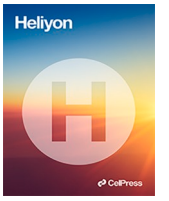
Heliyon 10 (2024) e24155

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

The detection efficacy of noninvasive prenatal genetic testing (NIPT) for sex chromosome abnormalities and copy number variation and its differentiation in pregnant women of different ages

Yimei Li, Xiaofeng Yang *, Ying Zhang, Huan Lou, Mingli Wu, Fang Liu, Wenjing Chang, Xueling Zhao

Department of Gynecology and Obstetrics, Zhengzhou Central Hospital Affiliated to Zhengzhou University, Zhengzhou, 450000, Henan, PR China

A R T I C L E I N F O

Keywords:
NIPT
Sex chromosome abnormalities
Copy number variation
Efficacy
Age

A B S T R A C T

Objective: To analyze the efficacy of noninvasive prenatal genetic testing (NIPT) in detecting fetal sex chromosome abnormalities and copy number variation (CNV), compare the efficacy between NIPT and serological screening alone, and further analyze the fetal sex chromosome abnormalities and CNV differentiation in pregnant women of different ages, so as to provide a reference for the prevention and control of fetal birth defects.

Methods: Clinical data from 22,692 pregnant women admitted to our hospital from January 2013 to December 2022 were retrospectively analyzed. All participants underwent serological screening and NIPT screening to compare fetal chromosomal abnormalities between the two screening modalities. 145 women whose fetus were diagnosed as sex chromosome abnormalities and 36 women whose fetus were diagnosed as CNV abnormalities based on NIPT screening were selected for prenatal diagnosis by amniocentesis or karyotyping. Taking prenatal diagnosis as the standard, the four-grid table method was used to detect the positive predictive value of NIPT screening for fetal sex chromosomal abnormalities and CNV. According to the age, pregnant women were divided into 18–30 years old (n = 9844), 31–35 years old (n = 7612), >35 years old (n = 5236), and then the detection rates of sexual fetal chromosomal abnormalities, CNV and total chromosomal abnormalities were compared in pregnant women.

Results: Among the 22,692 pregnant women in this study, the high-risk proportion of serologic screening with 4.38% was higher than that of NIPT screening with 1.93% ($P < 0.05$). Among the 145 women with fetal sex chromosome abnormalities screened by NIPT, 122 cases of fetal sex chromosome abnormalities were diagnosed prenatally, including 45, X/47, XXX/47, XYY/47, XXY. The positive predictive values of NIPT screening were 25.00%, 58.82%, 85.71%, and 85.71%, respectively, with an overall predictive value of 44.26%. The positive predictive value of fetal sex chromosome abnormalities in NIPT screening was higher than that of serological screening ($P < 0.05$). Among the 36 pregnant women with fetal CNV, NIPT screening showed that CNVs≤10 Mb and CNVs>10 Mb were 33.33% and 66.67%, respectively. There were 12 cases of prenatal diagnosis of fetal CNV, among which the NIPT-screened positive predictive values of fetal copy number deletion, duplicate, deletion and duplicate were 50.00%, 57.14% and 100.00%, respectively, with an overall predictive value of 58.33%. The positive predictive value

* Corresponding author.
E-mail address: yangxiaofeng@21cn.com (X. Yang).

https://doi.org/10.1016/j.heliyon.2024.e24155
Received 26 August 2023; Received in revised form 15 November 2023; Accepted 4 January 2024
Available online 7 January 2024

of CNV in NIPT screening was higher than that of serological screening without statistically significant difference ($P > 0.05$). The results of NIPT screening showed that the detection rate of fetal sex chromosome abnormalities and total abnormalities of pregnant women over 35 years of age was significantly higher than that of pregnant women aged 18–30 and 31–35 years ($P < 0.05$).

Conclusion: NIPT screening could greatly improve the detection efficacy of fetal sex chromosome abnormalities, CNV and other chromosome abnormalities, and decline the false positive rate. However, the positive predictive value of NIPT screening was relatively low, and further prenatal testing and genetic counseling are still required. In addition, NIPT screening for fetal sex chromosome abnormalities, and the detection rate of total abnormalities in pregnant women older than 35 years old were increased significantly, and pregnancy at an advanced age may be one of the risk factors for fetal chromosomal abnormalities.

1. Introduction

Chromosome diseases are caused by congenital chromosome number abnormalities or structural aberrations. Chromosomal abnormalities are a frequent cause of miscarriage, Down's syndrome, congenital multiple malformations, tumors, and infant death in clinical practice [1]. According to earlie data, less than 1% of newborns with chromosomal abnormalities survive [2]. Currently, clinical sex chromosomal anomalies such as Turner syndrome, Kerr's syndrome, 47, XYY syndrome, and others are widespread. Patients with 47, XXX syndrome and Turner syndrome often show symptoms such as short stature, delayed puberty, ovarian dysplasia, infertility, congenital heart malformation, and others. Male infertility and insufficient testicular development are both related to Kerr's syndrome, and the accompanying phenotype usually mainfests after puberty. Wolf-Hirschhorn syndrome (WHS), which is caused by an abnormal copy number, manifests as intellectual impairment, growth retardation, epilepsy, typical special features ("helmet-shaped visage") and other characteristic features [3]. Sex chromosome abnormalities and copy number variation seriously impact the positive growth and development of children as well as their quality of life. Early screening of fetal sex chromosome abnormalities and copy

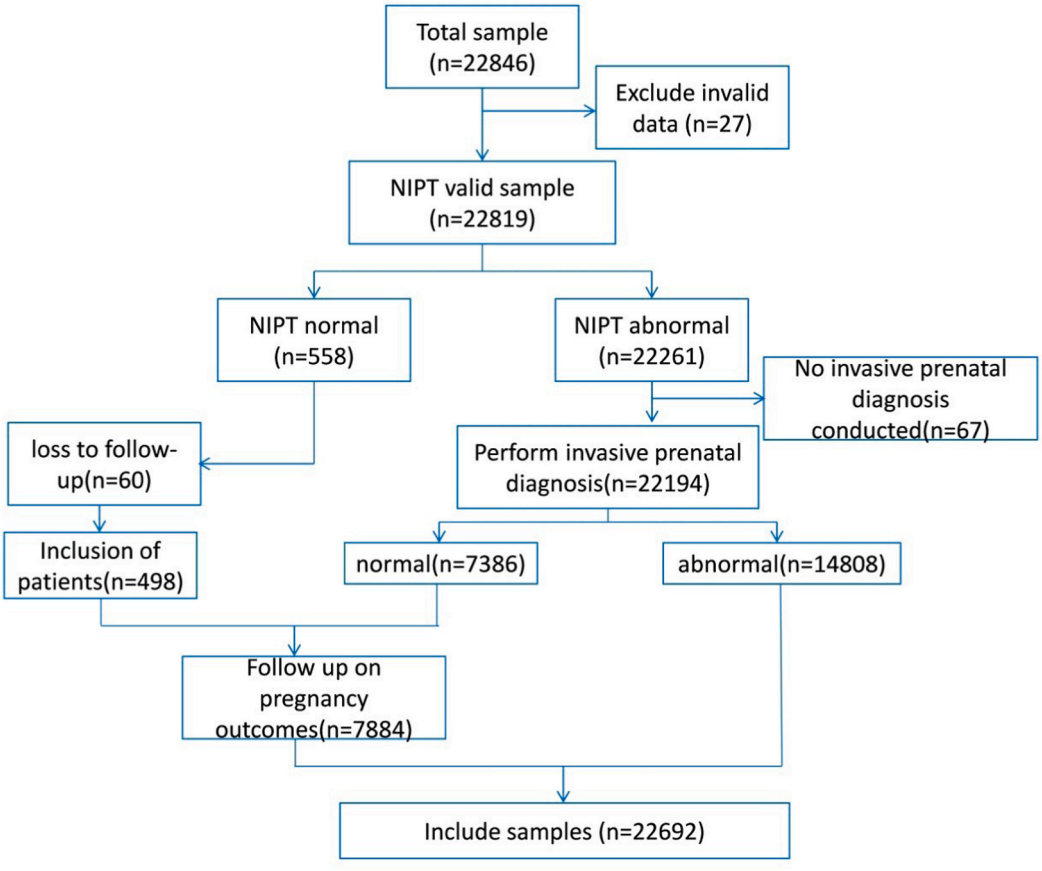


Fig. 1. Sample size inclusion flow chart.

number variation, as well as fast delivery outcomes selection, are therefore beneficial in easing the burden on pregnant women and improving the eugenic rate [4]. Amniocentesis or chromosome karyotype are currently the most used clinical approached for fetal chromosome abnormalities disgnosis, but both of these invasive procedures have the risk of consequences like fetal infection, fetal deformity, and abortion and cannot be widely used as screening tests [5]. It is reported that a total of 101 cases of chromosomal chimerism (0.54%, 100/18,369) are detected in 100 pregnant women, and it is concluded that although chromosome karyotype is the most sensitive method for detecting chromosomal chimera based on the results of postnatal follow-up of the infant at birth, culture-generated artifacts and biases should be considered, especially sex chromosome abnormalities involving X-monomer that require binding to uncultured fluorescence *in situ* hybridization [6]. The main prenatal screening method nowadays is clinical serological screening, with the advantages of rapid detection and inexpensive cost, but has poor detection rates and positive predictive values [7].

Non-invasive prenatal genetic testing (NIPT) has become a promising technology in recent years for diagnosing fetal chromosome diseases. High-throughput sequencing technology is used to find fetal free DNA in peripheral blood of pregnant women and determine the risk of fetal chromosome aneuploidy. NIPT is a method for screening common fetal chromosome aneuploidy abnormalities [8,9]. Currently, trisomy 21, trisomy 18 and trisomy 13 are the most common fetal diseases that can be screened by NIPT. The detection rate of trisomy 21 is higher than 99% with the false positive rate less than 1%, the detection rate of trisomy 18 is more than 85%, and trisomy 13 detection rate is more than 70% [10]. Therefore, non-invasive DNA is the most accurate and sensitive prenatal screening method for chromosomal aneuploidy diseases (trisomy 21-syndrome, trisomy 18-syndrome, and trisomy 13-syndrome) [11,12]. The evaluation of chromosome abnormalities and copy number variation (CNV) by NIPT has, however, received relatively little research.

In this study, we retrospectively analyzed the clinical data of 22,692 pregnant women admitted to our hospital from January 2013 to December 2022, to analyze the effect of NIPT on the detection of common chromosomal aneuploidy abnormalities in fetuses, and to determine the detection efficiency of NIPT for sex chromosome abnormalities and CNV, as well as the abnormal detection rate in pregnant women with different ages. It is of great significance to reduce the proportion of pregnant women using invasive prenatal diagnosis and improve the detection rate of fetal chromosomal diseases.

2. Data and methods

2.1. General data

Clinical data of 22,846 pregnant women admitted to our hospital from January 2013 to December 2022 were analyzed retrospectively. A total of 22,692 pregnant women were included after screening and exclusion of patients lost to follow-up according to inclusion and exclusion criteria, and the sample size inclusion process was shown in Fig. 1. The criteria for inclusion were as follows: (i) All of pregnant women were in line with China's prenatal screening procedures: For chromosomal abnormalities, early Tang screening, 11–14 weeks of pregnancy B ultrasound examination NT, Down screening, and non-invasive DNA examination; For Structural abnormalities: B-ultrasound at 22–24 weeks of pregnancy, and B-ultrasound for the second time at 28–34 weeks of pregnancy; (ii) Pregnant women aged 18 to 40; and (iii) Pregnant women and their families were informed, had good compliance, could cooperate with the inspection, and signed informed consent. Criteria for exclusion [13]: (i) Pregnant women with adverse pregnancy history, including pregnant women who had given birth to children with inherited metabolic diseases or single-gene genetic diseases, pregnant women who had given birth to children with chromosomal diseases, one of the spouses was a carrier of chromosomal abnormalities, history of previous fetal arrest or miscarriage, fetal death in utero, and dysplasia; (ii) Pregnant women with gestational diabetes mellitus, gestational hypertension, eclampsia, pregnancy with thyroid dysfunction, and pregnancy with nephrotic syndrome; (iii) Pregnant women with nervous system or mental illness; and (iiii) Pregnant women who developed premature rupture of membranes or late miscarriage after amniocentesis. The experimental procedures were approved by the hospital ethics committee.

2.2. Methods

Serological screening: 3 mL of peripheral blood was collected from all participants and left for 0.5 h. Serum was separated by the centrifugation at 3000 r/min for 10 min and stored at −20 °C until the use. The serum levels of Alpha-fetoprotein (AFP), Free β-human chorionic gonadotrophin (free-β-hCG), and Unconjugated estriol (uE3) in pregnant women were measured by time-resolved immunofluorescence assay. Lifecycle 4.0 prenatal screening risk calculation software, combined with age, body mass and gestational age of pregnant woman was used to analyze the risk values. The gestational age of pregnant women with irregular menstruation was assessed using ultrasound examination. Trisomy 21 syndrome (T21)$\geq$1/270 or Trisomy 18 syndrome (T18)$\geq$1/350 was considered as high risk; 1/1000 $\leq$ T21 $<$ 1/270 or 1/500 $\leq$ T18 $<$ 1/350 was considered as critical risk; and T21 $<$ 1/1000 or T18 $<$ 1/500 was considered as low risk.

NIPT screening: BGI SEQ500 sequencing platform and BGI HALOS platform were used for NIPT screening. 10 mL of peripheral blood of all pregnant women was collected and centrifuged at 4 °C for 10 min within 72 h to obtain the 1600 g of plasma. Then, the obtained plasma was centrifuged for 10 min at room temperature, and then the upper plasma was pipetted, aliquoted and stored at −80 °C. DNA was extracted from 1200 μL of plasma by magnetic bead-based method. The final elution volume was 42 U, and Qubit3.0 was used to calculate the DNA mass concentration. All DNA solutions were used for library construction (PCR-Free), and the process contained terminal repair, splicing, and real-time PCR quantification. Finally, sequencing was conducted using the BGI SEQ500 sequencing platform, followed by bioinformatics analysis of the sequencing data to calculate the Z value. The risk of predictive chromosome abnormalities and CNV was predicted according to the Z-value of each sample, which was divided into abnormal (Z-value

of −3.0-3.0) and normal (Z-value≥3.0). Classification of detection: sex chromosome abnormalities contained 45, X/47, XXX/47, XYY/47, XXY; NIPT fragment size included ≤10 Mb and >10 Mb.

Prenatal diagnosis: Amniocentesis or chromosome karyotype analysis were performed for pregnant women with high serological screening risk and abnormal NIPT screening. Under the guidance of B-ultrasound, an amniotic cavity puncture was punctured to retrieve 20 mL of amniotic fluid, which was then place in two aseptic centrifuge tubes. After the centrifugation, the precipitated amniotic fluid cells were aseptically inoculated in 5 mL amniotic fluid medium and cultured in a carbon dioxide incubator at 37 °C for 7–9 d. Cells were then harvested, and G-banding was performed for the karyotype analysis, in accordance with the International System for human Cytogenetic Nomenclature (ISCN) 2016 [14]. The cells from 5 mL amniotic fluid were pretreated, hybridized, washed and re-dyed with the kit of Abbott Laboratories using the probe CEP18/X/Y based on the standard operation procedures of the manual. The final results were evaluated under a fluorescence microscope. Fifty cells were randomly counted (The count was increased to 400 if mosaicism was present), and the test results were issued within 72 h. No abnormal cells were found, which was considered normal, and the presence of one abnormal cell was considered abnormal risk. Peripheral blood of pregnant women was collected to extract genomic DNA. After ultrasonic cracking, library was prepared, and finally high-throughput sequencing was completed by NextseqCN500 sequencer. The types of chromosome abnormalities were analyzed by bioinformatics analysis. By contasting the discovered CNVs with the international database of genomic variation, common polymorphic CNVs were excluded. The pathogenicity of CNVs was detemined by comparing with DECIPHER and OMIM databases. The standard used to assess the positive predictive values of pre-pregnancy screening was prenatal examination, where the positive predictive value was calculated as follows: sex chromosome abnormalities, copy number abnormalities detected by NIPT/prenatal diagnosed chromosome abnormalities, copy number abnormalities) × 100%. Evaluation of abnormal detection rates at different ages: Pregnant women were separated into 18–30 years old (n = 9844), 31–35 years old (n = 7612) and >35 years old (n = 5236), and the detection rates of sex chromosome abnormalities, pathogenic CNV and total chromosome abnormalities were compared.

2.3. Statistics

The SPSS 20.0 software was used to examine the experimental data was analyzed using. The *t*-test was used to assess the age and other measurement data, which were expressed as ($\bar{x} \pm s$). Data that were counted, such as screening results, were shown as (%). The Wilcoxon rank sum test was used for pairwise group comparisons, while the Friedman rank sum test was used for multi-group comparisons. Using the fourfold table method, the positive predictive values of NIPT screening for diagnostic chromosome abnormalities and CNV were evaluated. The results were regarded as statistically significant difference when $P < 0.05$.

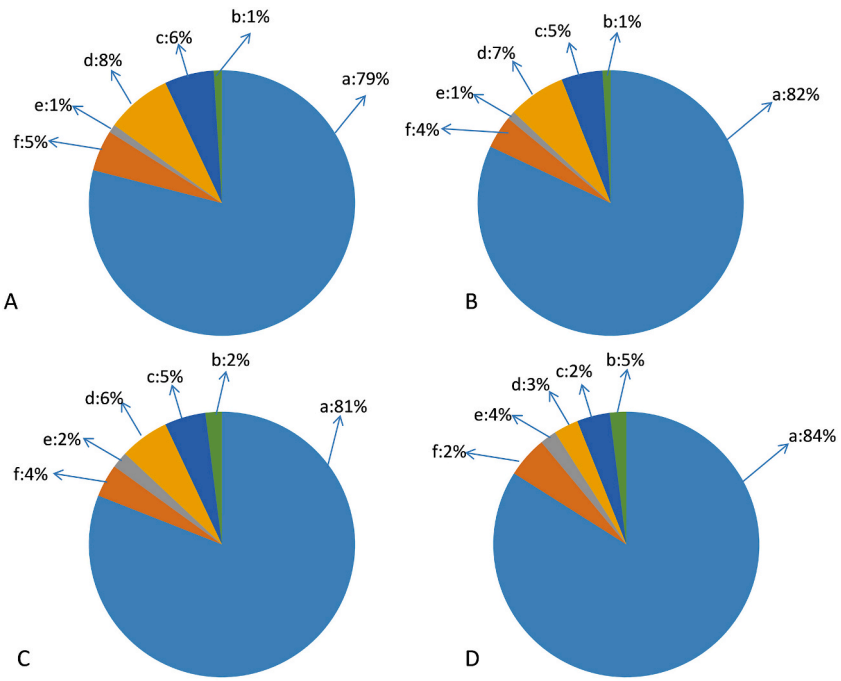


Fig. 2. The reasons for NIPT screening in pregnant women. A. In 2019. B. In 2020. C. In 2021. D. In 2022.
a. Voluntary request of pregnant women. b. Adverse pregnancy history. c. abnormal B-ultrasound results. d. Down's screening for trisomy syndrome. e. Abnormal NT value. f. Other reasons.

3. Results

3.1. Analysis of causes behind NIPT in pregnant women

For the 22,692 pregnant women, the mean age was 30.52 ± 1.85 years, and the time when NIPT was performed was 12.52 ± 2.12 weeks. A total of 312 of the 22,692 pregnant women were terminated, with a termination rate of 1.37%. In this study, the reasons for NIPT screening in pregnant women were examined. As shown in Fig. 2, the results indicated that the primary cause was the voluntarily requested testing made by pregnant women. This reason accounted for 84% of the total in 2022, up from 79% in 2019. The remaining reasons were abnormal B-ultrasound results, trisomy syndrome screening for high chance of Down syndrome, and other reasons.

3.2. Evaluation of diagnostic efficacy of NIPT and serological screening

The proportion of high risk serological screening was 4.38% among the 22,692 pregnant women in this study, while the rate of abnormal NIPT screening was 1.93%. With a rate of 4.38%, there was a total of 994 pregnant women who were at high risk for Down's by serological screening. A total of 185 chromosomal abnormalities were confirmed among the 815 patients who were followed up with an overall positive predictive value of 44.58%. With a rate of 1.93%, there was a total of 438 pregnant women who were at high risk for Down's by NIPT screening. A total of 296 chromosomal abnormalities were confirmed among the 415 patients who were followed up with an overall positive predictive value of 71.33%. In prenatal diagnosis, there were 122 patients with sex chromosome abnormalities, 12 with CNV, 243 with 21, 18 and 13 trisomy syndrome, and 39 with other autosomal abnormalities. The abnormal rate of NIPT screening was lower than the high risk rate of serological screening, and the positive predictive value was higher than serological screening ($P < 0.001$, Table 1 and Fig. 3).

3.3. Evaluation of the efficacy of NIPT in screening for sex chromosome abnormalities

Among 145 pregnant women with fetal sex chromosome abnormalities screened by NIPT, 122 cases with fetal sex chromosome abnormalities were detected by prenatal amniocentesis, among which the NIPT-screened positive predictive values of 45, X/47, XXX/47, XYY/47, XXY were 25.00%, 58.82%, 85.71%, 85.71%, respectively, with an overall predictive value of 44.26%. The positive predictive value of fetal sex chromosome abnormalities in NIPT screening was higher than that of serological screening ($P < 0.05$, Tables 2 and 3).

3.4. Evaluation of the efficacy of NIPT screening for fetal CNV

Among 36 pregnant women with fetal CNV screened by NIPT, the cases of fetal copy number deletion, duplication, and deletion and duplication were 11, 23 and 2, respectively. NIPT screening indicated that CNVs≤10 Mb and CNVs>10 Mb were 33.33% and 66.67%, respectively. Based on prenatal diagnosis of amniocentesis, 12 patients with CNV were examined. The NIPT-screened positive predictive values of fetal copy number deletion, duplication, and deletion and duplication were 50.00%, 57.14% and 100.00%, respectively, with an overall predictive value of 58.33%. Although the positive predictive value of fetal CNV in NIPT screening was higher than that in serological screening, without statistically significant difference ($P > 0.05$, Tables 4–6).

3.5. Comparison of abnormal detection rates of NIPT in pregnant women of different ages

The detection rate of fetal sex chromosome abnormalities was 85.29% in pregnant women over 35 years old during NIPT screening, which was higher than that in women between 31 and 35 years old with 76.19% ($P < 0.05$, Table 7). The detection rate of total abnormalities was 84.62% in pregnant women over 35 years old during NIPT screening, which was higher than that in women between 18-30 years old and 31–35 years old with 70.59% and 60.34%, respectively ($P < 0.05$, Table 9). There was no significant difference in the abnormal detection rates of fetal CNV among the three groups ($P > 0.05$, Table 8).

4. Discussion

Birth defects are abnormalities in structure, function, or metabolism that develop before a child is born. Genetic factors, such as chromosome aberration and gene mutation, environmental factors, as well as the combination of these two kinds of factors or other

Table 1
Evaluation of diagnostic efficacy of NIPT and serological screening (cases, %).

Groups	Cases	Test results	
		Abnormal (high risk)	Normal (low risk/critical risk)
Serological screening	22,692	994 (4.38)	21,698 (95.62)
NIPT	22,692	438 (1.93)	22,254 (98.07)
χ^2		222.911	
P		< 0.001	

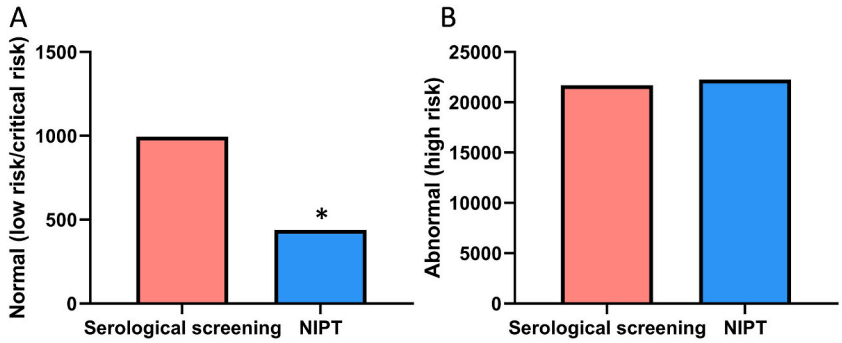


Fig. 3. Evaluation of diagnostic efficacy of NIPT and serological screening. A. Comparison of normal cases between the two groups. B. Comparison of abnormal cases between the two groups. Note: Compared with serological screening results *P < 0.001.

Table 2
Evaluation of the efficacy of NIPT in screening for fetal sex chromosome abnormalities (cases, %).

Abnormal sex chromosome types	NIPT (n)	Prenatal diagnosis (n)	Consistent	Inconsistent	Positive predictive values
45, X	88	72	18	54	25.00%
47, XXX	20	17	10	7	58.82%
47, XYY	8	7	6	1	85.71%
47, XXY	29	26	20	6	85.71%
Total	145	122	54	68	44.26%

Table 3
Evaluation of the efficacy of two screening methods for fetal sex chromosome abnormalities (cases, %).

Groups	Abnormal (high risk) (Cases)	Number of patients followed-up	Number of diagnosed cases	Positive predictive values
Serological screening	356	295	38	31.15%
NIPT	145	132	68	44.26%
χ^2				4.467
P				0.035

Table 4
Evaluation of NIPT screening for fetal CNVs (cases, %).

NIPT-screened fragment size	Depletion	Duplication	Depletion and duplication	Total
≤10 Mb	6	5	1	12
>10 Mb	5	18	1	24
Total	11	23	2	36

Table 5
Evaluation of fetal CNV detection efficiency of NIPT screening (cases, %).

Abnormal CNV types	NIPT (n)	prenatal diagnosis (n)	Consistent	Inconsistent	Positive predictive values
Depletion	11	4	2	2	50.00%
Duplication	23	7	4	3	57.14%
Depletion and duplication	2	1	1	0	100.00%
Total	36	12	7	5	58.33%

Table 6
Evaluation of the efficacy of two screening methods for fetal sex chromosome abnormalities (cases, %).

Groups	High risk (cases)	Number of patients followed-up	Number of diagnosed cases	Positive predictive values
Serological screening	58	49	4	33.33%
NIPT	36	31	7	58.33%
χ^2				1.151
P				0.219

Table 7
Comparison of the detection rate of fetal sex chromosome abnormalities by NIPT in pregnant women of different ages (cases, %).

Groups	Sex chromosome abnormalities			χ^2	*P*
	Total (cases)	Coincident (cases)	Coincidence rate (%)		
18-30 yeas old (n = 9844)	21	16	76.19	1.626	0.202
31-35 yeas old (n = 7612)	22	19	86.36	75.586	<0.001$^{\#\#\#}$
>35 yeas old (n = 5236)	102	87	85.29	113.223	<0.001***

Note: Compared with 18–30 years old, ***P < 0.001; Compared with over 35 years old, $^{\#\#\#}$P < 0.001.

Table 8
Comparison of NIPT detection rate of fetal CNV in pregnant women of different ages (cases, %).

Groups	CNV			χ^2	*P*
	Total (cases)	Coincident (cases)	Coincidence rate (%)		
18-30 yeas old (n = 9844)	16	5	31.25	0.121	0.728
31-35 yeas old (n = 7612)	11	3	27.27	0.779	0.377
>35 yeas old (n = 5236)	9	4	44.44	0.376	0.540

Table 9
Comparison of fetal total abnormal detection rates of NIPT in pregnant women of different ages (cases, %).

Groups	Overall detection			χ^2	*P*
	Total (cases)	Coincident (cases)	Coincidence rate (%)		
18-30 yeas old (n = 9844)	58	35	60.34	6.862	0.009 *
31-35 yeas old (n = 7612)	68	48	70.59	254.789	<0.001$^{\#\#\#}$
>35 yeas old (n = 5236)	312	264	84.62	386.269	<0.001 ***

Note: Compared with 18–30 years old, *P < 0.05, ***P < 0.001; Compared with over 35 years old, $^{\#\#\#}$P < 0.001.

unknown causes, can all contribute to its development. Congenital malformations, chromosome abnormalities, genetic metabolic diseases, and functional abnormalities (such as blindness, deafness and intellectual disabilities, and others) are examples of birth defects that not only increase the pain of newborns and families, but also causes a heavy medical economic burden [15,16]. The most common reason for birth malformations is chromosomal abnormalities. Genes are carried by chromosomes, and chromosomal diseases are chromosomal abnormalities, resulting in abnormal gene expression and abnormal body development. The pathogenesis of chromosome aberration is not clear, which may be caused by the non-separation of chromosomes in the late stage of cell division or breakage and reconnection of chromosome under the influence of various factors *in vivo* and *in vitro* [17]. DAI and colleagues [18] selected 69,608 pregnant women who underwent NIPT examination at the Genetics and Prenatal Diagnosis Center of the First Affiliated Hospital of Zhengzhou University as the study subjects, and found that the NIPT positive rate of high-risk pregnant women was 0.23% (161/69,608), and that 153 (95%) were successfully followed among 161 high-risk women with trisomy, and 139 fetuses were finally born, with a pregnancy termination rate of 13.66%. For fetuses with XXX syndrome or Turner syndrome without congenital heart malformations, such newborns may generally have abnormalities in the anatomical structure of other systems, and can also be accompanied by abnormal intellectual growth and development, so it is recommended to terminate the pregnancy clinically. Therefore, the popularization of prenatal screening and diagnosis of chromosomal diseases are crucial for reducing the occurrence of birth defects. There are now a variety of rapid, accurate, effective and feasible advanced methods for screening and diagnosing prenatal chromosomal diseases because to the development of medical genetics, molecular biology and imaging medicine. In order to reduce and avoid a large number of adverse consequences caused by birth defects, this study analyzed the detection efficiency of NIPT on sex chromosome abnormalities and CNV, and further explored the sex chromosome abnormalities and CNV in pregnant women of different ages, which was of great significance for early detection and early intervention of fetuses with chromosomal abnormalities.

Due to its fast detection and inexpensive cost, serological screening has long been a popular method of clinical prenatal screening. However, due to its low detection rate and low positive predictive value, it is now unable to meet clinical prenatal screening requirements [19,20]. For early detection at 10 weeks of pregnancy, NIPT can be used to assess cellular free fetal DNA (cff-DNA) from a simple blood sample taken from a pregnant woman. At 12 weeks of pregnancy, NIPT can be used with early pregnancy ultrasound to help increase the precision of prenatal screening. Early pregnancy detection and pregnancy termination are made easier by it [21,22]. The use of NIPT prenatal detection technology as a more precise form of Down's syndrome screening has important clinical ramifications for the prevention and management of newborn morbidity. In this trial, the positive predictive value was significantly greater, and the abnormal rate of NIPT screening was significantly lower than the high risk rate of serological screening [23,24]. It was suggested that NIPT had a high degree of accuracy and could lessen the need for intrusive procedures, which would diminish the chance of miscarriage. Alberry MS et al. [25] revealed that The accuracy of prenatal screening may be increased by using NIPT, which had a high sensitivity for aneuploid screening and could also enable the identification and study of the fetal genome from maternal

plasma. Another meta-analysis [26] showed that NIT had a sensitivity of 99.3%3 (95% CI 95.5%–99.9%) and a specificity of 99.9% (95% CI 99.8%–99.9%) for trisomy 21. Therefore, the extremely high sensitivity and specificity of NIPT are superior to the chromosomal aneuploidy screening methods to date.

Fetal chromosome abnormalities can be classified into two types, including aberrant chromosome number and abnormal chromosome structure. Among them, sex chromosome aneuploidy has a significant difference in detection results due to its particularity. The fetuses with 45, X generally experience spontaneous abortion, while other types have no obvious characteristics, and there are no obvious abnormalities on ultrasound detection [27]. Therefore, the clinical screening for sex chromosome abnormalities have always been a challenge. In this study, 122 had sex chromosome abnormalities among 145 women with sex chromosome abnormalities screened by NIPT, indicating that NIPT was of high value in screening for sex chromosome abnormalities. However, in the prenatal diagnosis of sex chromosome abnormalities, the positive predictive values of NIPT screening 45, X/47, XXX/47, XYY/47, XXY were 25.00%, 58.82%, 85.71%, 85.71%, respectively, with an overall predictive value of 44.26%, which were generally low. Therefore, when necessary, an invasive examination should be carried out to further establish abnormal screening. CNV generally results in disorders including fetal congenital malformation, neuropsychiatric development delay, and other diseases. Due to the complexity and diversity of CNV types and the prevalence of novel mutations, screening and diagnosis are challenging [28,29]. In this work, 12 cases of the 36 CNV women screened by NIPT had CNV. In the fetuses of CNV, the NIPT-screened positive predictive values of copy number deletion, duplication, and deletion and duplication were 50.00%, 57.14% and 100.00%, respectively, with an overall predictive value of 58.33%. These findings indicated that NIPT had certain value in the screening of CNV, and NIPT also had a certain suggestive effect on other chromosome abnormalities, microdeletions, and microduplications. In another large sample study of 12,979 cases, NPT had a positive coincidence rate of 83.33% for abnormalities with an increased number of sex chromosomes, but a decrease in the number of sex chromosomes (26.09%) and a positive coincidence rate for complex abnormalities (25.00%) were low [30]. Therefore, non-invasive prenatal genetic testing technology has certain efficacy in screening fetal sex chromosome aneuploidy. Because NIPT is not the definitive modality, the next step of interventional prenatal diagnosis should be performed when it suggests sex chromosomal aneuploidy abnormalities. At present, there is no effective treatment for chromosomal aneuploidy diseases in clinical practice, so early prenatal testing and diagnosis of fetal chromosomal malformations are extremely important in clinical work. Chromosome karyotype analysis or CMA comparison should be performed as soon as it is practical for fetuses with CNV detected by NIPT screening, and genetic status of both parents should be consulted for further diagnosis and selection of pregnancy outcomes. In addition, we found that the detection rate of NIPT screening for fetal sex chromosome abnormalities and total abnormalities in pregnant women over 35 years old was significantly higher than these of 18–30 years old and 31–35 years old. The increase in gestational age may enhance the risk of fetal chromosome abnormalities. Thus, early detection and diagnosis should be a priority for elderly pregnant women. Pregnant women with a false-positive NIPT test may be further performed with amniocentesis, which is invasive and includes risks of bleeding, infection, miscarriage, and injury to the fetus. The risk of such puncture is small with the greatest risk of miscarriage, which occurs in 1–3%. Without amniocentesis, it is possible to miss the opportunity to find chromosomal abnormalities before birth, and children with chromosomal abnormalities are mostly manifested as mental retardation, abnormal growth and development, etc., and there is currently no way to treat chromosomal diseases [31].

In summary, NIPT screening can greatly elevate the detection efficacy of fetal sex chromosome abnormalities, CNV and other chromosome abnormalities, while minimize the false positive rate and help reduce the birth defects. However, because of the relatively low positive predictive value of NIPT screening, additional prenatal examination and genetic counseling are still required. Furthermore, NIPT screening for pregnant women over 35 years of age has a considerably higher detection rates for fetal sex chromosome abnormalities and total abnormalities, and advanced pregnancy may be one of the risk factors for fetal chromosome abnormalities. In addition, NIPT is a prenatal screening test with the possibility for false positive and false negative, and it cannot diagnose or rule out the specific chromosomal disoder in the fetus. NIPT is still merely a screening technique, therefore there is a possibility of missing in the screening even though the accuracy rate is high. Therefore, it is still necessary to combine the comprehensive evaluation of four-dimensional system ultrasound and pregnancy examination, even if NIPT is non-invasive and low-risk.

At present, there is no clinical discovery of the treatment modalities for such chromosomal diseases. It is necessary to detect and intervene as early as possible, and detect and terminate the pregnancy of such chromosomal abnormalities through prenatal screening and diagnosis. NIPT also has an advantage in its positive predictive value compared to traditional screening methods. In this study, a comprehensive analysis of sex chromosomal abnormalities and CNV screening by NIPT showed that NIPT detection had high accuracy and specificity for common chromosomal aneuploidy abnormalities, and a low false positive rate. Especially for trisomy 21, NIPT had a certain detection efficiency for sex chromosome abnormalities and copy number variation. Secondly, NIPT had clinical application value in the prenatal screening of chromosomal abnormalities of twins, and NIPT was also effective for pregnant women of advanced age and different gestational weeks. In addition, a large sample size of 22,846 pregnant women was analyzed in the present study, which provided more reliable and representative results and included more data points, thus reducing the effect of random errors and potentially reflecting the population being studied, rather than just a portion of the sample.

Non-invasive prenatal genetic testing technology has important economic and social benefits, which are mainly reflected in the following aspects. (1) Reduction in the rate of birth defects: Through genetic testing of the fetus, the genetic defects of the fetus can be detected in time, the birth defect rate can be reduced, and eugenics can be promoted. (2) Reduction in medical costs: Non-invasive prenatal genetic testing technology can reduce unnecessary invasive tests such as amniocentesis and chorionic villus sampling, reduce the medical risks of pregnant women and fetuses, and reduce medical costs. (3) Improvement in fertility: Non-invasive prenatal genetic testing technology can predict the genetic status of the fetus in advance, provide scientific fertility guidance for families, and improve fertility. (4) Promotion in the progress of medical science and technology: Non-invasive prenatal genetic testing technology is an important application of genetic testing technology, and its development has promoted the progress of genetic testing technology.

Ethics approval and consent to participate

This study was approved by The Ethics Committee of Zhengzhou Central Hospital affiliated to Zhengzhou University (201,336). Informed consent was obtained from participants for the participation in the study and all methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Informed consent was obtained from all individual participants included in the study. The patients participating in the study all agree to publish the research results.

Availability of data and materials

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

Funding

Not applicable.

CRediT authorship contribution statement

Yimei Li: Data curation, Formal analysis, Methodology, Software. **Xiaofeng Yang:** Conceptualization, Resources, Supervision, Writing - review & editing. **Ying Zhang:** Data curation, Software. **Huan Lou:** Data curation, Software. **Mingli Wu:** Investigation, Visualization. **Fang Liu:** Investigation, Visualization. **Wenjing Chang:** Investigation, Validation. **Xueling Zhao:** Investigation, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] M. Viotti, Preimplantation genetic testing for chromosomal abnormalities: aneuploidy, mosaicism, and structural rearrangements, Genes 11 (6) (2020 May 29) 602.
[2] L.M. El-Attar, A.A. Bahashwan, A.D. Bakhsh, Y.M. Moshrif, The prevalence and patterns of chromosome abnormalities in newborns with major congenital anomalies: a retrospective study from Saudi Arabia, Intractable Rare Dis Res 10 (2) (2021 May) 81–87.
[3] N.S. Sahajpal, H. Barseghyan, R. Kolhe, A. Hastie, A. Chaubey, Optical genome mapping as a next-generation cytogenomic tool for detection of structural and copy number variations for prenatal genomic analyses, Genes 12 (3) (2021 Mar 11) 398.
[4] B. Levy, R. Wapner, Prenatal diagnosis by chromosomal microarray analysis, Fertil. Steril. 109 (2) (2018 Feb) 201–212.
[5] S. Goto, N. Suzumori, K. Kumagai, A. Otani, S. Ogawa, Y. Sawada, S. Inuzuka, M. Sugiura-Ogasawara, Trends of fetal chromosome analysis by amniocentesis before and after beginning of noninvasive prenatal testing: a single-center experience in Japan, J. Obstet. Gynaecol. Res. 47 (11) (2021 Nov) 3807–3812.
[6] H. Kang, L. Wang, Y. Xie, Y. Chen, C. Gao, X. Li, Y. Hu, Q. Liu, Prenatal diagnosis of chromosomal mosaicism in 18,369 cases of amniocentesis, Am. J. Perinatol. (2023 Jun 19).
[7] L. Yin, Y. Tang, Q. Lu, M. Jiang, A. Pan, X. Zhu, Application of non-invasive prenatal tests in serological preclinical screening for women with critical-risk and low-risk pregnancies but abnormal multiple of the median values, Clin. Lab. 66 (7) (2020 Jul 1).
[8] L. Carbone, F. Cariati, L. Sarno, A. Conforti, F. Bagnulo, I. Strina, L. Pastore, G.M. Maruotti, C. Alviggi, Non-invasive prenatal testing: current perspectives and future challenges, Genes 12 (1) (2020 Dec 24) 15.
[9] P. Kleinfinger, L. Lohmann, A. Luscan, D. Trost, L. Bidat, V. Debarge, et al., Strategy for use of genome-wide non-invasive prenatal testing for rare autosomal aneuploidies and unbalanced structural chromosomal anomalies, J. Clin. Med. 9 (8) (2020 Aug 1) 2466.
[10] I. Bedei, A. Wolter, A. Weber, F. Signore, R. Axt-Fliedner, Chances and challenges of new genetic screening technologies (NIPT) in prenatal medicine from a clinical perspective: a narrative review, Genes 12 (4) (2021 Mar 29) 501.
[11] P. Mohan, J. Lemoine, C. Trotter, et al., Clinical experience with non-invasive prenatal screening for single-gene disorders, Ultrasound Obstet. Gynecol. 59 (1) (2022 Jan) 33–39.
[12] N. Fries, S. Le Garrec, M. Egloff, et al., Non-invasive prenatal testing: what are we missing? Ultrasound Obstet. Gynecol. 57 (2) (2021 Feb) 345–346.
[13] A. Corcillo, D.Y. Quansah, C. Kosinski, K. Benhalima, J.J. Puder, Impact of risk factors on short and long-term maternal and neonatal outcomes in women with gestational diabetes mellitus: a prospective longitudinal cohort study, Front. Endocrinol. 13 (2022 Jun 20) 866446.
[14] T. Liehr, International system for human cytogenetic or cytogenomic nomenclature (ISCN): some thoughts, Cytogenet. Genome Res. 161 (5) (2021) 223–224.
[15] R.J. Lipinski, R.S. Krauss, Gene-environment interactions in birth defect etiology: challenges and opportunities, Curr. Top. Dev. Biol. 152 (2023) 1–30.
[16] T.G. Beames, R.J. Lipinski, Gene-environment interactions: aligning birth defects research with complex etiology, Development 147 (21) (2020 Jul 17) dev191064.
[17] X. Chen, D. Lin, Y. Ye, X. Zhang, D. Chen, Trends in the prevalence, prenatal diagnosis, and outcomes of births with chromosomal abnormalities: a hospital-based study in Zhejiang Province, China during 2014-2020, Orphanet J. Rare Dis. 17 (1) (2022 Dec 22) 446.
[18] P. Dai, G. Zhao, S. Hu, N. Liu, X. Kong, [Prenatal diagnosis and pregnancy outcome of fetuses with rare autosomal trisomies indicated by non-invasive prenatal testing], Zhonghua Yi Xue Yi Chuan Xue Za Zhi 40 (5) (2023 May 10) 513–518.
[19] J. Shaw, E. Scotchman, N. Chandler, et al., Preimplantation genetic testing: non-invasive prenatal testing for aneuploidy, copy-number variants and single-gene disorders, Reproduction 160 (5) (2020 Nov) A1–A11.
[20] O. Samura, A. Okamoto, Causes of aberrant non-invasive prenatal testing for aneuploidy: a systematic review, Taiwan. J. Obstet. Gynecol. 59 (1) (2020 Jan) 16–20.

[21] S.R. van Boeckel, H. Macpherson, J.E. Norman, D.J. Davidson, S.J. Stock, Inflammation-mediated generation and inflammatory potential of human placental cell-free fetal DNA, Placenta 93 (2020 Apr) 49–55.
[22] K.R.M. van der Meij, E.A. Sistermans, M.V.E. Macville, S.J.C. Stevens, C.J. Bax, M.N. Bekker, et al., TRIDENT-2: national implementation of genome-wide non-invasive prenatal testing as a first-tier screening test in The Netherlands, Am. J. Hum. Genet. 105 (6) (2019 Dec 5) 1091–1101.
[23] D. Kimelman, M.E. Pavone, Non-invasive prenatal testing in the context of IVF and PGT-A, Best Pract. Res. Clin. Obstet. Gynaecol. 70 (2021 Jan) 51–62.
[24] P. Benn, A. Rebarber, Non-invasive prenatal testing in the management of twin pregnancies, Prenat. Diagn. 41 (10) (2021 Sep) 1233–1240.
[25] M.S. Alberry, E. Aziz, S.R. Ahmed, et al., Non invasive prenatal testing (NIPT) for common aneuploidies and beyond, Eur. J. Obstet. Gynecol. Reprod. Biol. 258 (2021 Mar) 424–429.
[26] C. Deng, S.W. Cheung, H. Liu, Noninvasive prenatal screening for fetal sex chromosome aneuploidies, Expert Rev. Mol. Diagn 21 (4) (2021 Apr) 405–415.
[27] E. Scotchman, J. Shaw, B. Paternoster, et al., Non-invasive prenatal diagnosis and screening for monogenic disorders, Eur. J. Obstet. Gynecol. Reprod. Biol. 253 (2020 Oct) 320–327.
[28] Y. Onodera, H. Miura, A. Fujishima, et al., A survey of genetic counseling for non-invasive prenatal testing examinees in akita, JMA J 5 (3) (2022 Jul 15) 349–355.
[29] V. da Silva, M. Ramos, M. Groenen, R. Crooijmans, A. Johansson, L. Regitano, L. Coutinho, R. Zimmer, L. Waldron, L. Geistlinger, CNVRanger: association analysis of CNVs with gene expression and quantitative phenotypes, Bioinformatics 36 (3) (2020 Feb 1) 972–973.
[30] E. Iwarsson, B. Jacobsson, J. Dagerhamn, T. Davidson, E. Bernabé, M. Heibert Arnlind, Analysis of cell-free fetal DNA in maternal blood for detection of trisomy 21, 18 and 13 in a general pregnant population and in a high risk population - a systematic review and meta-analysis, Acta Obstet. Gynecol. Scand. 96 (1) (2017 Jan) 7–18.
[31] C.P. Chen, L.K. Wang, S.R. Chern, P.S. Wu, S.W. Chen, F.T. Wu, Y.Y. Chen, C.W. Pan, W. Wang, Prenatal diagnosis of pseudomosaicism for trisomy 20 at amniocentesis with a negative non-invasive prenatal testing (NIPT) result in a pregnancy with a favorable outcome, Taiwan. J. Obstet. Gynecol. 61 (4) (2022 Jul) 675–676.