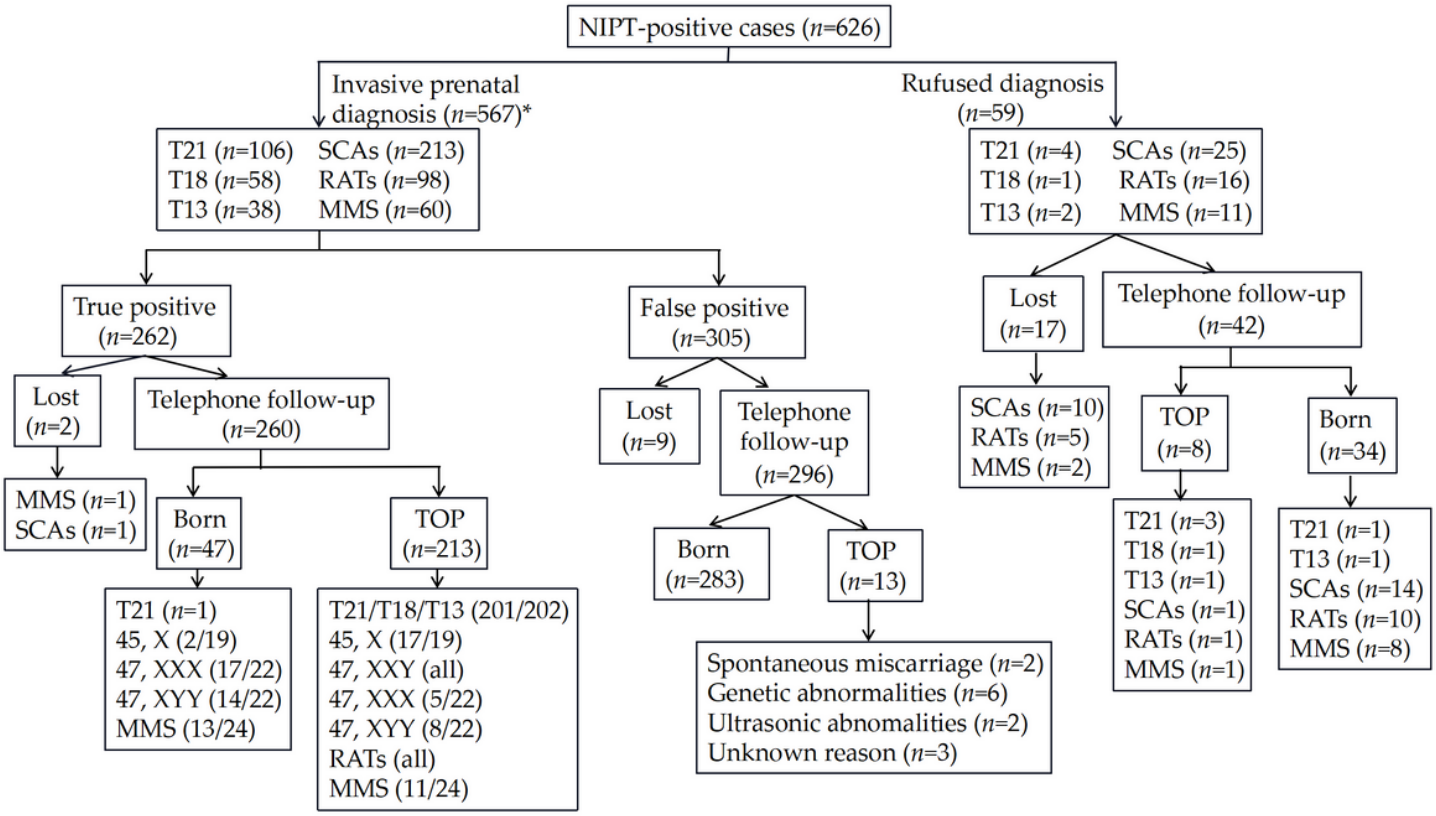

Figure 1

Pregnancy outcomes of all NIPT-positive cases

NIPT: non-invasive prenatal screening; SCAs: sex chromosome aneuploidies; RATs: rare autosomal trisomies; MMS: microdeletion and microduplication syndromes; TOP: Termination of pregnancy ; *: Six cases suggested abnormalities on two chromosomes, so the sum in the box is 6 more than 567.

Supplementary Files

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