Clinical Experience with Fetal Sex Chromosome Aneuploidy Identified by Non-Invasive Prenatal Testing in 45773 Cases

Xinran Lu
Affiliated Maternity and Child Health Hospital of Anhui Medical University https://orcid.org/0000-0001-5648-820X

Chaohong Wang
Maternity and Child Health Hospital of Anhui Province

Yuxiu Sun
Maternity and Child Health Hospital of Anhui Province

Junxiang Tang
Maternity and Child Health Hospital of Anhui Province

Keting Tong
Maternity and Child Health Hospital of Anhui Province

Jiansheng Zhu (✉️ 593130772@qq.com)

Research

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Abstract

**Objective:** To investigate the positive predictive value (PPV) and clinical features of non-invasive prenatal testing (NIPT) as a screening method in detecting sex chromosome aneuploidy (SCA) within a high-risk population at the Maternity and Child Health Hospital of Anhui Province.

**Methods:** From June 2015 to June 2019, 45773 women with singleton pregnancies volunteered to take an NIPT. Cell-free fetal DNA was extracted for high-throughput sequencing and amniocentesis karyotype analysis was performed in pregnant women.

**Results:** 314 high-risk pregnant women underwent NIPT and 143 chose invasive prenatal diagnosis. Karyotype analysis was performed in amniotic fluid cells, wherein 7 cases of 45,X (PPV: 12.50%), 16 cases of 47,XXX (PPV: 55.17%), 25 cases of 47,XXY (PPV: 71.43%), and 10 cases of 47,XYY (PPV: 76.92%) were confirmed. The PPV of NIPT for SCA was 40.56%. The rate of SCA detected in women aged 40 years and older was 0.39%, which was significantly different from that detected in women aged <30, 30–34, and 35–39 years (P < 0.05). The detection rates of 47,XXX and 47,XXY were significantly correlated with maternal age (P < 0.05), but those of 45,X and 47,XYY showed no significant correlation with maternal age.

**Conclusion:** NIPT can be applied for the detection of SCA, but the detection accuracy is low. Genetic counseling and further prenatal diagnosis should be provided.

Background

Birth defects are structural deformities or functional abnormalities due to congenital, genetic or environmental factors. There are a main cause of prenatal death and child disability in China, affecting children's health and quality of life and placing a mental burden on families and society. Chromosomal abnormalities are one of the most serious birth defects\(^1\). Thus, prenatal testing has become critical in preventing birth defects.

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. Nowadays, with the development of high-throughput sequencing technology, NIPT is now recommended as the most accurate screening test for fetal trisomy 21 (T21), trisomy 18 (T18) and trisomy 13 (T13) of pregnant women with high risk of serum biochemistry screening results in the second trimester in clinical practice\(^1,3\). 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\(^4\). Research has suggested that the PPV of T21, T18, and T13 were 65–94%, 47–85%, and 12–62%, respectively\(^5\).

SCAs refer to conditions caused by numerical abnormalities in X and Y chromosomes, including Turner syndrome (45,X), triple X syndrome (47,XXX), Klinefelter syndrome (47,XXY) and 47,XYY syndrome (47,XYY)\(^1\). In general, SCA are usually experienced with infertility, short or tall stature, behavioral
problems, mental disorder and developmental delay in intelligence and sexual\cite{1, 12}. However, the phenotype of patients span a large range of associated symptoms that vary in severity depending on the timing of diagnosis and types of SCA\cite{6, 7, 8, 9, 10, 11}. The incidence of SCA is estimated to be 1 in every 500 live births and about 75–90% cases are undiagnosed during their lifetime\cite{12}. Despite having a combined prevalence greater than T21, SCA testing have not been included in prenatal screening programs, due to the frequently mild phenotype and coupled with uncertainty over the benefits of early treatments\cite{13}. However, none of the screening methods was specifically used for SCA, except where an ultrasound finding of cystic hygroma, fetal hydrops and enlarged nuchal translucency may raise suspicion of 45,X\cite{13}. Before NIPT technology was launched, the detection of sex chromosome abnormalities was diagnosed only through culture of amniotic fluid cells of high-risk pregnant women or newborn screening. It may increase the risk of procedure-related miscarriage and maternal anxiety. In the pre-NIPT era, about less than 50% of expected SCA were diagnosed during pregnancy\cite{13, 14, 15}.

PPV was calculated as the cases for which NIPT and confirmatory diagnostic testing were concordant (true-positive), divided by the cases with positive NIPT screening (true-positive plus false-positive), multiplied by 100. PPV is important because it indicates the probability that a positive result represents a true positive and is an index to evaluate the screen's value in clinical. We used PPV to evaluate the efficiency of NIPT for SCA detection in our study. Recently, many laboratories offering NIPT included sex chromosome testing, because of the low number of samples, only few studies have investigated the efficiency of NIPT in detecting SCA, which remains limited. To our knowledge, this study has enrolled the largest number of pregnant women to research the clinical application value of SCA detected by NIPT in domestic.

With the implementation of the second-child policy in China in 2014, the rate of pregnancy in women aged $\geq$ 35 years has gradually increased\cite{5}. At present, it is generally believed that advanced maternal age is an important risk factor in chromosomal abnormalities\cite{16}. Studies have shown that the incidence of SCA may be related to the age of the father and mother and the mother's parity, but only few relevant studies and the conclusions are also quite different\cite{17}. An understanding of the correlation between SCA incidence and age would provide a solid basis for the development of appropriate prenatal screening and diagnostic methods.

This study aimed to evaluate the screening value of NIPT for SCA testing and compare the association of maternal age with fetal SCA.

**Results**

**Maternal characteristics**

From June 1, 2015 to June 30, 2019, a total of 45773 maternal blood samples from singleton pregnancies were collected in Maternity and Child Health Hospital of Anhui Province. In our study, the gestational age of pregnancy at the time of amniocentesis ranged from $12^{+0} - 26^{+6}$ and the maternal age
ranged from 16–45 years, with the < 30 age group forming the majority (17449/45773, 38.12%). Among the 45773 cases of NIPT, there were 17421 cases of advanced maternal age (38.06%), 19820 cases with high or critical risk (43.30%), 503 cases of fetal structural abnormalities or soft markers by B-ultrasound (1.10%), 86 cases of increased NT (NT ≥ 3 mm) (0.19%) and 7943 cases of volunteering NIPT with applicable people or people with caution (17.35%).

<table>
<thead>
<tr>
<th>Maternal age (years)</th>
<th>Number</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td>17449</td>
<td>38.12</td>
</tr>
<tr>
<td>30–34</td>
<td>11403</td>
<td>24.91</td>
</tr>
<tr>
<td>35–39</td>
<td>14353</td>
<td>31.36</td>
</tr>
<tr>
<td>≥ 40</td>
<td>2568</td>
<td>5.61</td>
</tr>
<tr>
<td>Advanced maternal age (≥ 35 years old)</td>
<td>16921</td>
<td>36.97</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gestational age at NIPT (weeks)</th>
<th>Number</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 12+0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>12–15</td>
<td>4853</td>
<td>10.60</td>
</tr>
<tr>
<td>16–19</td>
<td>33066</td>
<td>72.24</td>
</tr>
<tr>
<td>20–23</td>
<td>6740</td>
<td>14.72</td>
</tr>
<tr>
<td>24–26</td>
<td>1037</td>
<td>2.27</td>
</tr>
<tr>
<td>&gt; 26</td>
<td>77</td>
<td>0.17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Number</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced maternal age (age ≥ 35 years)</td>
<td>17421</td>
<td>38.06</td>
</tr>
<tr>
<td>High or critical risk of serological screening</td>
<td>19820</td>
<td>43.30</td>
</tr>
<tr>
<td>Fetal structural abnormalities or soft markers by B-ultrasound</td>
<td>503</td>
<td>1.10</td>
</tr>
<tr>
<td>Increased NT</td>
<td>86</td>
<td>0.19</td>
</tr>
<tr>
<td>Voluntary demand</td>
<td>7943</td>
<td>17.35</td>
</tr>
<tr>
<td>Othera</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 1
Maternal characteristics of pregnant women who underwent NIPT

\[ a \text{ Patients with interventional surgery contraindications: placenta previa, reoperative infection.} \]

The screening value of SCA detection by NIPT
The total cases of SCA abnormal results were 314, including 58 cases of true-positive, 85 cases of false-positive and 171 cases of unveried. 147 pregnancies received positive results for 45,X. Among them, 56 pregnancies underwent invasive prenatal testing (38.10%). 7 cases were found to be true-positive, with the PPV of 12.50%. The NIPT and karyotype analysis was fully concordant in 1 case with the increased NT of 7.0 mm, 6 cases were mosaic 45,X. Karyotype information was available for 29 out of 61 NIPT results for 47,XXX (47.54%). There were 16 cases of true-positive. The PPV of 47,XXX was 55.17%. For 35 out of 71 NIPT results of 47,XXY with prenatal diagnostic (49.30%), 25 cases were identified to be true-positive with the PPV of 71.43%. Of those 35 cases with positive NIPT screening results for sex chromosome abnormalities of 47,XYy, karyotype information was available in 13 cases (37.14%), 10 cases were confirmed as true-positive. The PPV of 47,XYy was 76.92% (see Table 2). Moreover, the proportion of sex chromosome trisomy and monosomy were highly different. Of those 167 cases with positive screening results for sex chromosome trisomy, 52 out of 80 NIPT results were confirmed to be true-positive detected by invasive prenatal testing (65.00%). 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 carried out to examine the associations between sex chromosome trisomy and monosomy X. Statistical significance was observed between the two groups ($\chi^2 = 16.166, P = 0.000$).

The PPV of SCA detected by NIPT was 40.56% (58/143). The PPV of SCA in pregnant women with advanced age group ($\geq 35$ years) was 48.44% (31/64), in high or critical risk of serological screening group was 27.5% (11/40), in voluntary demand for NIPT group was 45.16% (14/31) and in increased NT group was the high up to 44.44% (4/9). To our knowledge, the incidence of SCA with high or critical risk of serological screening in pregnant women aged < 30 year age group was 51.16% (22/43), while in pregnant women aged 30–34 year age group was 48.65% (18/37). There were no statistical significance between two groups ($P < 0.05$). The incidence of SCA with increased NT in pregnant women aged < 30 year age group and 30–34 year age group were 9.30% (5/43), 8.11% (4/37) and no statistical significance were found. ($P < 0.05$).

<table>
<thead>
<tr>
<th>SCA type</th>
<th>Positive NIPT cases</th>
<th>Amniocentesis cases</th>
<th>True-positive cases</th>
<th>False-positive cases</th>
<th>PPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45,X</td>
<td>147</td>
<td>56</td>
<td>7</td>
<td>49</td>
<td>12.50</td>
</tr>
<tr>
<td>47,XXX</td>
<td>61</td>
<td>29</td>
<td>16</td>
<td>13</td>
<td>55.17</td>
</tr>
<tr>
<td>47,XXY</td>
<td>71</td>
<td>35</td>
<td>25</td>
<td>10</td>
<td>71.43</td>
</tr>
<tr>
<td>47,XYy</td>
<td>35</td>
<td>13</td>
<td>10</td>
<td>3</td>
<td>76.92</td>
</tr>
</tbody>
</table>

**Table 2**

Overall performance of NIPT for SCA detection

**Relationship between SCA detection rate and maternal age**

The pregnant women were classified by age at due date as follows: <30, 30–34, 35–39, and $\geq 40$ years, and the incidence of SCA was evaluated in each age group. There was an increasing trend with maternal...
However, the difference was statistically significant for the detection rate of SCA in different aged groups ($\chi^2 = 17.541, P < 0.05$). The incidences of 45,X and 47,XYY did not differ in pregnant women among all age groups ($\chi^2 = 4.335, P > 0.05$ for 45,X and $\chi^2 = 1.076, P > 0.05$ for 47,XYY). Meanwhile, the incidences of 47,XXX and 47,XXY were different in pregnant women among all age groups ($\chi^2 = 11.370, P < 0.05$ for 47,XXX and $\chi^2 = 12.704, P < 0.05$ for 47,XXY). (see Table 3). The incidence of 45,X in pregnant women aged 30–34 years was lower, which was no significantly different from that of the < 30 year age group. The incidence of 47,XXX in women aged ≥ 40 years was significantly different from that of the < 30 year, 30–34 year, 35–39 year age groups ($P < 0.05$). The incidence of 47,XXY in pregnant women aged ≥ 40 years was 0.19% (5/2568), which was significantly different from that of the < 30 year age group ($P < 0.05$). However, there was no statistically significant difference in the incidence of 47,XYY among different age groups. The incidence of SCA in pregnant women aged ≥ 40 years was significantly different from that of the 35–39 years age group ($\chi^2 = 5.773, P = 0.016$). Among 62 advanced-age pregnant women with positive results for SCA detected by NIPT that underwent invasive prenatal diagnosis, 30 were identified as true-positive. The results showed that the positive coincidence rate between NIPT technique and amniotic fluid karyotyping analysis was 48.39%.

### Table 3
SCA screening in pregnant women of different age groups

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number</th>
<th>45,X&lt;sup&gt;a&lt;/sup&gt;</th>
<th>47,XXX&lt;sup&gt;b&lt;/sup&gt;</th>
<th>47,XXY&lt;sup&gt;c&lt;/sup&gt;</th>
<th>47,XYY&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td>17449</td>
<td>2 (0.01)</td>
<td>5 (0.03)</td>
<td>4 (0.03)</td>
<td>3 (0.02)</td>
</tr>
<tr>
<td>30–34</td>
<td>11403</td>
<td>0 (0.00)</td>
<td>3 (0.03)</td>
<td>7 (0.06)</td>
<td>3 (0.03)</td>
</tr>
<tr>
<td>35–39</td>
<td>14353</td>
<td>4 (0.03)</td>
<td>4 (0.03)</td>
<td>9 (0.06)</td>
<td>4 (0.03)</td>
</tr>
<tr>
<td>≥ 40</td>
<td>2568</td>
<td>1 (0.04)</td>
<td>4 (0.16)</td>
<td>5 (0.19)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Total</td>
<td>45773</td>
<td>7 (0.02)</td>
<td>16 (0.03)</td>
<td>25 (0.05)</td>
<td>10 (0.02)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The incidence of 45,X was not significantly associated with maternal age ($\chi^2 = 4.335, P > 0.05$)

<sup>b</sup> The incidence of 47,XXX was significantly associated with maternal age ($\chi^2 = 11.370, P < 0.05$)

<sup>c</sup> The incidence of 47,XXY was significantly associated with maternal age ($\chi^2 = 12.704, P < 0.05$)

<sup>d</sup> The incidence of 47,XYY was not significantly associated with maternal age ($\chi^2 = 1.076, P > 0.05$)

### Discussion

NIPT has been widely applied in clinical for screening T21, T18 and T13 in the last few years. But, it is still lacking large-scale studies on the efficiency of NIPT for SCA. The overall PPV of SCA detected by NIPT was 40.56%, which displayed a lower PPV compared with other studies. One possible reason was that the study included cases that were not confirmed but rather suspected because of the clinical
findings, which could have an impact on the calculated PPV\[^{1,18,19,20}\]. When classified into individual SCAs, the PPVs were 12.50% for 45,X, 55.17% for 47,XXX, 71.43% for 47,XXY, and 76.92% for 47,XYY, similar to previous studies\[^{17,18}\]. We have also evaluated the different PPV of NIPT with different pregnancies characteristics. Advanced maternal age (\(\geq 35\) years) is a high risk factor for SCA. Our study showed that NIPT performed better in predicting sex chromosome trisomies than monosomy X. This is potentially because the low guanosine-cytosine content of the X chromosome leads to highly variable amplification of the X chromosome, as well as the occurrence of age-related X chromosome loss in normal female white blood cells\[^{5}\]. The PPV of SCA is lower than other common chromosome aneuploidy. The reason is that sex chromosome abnormalities are less prevalent\[^{5}\]. With regard to the discordance between NIPT and invasive prenatal testing, the explanations are as follows. First, a study by Wang et al. reported that 8.6% positive results for SCA were due to maternal mosaicism\[^{21}\], with other studies supporting this opinion\[^{1,2,18}\]. Previous studies have demonstrated that identification of maternal karyotype will decrease the rate of false-positive SCA and can offer an explanation for the false-positive results for SCA\[^{2,4,22,23}\]. Another reason for the discordant results is confined placental mosaicism, which happens in approximately 1% of all pregnancies\[^{2}\]. 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 impacting the performance of NIPT in detecting fetal aneuploidies. In our study, there were 2 false-positive cases due to maternal mosaicism, with z-scores of 62.09 and 101.41 (normal range: \(-3 < z\)-score < 3). The results were confirmed by the maternal peripheral blood karyotyping.

The clinical symptoms of SCA include impaired fertility, characteristic physical features and development abnormal in intelligence and sexual features\[^{13}\]. Recent studies demonstrated that early interventions such as postnatal hormone therapy, physical therapy and occupational therapy can have positive influences on the behavioral phenotype or neurodevelopmental outcomes if applied earlier to SCA patients\[^{11}\]. Prenatal screening and diagnosis of SCA can provide the opportunity for early intervention, postnatal comprehensive management and improve the quality of life of the affected child\[^{12,26,27,28}\]. Sex chromosome abnormalities are more common than the major trisomies at birth and the neonates are often phenotypically normal\[^{30}\]. Conventional prenatal screening can not directly identified sex chromosome abnormalities and these can only be identified in postnatal karyotyping. And these would delay the best period of treatment of SCA patients. With the application of NIPT, SCA can be prenatally detected. Although it increases the number of invasive prenatal diagnosis, the benefit of detection for fetal SCA is outweigh the risk related to the invasive procedures. The application of NIPT are able to provide an alternate option for pregnant women to invasive prenatal testing for identification of fetal sex chromosome abnormalities. There are still some issues that require further consideration. Due to the low PPV of NIPT screening for SCA, this will increase unnecessary invasive prenatal diagnosis rate, especially for 45,X\[^{2}\]. Some pregnant women will terminate pregnancy when their chromosomal abnormalities were accidentally discovered by SCA screening\[^{31,32,33}\], and this involves ethical issues regarding the mild phenotype of SCA and the potential increase in the rate of gender selection\[^{2}\].
The differences in the incidence of SCA were statistically significant among the age groups and the incidence was significantly higher in the ≥ 40 years age group (P < 0.05). The lowest detection rate was 0.08% in women age < 30 years, which is considered as the ideal age for childbearing. Advanced-age pregnant women have an increased risk of bearing a fetus with an SCA. Therefore, genetic counseling combined with serum biochemical screening and ultrasound detection of abnormalities, should be fully carried out for advanced-age pregnant women. The incidences of 45,X and 47,XYY were significantly correlated with maternal age, whereas those of 47,XXX and 47,XXY did not show significant correlations\(^5\),\(^{35}\). Although there was no statistical significance between the incidence of 47,XYY and the maternal age, but it observed that the incidence 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 incidence of fetus 47,XXY, it still needs to expand the sample size to further research\(^16\). In summary, advanced-age pregnant women have a risk to have a fetal with sex chromosome trisomy, especially 47,XXX or 47,XXY\(^5\),\(^{16}\). There were some studies have showed that for 45,X syndrome, the maternal age coefficient is negative and imply a decreasing incidence in older mothers\(^16\), but our study did not find. Understanding the incidence rate of SCA have proved valuable in counseling couples who seek advice about the risk of fetal sex chromosome abnormalities with advanced maternal age and are considering the option of prenatal diagnosis and termination of pregnancy.

In this study, we analysis the results of NIPT and prenatal diagnosis results as well as the association of maternal age with fetal SCA. There are some limitations in our study. First, false-negative samples remain unavailable because of the absence of neonatal karyotype information, and most children do not show symptoms in the neonatal period. Therefore, we do not have access to compute sensitivity and specificity. Second, our study did not include such as gravidity and parity history, paternal age, which might be associated with fetal sex chromosome abnormalities as relevant studies reported.

**Conclusions**

We herein reported a large-scale retrospective study investigating the application of NIPT as a screening technique for SCA detection in singleton pregnancies and the association of maternal age with fetal SCA. NIPT performed better in predicting sex chromosome trisomies than monosomy X. As noted above, false-positive cases do exist and are detected by NIPT. Hence, it is necessary to highlight the importance of pre-test and post-test counseling before a choice is made to terminate the pregnancy. The results of this study showed that the incidences of 47,XXX and 47,XXY were correlated with maternal age, but correlations were not observed with 45,X and 47,XYY. To further investigate and validate the clinical application of NIPT in screening SCA, large long-term studies will be required.

**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 enrolled. The maternal age, serum biochemical screening results, and ultrasonic findings were recorded. The NIPT indications were as follows: pregnant women aged ≥35 years who refused to undergo invasive prenatal diagnosis; high or critical risk identified by serum biochemical screening; ultrasonography abnormalities; applicable or careful use of pregnant women who volunteered to undergo NIPT. Each pregnant women accepted comprehensive and detailed prenatal genetic counseling before NIPT. Collection of personal information, specimen preparation, and testing were performed with the consent of the participating pregnant women and informed written consent was obtained from all participants who received NIPT. The study was approved by the Ethics Committee of the Anhui Medical University. If a pregnant woman has received a positive NIPT result, invasive prenatal testing (such as amniocentesis) would be recommended by a clinical geneticist or obstetrician.

NIPT

With the informed consent of the pregnant women, 10 mL of maternal peripheral blood was collected in a Cell-Free DNA BCT™ 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 and the supernatant was then 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 CN500 with the Bambni Plus Test Data Analysis System for bioinformatic analysis of the sequencing data. The sequencing reads were filtered and aligned to the human reference genome (hg19)[5]. The z-score values were calculated for each chromosome and the results were interpreted with a cut-off value of ±3. Chromosome z-score values that were between -3.0 and 3.0 were classified as normal. Z-scores with an absolute value of greater than 3.0 were classified as chromosomal abnormalities.

Karyotype analysis of amniotic fluid

Pregnant women who received positive results for SCA detection by NIPT agreed to undergo invasive prenatal diagnosis, which is the gold standard for chromosome aneuploidy testing. Under ultrasound guidance, 20 mL of amniotic fluid was aseptically withdrawn from the pregnant women by amniocentesis. Inoculation, culture, G-banding, and karyotype scanning using a GSL-120 automatic karyotype scanner were performed. According to the principle outlined by “An International System for Human Cytogenetic Nomenclature, ISCN2013”, 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.

Data and statistical analysis
Statistical analysis was performed using SPSS version 23 (SPSS Inc., Chicago, IL, 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 as statistically significant.

**Abbreviations**


**Declarations**

**Acknowledgements**

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**Author’s contribution**

LU Xinran worked out the ideas of the article and drafted the manuscript. Wang Chaohong and SUN Yuxiu collected datas. TANG Junxiang analysed the data and interpretation. TONG Keting participated 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 analysed 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.
References


