Non-invasive Prenatal Testing for Assessing Fetal Sex Chromosome Aneuploidy: A Retrospective Study of 45773 Cases

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Research

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Abstract

Objective: To assess the positive predictive value (PPV) of non-invasive prenatal testing (NIPT) as a screening test for sex chromosome aneuploidy (SCA) with different maternal characteristics and prenatal decision in positive cases.

Materials and Methods: We retrospectively analyzed 45773 singleton pregnancies with different characteristics that were subjected to NIPT in Maternity and Child Health Hospital of Anhui Province. The results were validated by karyotyping. Clinical data, diagnostic results, and pregnancy outcomes were collected.

Results: A total of 314 cases were SCA positive by NIPT; among those, 143 underwent invasive prenatal diagnostic testing, and 58 resulted as true-positive. Overall, the PPV for 45,X, 47,XXX, 47,XXY and 47,XYY was 12.5%, 51.72%, 66.67% and 83.33%, respectively. Interestingly, when screening only pregnant women in advanced maternal age (AMA), the PPV for 45,X, 47,XXX, 47,XXY and 47,XYY was 23.81%, 53.33%, 78.95%, and 66.67%, respectively. AMA was a high-risk predictor of having a fetus with SCA. The frequencies of 47, XXX, and 47,XXY were significantly correlated with maternal age.

Conclusion: NIPT performed better in predicting sex chromosome trisomies than monosomy X, and patients with 45,X positive fetus were more eager to terminate pregnancy compared to those with 47,XXX and 47, XYY. Our findings may assist in genetic counseling of AMA pregnant women. Our Pre- and post-test counseling are essential for familiarizing pregnant women with the benefits and limitations of the NIPT, which may ease their anxiety and provide them with the informed choice for further diagnosis and pregnancy decision.

Background

Birth defects are structural deformities or functional abnormalities caused by congenital, genetic, or environmental factors[1]. Chromosomal abnormalities can be divided into chromosomal numerical abnormalities and chromosomal structural abnormalities. Chromosomal numerical abnormalities are usually a result of damage to the mitotic mechanism of cells, while structural abnormalities are a product of chromosome breaks and relocations. The phenotype of chromosomal abnormalities usually includes male infertility, spontaneous abortion, stillbirth, neonatal death, and congenital malformations. Thus, prenatal testing has become critical in preventing congenital disabilities[2].

Sex chromosome aneuploidy (SCA) refers to conditions caused by numerical abnormalities in X and Y chromosomes, such as Turner syndrome (45,X), triple X syndrome (47,XXX), Klinefelter syndrome (47,XXY), and Jacob's syndrome (47,XYY)[1]. 45,X is a common chromosomal disorder affecting approximately 1 in 2500 to 1 in 2000 of live-born female infants[3], the common clinical manifestations of which include congenital cardiac, renal anomalies, and acquired metabolic syndrome[3, 4]. 47,XXX occurs in 1 in 1000 female births[5]. In contrast to other trisomies, most of the girls born with triple X chromosomes do not have characteristic physical appearance at birth, but their height tends to be variable. In addition, some individuals may exhibit developmental delays (speech and motor), learning or intellectual disability, and psychiatric problems[4, 5]. 47,XXY is the most common sex chromosome aneuploidy, and it occurs in 1 of every 660 males[6]. When they come to adults, the extra X chromosome may affect testicular development which will result in infertility and hypogonadotropic hypogonadism[6]. The males with 47,XXY also have a higher risk of learning disabilities, developmental delays, cardiometabolic disease, typical physical symptoms and neurodevelopmental manifestations[6, 7]. 47,XYY occurs in 1 in 1000 males[8] who tend to have tall stature, social obstacles, behavioral problems, and language impairment. The cognitive phenotype in males with 47,XYY typically includes normal mildly diminished general intelligence[8].

The phenotype of SCA patients spans a broad range of associated symptoms that vary in severity, depending on the timing of diagnosis and types of SCA[4, 5, 7, 8, 9]. The frequency of SCA is estimated to be 1 in every 500 live births. About 75–90% of cases are undiagnosed during their lifetime[10]. Recently, studies reported that postnatal hormone therapy could have positive effects on behavioral phenotype if applied earlier to SCA patients. Moreover, screening and prenatal detection of SCA can provide an opportunity for early management, improving the life quality of the affected child.

Before the introduction of non-invasive prenatal testing (NIPT), amniocentesis, chorionic villus sampling, and cord blood collection were the most common tests for assessing sex chromosome abnormalities. However, this method has been associated with an increased risk of procedure-related miscarriage (0.5–1.0%) and maternal anxiety[15]. In 1997, Lo *et al* [1]. discovered cell-free DNA derived from the Y-chromosome in the maternal plasma of pregnant women carrying male fetuses. NIPT analyzes cell-free DNA, which is a mixture of maternal DNA and a low percentage of fetal DNA believed to originate from the trophoblasts, in a pregnant woman's blood[1, 11]. NIPT uses massively parallel sequencing for prenatal fetal aneuploidies screening. Nowadays, with the development of high-throughput sequencing technology, NIPT has been highly recommended for screening test for fetal trisomy 21 (T21), trisomy 18 (T18), and trisomy 13 (T13) of pregnant women with a high risk of serological screening tests results in the second trimester in clinical practice[1, 12]. Extensive studies have demonstrated the high sensitivity and specificity of NIPT in screening T21, T18, and T13, with sensitivities of 95.9%, 86.5%, and 77.5% and specificities of 99.9%,

99.8%, and 99.9%, respectively[13]. Moreover, studies have suggested that the positive predictive value (PPV) of T21, T18, and T13 is 65–94%, 47–85%, and 12–62%, respectively[14]. In addition, NIPT has the advantage of being non-invasive, avoiding the 0.5–1.0% risk of miscarriage associated with amniocentesis/chorionic villus sampling[15, 16].

With the implementation of the second-child policy in China in 2014, the pregnancy rate among women aged ≥ 35 years has gradually increased[5]. At present, it is generally believed that advanced maternal age (AMA) is an important risk factor in chromosomal abnormalities[2]. An understanding of the correlation between SCA frequency and age would provide a solid basis for the development of appropriate prenatal screening and diagnostic methods.

The aim of this study was to assess the positive predictive value (PPV) of NIPT as a screening test for SCA with different maternal characteristics and prenatal decisions.

Results

Maternal characteristics

A total of 45773 maternal blood samples from singleton pregnancies were collected in Maternity and Child Health Hospital of Anhui Province between June 1, 2015 and June 30, 2019. Gestational age at the time of amniocentesis ranged from 12^{+0}–26^{+6}, and the maternal age ranged from 16–45 years. Before undergoing NIPT, screening tests were conducted among pregnant women, which included serological screening tests and fetal ultrasonography.

In total, 16921 (36.97%) cases were AMA (age ≥ 35 years). There were 19820 (43.30%) cases at high or critical risk for developing SCA. B-ultrasound showed that 503 (1.10%) fetuses were soft markers, and 86 (0.19%) fetuses had increased NT (NT ≥ 3 mm). Moreover, 500 (1.09%) pregnant women missed other screening opportunities, and 7943 (17.35) pregnant women with no clinical indications underwent NIPT (Table 1).

Table 1
Maternal characteristics of pregnant women who underwent NIPT

Maternal age (years)	Cases (%)
< 30	17449 (38.12%)
30 ≤ year ≤ 34	11403 (24.91%)
35 ≤ year ≤ 39	14353 (31.36%)
> 39	2568 (5.61%)
Gestational age at NIPT (weeks)	
12–15	4853 (10.60%)
16–19	33066 (72.24%)
20–23	6740 (14.72%)
24–26	1037 (2.27%)
> 26	77 (0.17%)
Clinical features	
Advanced maternal age (age ≥ 35 years)	16921 (36.97%)
High or critical risk of serological screening	19820 (43.30%)
Fetal soft markers by B-ultrasound	503 (1.10%)
Increased NT	86 (0.19%)
Missed other screening opportunities	500 (1.09%)
No clinical indications	7943 (17.35%)

The clinical value of NIPT for screening for fetal SCA and pregnancy outcome

As shown in Table 2, 143 pregnant women (45.54%) accepted the prenatal diagnosis. All patients received the results within 3 weeks. After prenatal diagnosis, 58 pregnant women were true-positive. One hundred forty-seven pregnancies were positive for 45,X. Of them, 56 pregnancies underwent invasive prenatal testing (38.10%). Seven cases were true-positive, with 12.50% PPV of NIPT. Six patients terminated their pregnancies, with the termination rate of 85.71%. For patients with 47,XXX, karyotype information was available for 29 out of 61 NIPT results (47.54%). Among them, 15 cases were true-positive, with a PPV of 51.72%. The NIPT and karyotype analysis were fully concordant in 15 patients, of which 3 decided to terminate their pregnancy (20%). For patients with 47,XXY, karyotype information was available for 39 out of 71 NIPT results with prenatal diagnostic (54.93%). Twenty-six cases were true-positive, with the PPV of 66.67%. Nineteen pregnant women decided to terminate their pregnancies (73.08%). Karyotype information for patients with 47,XYY, was available for 12 out of 35 NIPT results with prenatal diagnostic (34.29%). Ten cases were confirmed to be true-positive, with the PPV of 83.33%. Of these ten patients, one decided to terminate their pregnancies (10%).

Moreover, the proportion of sex chromosome trisomy and monosomy were highly different. Of 167 cases with positive screening results for sex chromosome trisomy, 51 out of 80 NIPT results were confirmed to be true-positive by invasive prenatal testing (63.75%). For 56 out of 147 cases for sex chromosome monosomy with prenatal diagnosis, 7 cases were consistent with NIPT results (12.50%). Chi-square tests were used to further examine the associations between sex chromosome trisomy and monosomy X. Statistical significance was observed between two groups ($\chi^2 = 12.412$, $P < 0.05$).

After prenatal diagnosis, all patients received prenatal genetic counseling. Among the 85 false-positive patients, 67 were successfully followed up. They all successfully delivered their babies. A total of 171 pregnant women refused prenatal diagnosis, 126 had effective follow-up results. Moreover, 86 pregnant women refused prenatal diagnosis due to abortion-related anxiety, and 33 pregnant women because of the severe B-ultrasound results. Another 7 pregnant women had spontaneous abortion and stillbirths in gestation.

The PPV of SCA detected by NIPT was 40.56% (58/143). The PPV of SCA in pregnant women with advanced age group (≥ 35 years) was 50.79% (32/63); in high or critical risk of the serological screening group was 30.43% (14/46); in no clinical indications group was 40% (10/25); in missed other screening opportunities group was 25% (1/4), and in increased NT group was up to 33.33% (1/3).

A total number of 85 false positive NIPT cases were identified. Importantly, 32 cases were suspicious of an abnormal maternal ChrX karyotype, detected by NIPT, including 23 cases with an abnormal ChrX gain and 9 cases with an abnormal ChrX loss. Using genetic counselling, 32 cases underwent maternal peripheral blood karyotyping. Moreover, 2 cases among 23 cases with an abnormal ChrX gain were diagnosed with 47,XXX, while 2 cases among 9 cases with an abnormal ChrX loss was diagnosed with 45,X/47,XXX mosaicism and 47,XXX/45,X/46,XY mosaicism.

Table 2
The results of prenatal diagnosis and clinical outcome

SCA type	NIPT positive (N)	Karyotype analysis			Unverified (N)	Termination rate (%)
		True positive (N)	False positive (N)	PPV (%)		
45,X	147	7	49	12.5	91	85.71
47,XXX	61	15	14	51.72	33	20
47,XXY	71	26	13	66.67	32	73.08
47,XYY	35	10	2	83.33	22	10
SCA sex chromosome aneuploidy, PPV positive predictive value						

Relationship between SCA frequency and maternal age

We evaluated the relationship between different PPV and maternal age. For SCA, the PPV increased with maternal age (Table 3) and was the highest in women aged > 39 years. In our study, there were 16921 (36.97%) patients with AMA (≥ 35 years), accounting for a relatively large population. The PPV, which was 50.79% for SCA in AMA women, was slightly higher than the total samples (40.56%). The frequency of SCA in AMA women was higher compared with the total samples. The frequencies of 45,X and 47,XYY did not differ in pregnant women among all

age groups ($\chi^2 = 4.254$, $P > 0.05$ for 45,X, and $\chi^2 = 1.120$, $P > 0.05$ for 47,XYY). Meanwhile, the frequencies of 47,XXX and 47,XXY were different in pregnant women between different age groups ($\chi^2 = 13.453$, $P < 0.05$ for 47,XXX, and $\chi^2 = 12.163$, $P < 0.05$ for 47,XXY).

Table 3
NIPT results for SCA screening in pregnant women of different age groups

NIPT results	Total samples			AMA women			< 30			30 ≤ year ≤ 34			35 ≤ year ≤ 39			> 39		
	TP	FP	PPV	TP	FP	PPV	TP	FP	PPV	TP	FP	PPV	TP	FP	PPV	TP	FP	PPV
45,X	7	49	12.5	5	16	23.81	2	18	10	0	15	0	4	14	22.22	1	2	33.33
47,XXX	15	14	51.72	8	7	53.33	4	4	50	3	3	50	4	5	44.44	4	2	66.67
47,XXY	26	13	66.67	15	4	78.95	4	6	40	7	3	70	10	4	71.43	5	0	100
47,XYY	10	2	83.33	4	2	66.67	3	0	100	3	0	100	4	2	66.67	/	/	/
SCA suspected abnormal	0	7		0	2		0	2		0	3		0	2		/	/	/
Total	58	85	40.56	32	31	50.79	13	30	30.23	13	24	35.14	22	27	44.9	10	4	71.43

NIPT non-invasive prenatal testing, SCA sex chromosome aneuploidy, AMA advanced maternal age, TP true positive, FP false positive, PPV positive predictive value

Discussion

Several recent studies reported the sensitivity, specificity, and PPV of NIPT for T21, T18, and T13 screening. Yet, so far, no large-scale clinical studies have been conducted to assess the efficiency of NIPT for detecting SCA. To the best of our knowledge, this is the largest study (45773 cases) that evaluated NIPT for SCA. According to our data, the overall PPV of SCA detected by NIPT was 40.56%, within the rage of PPV for SCA screening between 30–60%[12, 14, 17, 18, 19]. When categorized by individual SCAs, the PPVs were 12.5% for 45,X, 51.72% for 47,XXX, 66.67% for 47,XXY, and 83.33% for 47,XYY, which was similar to those reported by other studies[1, 12, 20]. Yet, in our study, we noticed that different pregnancies characteristics showed different PPV, e.g., the PPV of SCA in AMA pregnant women (50.79%) was higher than in the total samples (40.56%). Therefore, we believe that AMA is a high-risk factor for SCA. Moreover, the overall termination rate was 50% for SCA (including mosaic cases), and 41.38% (not including mosaic cases); the termination rate for fetal SCA was 85.71% for 45,X, 20% for 47,XXX, 73.08% for 47,XXY, and 10% for 47,XYY.

Our study showed that NIPT performed better in predicting sex chromosome trisomies than monosomy X. This may be due to the following: (1) there are 1098 genes on the X chromosome and 78 genes on the Y chromosome; 58 genes are homologous genes on both sex chromosomes. The majority of the genes (29 genes) are at the end of the sex chromosomes. (2) the low guanosine-cytosine content of the X chromosome leads to highly variable amplification of the X chromosome. (3) the non-random inactivation of the X chromosome in placental tissue might be the reason for the low PPV of Turner syndrome, with the paternal X chromosome tending to inactivate in XX female trophoblasts[1, 12]. Besides, it was reported that there is an age-related X chromosome loss in normal female white blood cells, which may influence the effectiveness in predicting fetal 45,X[12]; although this was not observed in our study.

The PPV of SCA is lower than other common chromosome aneuploidy. The reason is that sex chromosome abnormalities are less prevalent[14]. Wang *et al* reported that 8.6% positive results for SCA were due to maternal mosaicism[21], which was later confirmed by other studies[1, 12, 22]. With the combination of NIPT and maternal peripheral blood karyotype analysis, we found that about 12.5% of the discordant NIPT SCA results are due to maternal mosaicism in our study. Previous studies have demonstrated that the identification of maternal karyotype tends to decrease the rate of false-positive SCA and can offer an explanation for the false-positive results for SCA[12, 13, 23]. With regard to the discordance between NIPT and invasive prenatal testing, another reason is confined placental mosaicism, which occurs approximately in 1–2% of all pregnancies[18]. The origin of most cell-free fetal DNA in the maternal plasma is mostly from the apoptosis of placental cells from the cytotrophoblast[24]. The mosaicism degree reduces the effective cell-free fetal DNA concentration in maternal plasma, thus affecting the performance of NIPT in detecting fetal aneuploidies. Our results verified the value of determining the maternal karyotype in increasing the accuracy of reporting NIPT results for chromosomes X and Y; however, further studies are needed to provide more clinical data in support of this premise.

With the prenatal diagnosis of fetal SCA, some pregnant women were willing to continue their pregnancy, and there were differences in the rate of pregnancy termination rate between different types of SCAs. Pregnant women with fetuses of 45,X and 47,XXY were more eager to terminate a pregnancy than those with 47,XXX and 47,XYY, which was consistent with other studies[12, 25]. Gruchy *et al*[26] reported that with

regard to Turner syndrome, the rate of pregnancy termination was closely related to the 45,X karyotype, mosaic karyotype, and structural abnormalities of the X chromosome. In our study, pregnant women with a fetus of 45,X with the mosaic of 23.08% had a stronger tendency to continue the pregnancy, while those with a mosaic of 28.38%, 48.53%, 59.38%, 76.67%, and 80.49% were more inclined to terminate a pregnancy. Nowadays, studies report that almost all pregnant women carrying fetuses with 45,X decide to terminate their pregnancy[27]. To some extent, the parental decisions for the pregnancy termination may have been related to the types of SCA, level of prenatal genetic counseling, history of infertility, parental and social acceptance, economic condition, and similar.

In clinical practice, AMA pregnant women are willing to accept NIPT as a non-invasive and accurate screening. In this study, the proportion of AMA pregnant women was the second-highest in the NIPT screening population, reaching 36.97%. Among them, NIPT detected 108 cases SCA; 63 cases underwent amniocentesis, and 32 (50.79%) cases were confirmed to be true-positive. Of all the confirmed SCA cases, 55.17% (32/58) were from AMA pregnant women. The study showed that NIPT could be used as a useful screening test for SCA in AMA pregnant women, which was similar to the results reported by Zheng *et al* [28]. The differences in the frequency of SCA were statistically significant among the age groups, and the frequency was significantly higher in the > 39 years age group ($P < 0.05$). Therefore, genetic counseling, combined with serological screening tests and B-ultrasound detection of abnormalities, should be fully carried out for AMA pregnant women. The frequencies of 47,XXX and 47,XXY were significantly correlated with maternal age, whereas frequencies of 45,X and 47,XYY did not show significant correlations. Although there was no statistical significance between the frequency of 47,XYY and the maternal age, the frequency of 47,XYY decreased with maternal age in the advanced aged group. To determine whether there is a correlation between the maternal age and the frequency of fetus 47,XYY, further studies with bigger sample size are warranted[2]. The risk of 47,XXX increased with AMA, which was consistent with Zhu *et al* study[2]. Previous studies showed that for 45,X syndrome, the maternal age coefficient is negative and implies a decreasing incidence in older mothers[2]. Yet, this was not consistent with our results. Understanding the frequency of SCA has proven valuable in counseling couples who seek advice about the risk of fetal sex chromosome abnormalities with AMA and are considering the option of prenatal diagnosis and termination of pregnancy.

This study has a few limitations. First, the sensitivity, specificity, and negative predictive value were not calculated. Newborns with SCA usually appear phenotypically normal. Therefore, it was difficult to confirm the results of SCA without karyotype analysis during their neonatal period. Second, the number of SCA cases in our study was not big enough to discuss its frequency across different age groups. Therefore, more pregnancies must be evaluated in order to further understand the association of maternal age with fetal SCA.

Recent studies have demonstrated that early interventions such as postnatal hormone therapy, physical therapy, and occupational therapy could have positive effects on the behavioral phenotype or neurodevelopmental outcomes if applied earlier to SCA patients[9, 29]. Prenatal screening and diagnosis of SCA can provide the opportunity for early intervention, comprehensive postnatal management, and improve the quality of life of the affected child[10]. Sex chromosome abnormalities are more common than the major trisomies at birth, and the neonates are often phenotypically normal[30]. Conventional prenatal screening cannot be used to directly identify sex chromosome abnormalities that can only be identified using postnatal karyotyping, which in turn may delay the treatment of SCA patients. NIPT allows prenatal screening of SCA. The application of NIPT can provide an alternate option for pregnant women to invasive prenatal testing for the identification of fetal sex chromosome abnormalities. However, there are still some issues that require further consideration. Due to the existing false positive rate of NIPT screening for SCA, the number of unnecessary invasive prenatal diagnosis may increase, especially for 45,X[12]. Still, the benefit of detection for fetal SCA outweighs the risk related to invasive procedures. Some pregnant women may decide to terminate a pregnancy if the chromosomal abnormalities are accidentally discovered by SCA screening, which involves ethical issues regarding the mild phenotype of SCA and the potential increase in the rate of gender selection[12].

Conclusions

Our data suggest that NIPT can be used to identify fetal SCA by analyzing cffDNA obtained from the maternal plasma using massively parallel sequencing technology. We found that patients with 45,X fetuses were more eager to terminate their pregnancies. Different maternal characteristics have different PPV. AMA was a risk for SCA. Also, the frequencies of 47,XXX, and 47,XXY were correlated with maternal age. Pre- and post-test counseling are essential in familiarizing women with the benefits and limitations of the NIPT, which may ease their anxiety and provide them with the informed choice for further diagnosis and pregnancy decisions.

Materials And Methods

Subjects

In this study, 45773 women with singleton pregnancies who underwent NIPT at the Maternity and Child Health Hospital of Anhui Province between June 1, 2015 and June 30, 2019 were recruited. Maternal age, serological screening results, nuchal translucency, and B-ultrasound

results were recorded. Inclusion criteria were as follows: (1) gestational week between 12^{+0}~26^{+6}; (2) singleton pregnancy. The exclusion criteria were: (1) gestational age < 12 weeks; (2) multiple pregnancies; (3) the couple had definite chromosomal abnormalities; (4) pregnant women who received an allogeneic blood transfusion, stem cell therapy, transplant surgery, etc.; (5) having a family history of genetic disease or suggesting a high risk of genetic disease in the fetus; (6) pregnant women with malignant tumor; (7) other conditions that might affect the accuracy of the results.

The study was approved by the Ethics Committee of the Anhui Medical University. Informed consent was obtained from all patients. Before testing, all patients received a consultation with a genetic counselor or clinical geneticist, after which, a prenatal diagnosis was recommended.

Maternal serum screening tests and ultrasonography

Maternal age ≥ 35 years was defined as an advanced maternal age (AMA)[2]. We used a combination of the first trimester screening (from 9 weeks to 13+6 weeks) and the second trimester screening (from 15 weeks to 20+6 weeks). The serological screening tests used were the pregnancy-associated plasma protein A (PAPP-A) (PAPP-A) and free β-HCG for the first trimester screening, and AFP, free β-HCG, and free E3 for the second trimester screening. Time-resolved immunofluorescence assay was used for detection. B-ultrasound soft markers refer to non-specific ultrasound image manifestations that are often observed in normal fetuses and are not serious[34,35]. Most of them are transient and are more common in fetuses with congenital anomalies and are associated with chromosomal abnormalities[35]. The following signs are mainly used as B-ultrasound soft markers: absent or shortened nasal bone, thickened nuchal fold, single umbilical artery, echogenic bowel, pyelectasis, and choroid plexus cysts[35]. 'NT was measured by a trained sonographer following the Fetal Medicine Foundation protocol, and NT ≥ 3 mm was defined as increased NT[20]. Volunteering NIPT with applicable people or people with caution ' referred to the two categories pregnant women. In our study, we divided the participants into two categories, including applicable people and people with caution. The “applicable people” referred to pregnant women with no clinical indications and to people who had missed other screening opportunities. “People with caution” include: (1) advanced maternal age (age ≥ 35 years); (2) high or critical risk of serological screening; (3) fetal soft markers by B-ultrasound,;(4) increased NT. The risk values were calculated by Lifecycle software (4.0): high risk, T21 > 1/300, T18 > 1/350; critical risk, T21 = from1/300 to 1/1000, T18 = from 1/350 to 1/1000.

Non-invasive prenatal testing (NIPT)

A total of 10 mL of maternal peripheral blood was collected in a Cell-Free DNA BCTTM tube (EDTA). Plasma was separated from the maternal plasma by two rounds of centrifugation within 48 h. Whole blood was centrifuged at 1600 × g for 10 min at 4°C, after which the supernatant was centrifuged at 16000 × g for 10 min. The maternal plasma was immediately stored at -80°C until DNA extraction. Extraction of cell-free fetal DNA, library construction, quality control, and pooling were performed in the laboratory of Maternity and Child Health Hospital of Anhui Province. DNA was extracted by the QIAamp Circulating Nucleic Acid Kit (Qiagen). The pooled library was sequenced using an Illumina with the Data Analysis System for bioinformatic analysis of the sequencing data. The sequencing reads were filtered and aligned to the human reference genome (hg19)[14]. Calculating a Z score per chromosome and chromosome with an absolute value of z-score > 3 was identified with chromosome aneuploidies or microdeletions/microduplications[36]. Also, samples with a normalized chromosome value of 3.0 or less were marked with normal.

Karyotype analysis of amniotic fluid

Karyotype analysis of amniotic fluid was performed for samples collected from pregnant women who were positive for SCA by NIPT, and who agreed to undergo invasive prenatal diagnosis, which is the gold standard for chromosome aneuploidy testing. Briefly, under B-ultrasound guidance, 20 mL of amniotic fluid was aseptically withdrawn using amniocentesis. Inoculation, culture, G-banding, and karyotype scanning using a GSL-120 automatic karyotype scanner were then performed. The karyotype was described according to the “International System for Human Cytogenetic Nomenclature, ISCN2016” guidelines. According to the principle outlined by the American College of Medical Genetics and Genomics, 2018, a total of 30 dividing phases were counted per sample using an AI chromosome image analysis system (CytoVision, Switzerland). Five karyotypes were analyzed, and double counts were obtained in the case of chimeras.

Statistical analysis

Statistical analysis was performed using SPSS version 23 (IBM Corp., Armonk, NY, USA). A chi-square test was applied to compare the incidence of SCA among pregnant women in different age groups. A P value of <0.05 was considered to be statistically significant.

Declarations

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Author's contribution

LU Xinran designed the study and drafted the manuscript. WANG Chaohong and SUN Yuxiu collected all the data. TANG Junxiang analyzed the data and interpretation. TONG Keting participated in the laboratory workflow. ZHU Jiansheng oversaw the work and revised the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Anhui Medical University.

Consent for publication

Not Applicable

Competing interests

The authors have declared no conflict of interest.

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