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Association Between Fetal Fraction and Positive Predictive Value of Noninvasive Prenatal Testing for Sex Chromosome Aneuploidies: A Retrospective Study of 68,290 Cases

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Research Article

Keywords: noninvasive prenatal testing, cell-free DNA, fetal fraction, maternal age, sex chromosome aneuploidy, positive predictive value

Posted Date: September 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10758584/v1

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Additional Declarations: No competing interests reported.

Abstract

Background

Noninvasive prenatal testing (NIPT) for sex chromosome aneuploidies (SCAs) has consistently demonstrated lower positive predictive values than for common autosomal trisomies, and the extent to which fetal fraction and maternal characteristics are independently associated with true-positive sex chromosome aneuploidy results remains incompletely characterized.

Methods

This retrospective cohort study included 68,290 consecutive pregnancies undergoing NIPT between January 2020 and December 2024, from which 399 cases with high-risk NIPT results for fetal sex chromosome aneuploidies (SCAs) were identified. Of these, 276 cases accepted prenatal diagnosis via one or more platforms, while cases with inconclusive confirmatory results were independently adjudicated.

Results

Of 399 SCA screen-positive cases identified among 68,290 NIPT examinations, 276 (69.2%) underwent confirmatory invasive prenatal diagnosis. The overall PPV was 39.4%. PPV varied markedly by SCA subtype: 47, XYY 80.0% (8/10), 47, XXY 62.9% (22/35), 47, XXX 57.7% (15/26), and 45, X 18.9% (25/132). The mean FF was significantly higher in the SCA cohort compared with the non-SCA population and in true-positive cases compared with false-positive cases ($P = 0.001$). PPV exhibited a significant dose-response relationship with FF: 14.3% at FF 4–8%, 25.0% at FF 8–12%, and 42.3% at FF ≥ 12% ($P = 0.015$). Stratification by four maternal age groups showed no significant overall trend in PPV ($P = 0.876$), although subtype-specific analysis revealed age-dependent patterns for 45, X and 47, XXX.

Conclusions

Subtype-specific PPVs for NIPT-based SCA screening are reported. Fetal fraction showed an independent association with true-positive status, with a dose-response relationship across FF categories, whereas maternal age and twin pregnancies were not associated with PPV. These findings suggest a potential role for FF in post-test SCA risk stratification, though invasive confirmation remains essential.

INTRODUCTION

Sex chromosome abnormalities (SCAs) represent a clinically significant category of fetal chromosomal disorders, with a collective incidence of approximately 1 in 400 live births, exceeding that of any single autosomal trisomy[1, 2]. The most common SCAs include monosomy X (45, X; Turner syndrome), 47, XXY (Klinefelter syndrome), 47, XXX (Triple X syndrome), and 47, XYY (Jacobs syndrome). Unlike autosomal aneuploidies, the phenotypic spectrum of SCAs is highly variable, ranging from mild or even subclinical manifestations to significant congenital anomalies, neurodevelopmental deficits, and fertility impairment[3, 4]. This phenotypic heterogeneity, combined with a high prevalence of mosaicism, poses unique challenges for prenatal screening and counseling[5].

Since its clinical introduction in 2011, non-invasive prenatal testing (NIPT) based on cell-free fetal DNA (cffDNA) analysis in maternal plasma has revolutionized prenatal screening for common autosomal aneuploidies, with well-established high sensitivity and specificity for trisomies 21, 18, and 13[6, 7]. However, the clinical performance of NIPT for SCA detection has been consistently reported to be inferior to that for common autosomal trisomies[8, 9, 10]. This discrepancy is largely attributable to biological confounders including confined placental mosaicism (CPM), maternal constitutional or somatic sex chromosome mosaicism, maternal copy number variations (CNVs), vanishing twin syndrome, among others[11, 12, 13]. Among these, maternal age-related somatic loss of the X chromosome is a well-documented contributor to false-positive monosomy X results[14, 15].

However, several key clinical questions remain unresolved. First, whether fetal fraction within the reportable range influences the likelihood of a true-positive versus false-positive SCA result has received limited attention beyond its recognized role in test failure[16]. Second, the impact of maternal age on SCA subtype-specific PPV has not been systematically quantified, and existing data from predominantly high-risk populations may not generalize to unselected cohorts. Thus, we conducted a comprehensive retrospective analysis of cases at the Prenatal Diagnosis Center of Guangdong Women and Children Hospital between January 2020 and December 2024. The primary objectives were to: (1) determine the positive predictive value (PPV) of NIPT for each SCA subtype; (2) evaluate whether fetal fraction and maternal characteristics are independently associated with true-positive SCA results; and (3) based on these findings, inform clinical genetic counseling and subsequent management of pregnancies with high-risk NIPT results for fetal SCAs.

METHOD

Study design and population

This retrospective observational study enrolled 68,290 pregnant women who underwent non-invasive prenatal testing at the Department of Medical Genetics, Guangdong Women and Children Hospital between January 2020 and December 2024. Among them, 399 cases received high-risk results for fetal sex chromosome abnormalities.

Inclusion and exclusion criteria

Women were included in the primary analysis if they met the following criteria: (1) gestational age ≥ 12 weeks at the time of NIPT blood collection; (2) NIPT result indicating a high risk for one or more SCAs.

Exclusion criteria were as follows: (1) pregnancy complicated by maternal malignancy (known or suspected at the time of NIPT); (2) known maternal chromosomal abnormality prior to NIPT; (3) allogeneic blood transfusion, stem cell therapy, solid organ or bone marrow transplantation within one year prior to NIPT blood collection.

NIPT procedures

After obtaining written informed consent, maternal peripheral blood (10 mL) was collected in EDTA-K2 anticoagulant tubes. Plasma was separated using a two-step centrifugation protocol within 72 hours of collection. Cell-free fetal DNA (cffDNA) was extracted from plasma using a fetal chromosomal aneuploidy (T21, T18, and T13) detection kit according to the manufacturer's instructions, followed by sequencing library construction. Sequencing was performed on the BioelectronSeq 4000 high-throughput sequencing platform using

semiconductor sequencing technology with the fetal chromosomal aneuploidy (T21, T18, T13) detection kit. After quality control filtering, the sequencing reads were aligned to the human reference genome. Fetal fraction and Z-scores for target chromosomes were calculated through bioinformatics analysis. A fetal fraction ≥ 4% was adopted as the quality control threshold for reportable results.

SCA classification and case ascertainment

High-risk NIPT results for SCAs were stratified into the following categories based on the reported finding: (1) 45, X (monosomy X / Turner syndrome); (2) 47, XXX (Triple X syndrome); (3) 47, XXY (Klinefelter syndrome); (4) 47, XYY (Jacobs syndrome); (5) X-chromosome copy number variants; and (6) SCA atypical/other (including cases where the specific SCA could not be definitively classified from NIPT alone).

Confirmatory diagnostic testing

All pregnant women with NIPT results indicating a high risk for SCAs were managed according to a standardized clinical protocol. In accordance with established guidelines, termination of pregnancy based solely on a positive screening result was strictly prohibited. All women received formal post-test genetic counseling and were advised to undergo invasive prenatal diagnosis to obtain confirmatory fetal cytogenetic evidence prior to making any pregnancy management decisions.

Invasive prenatal diagnostic methods were selected based on clinical indications and technical availability and included the following modalities: (1) G-banding karyotype analysis; (2) chromosomal microarray analysis (CMA); (3) short tandem repeat (STR) genotyping; (4) copy number variation sequencing (CNV-seq); (5) fluorescence in situ hybridization (FISH).

Pregnancy outcome follow-up

Pregnancy outcomes were ascertained through a combination of medical record review, telephone follow-up, and linkage to the institutional delivery database. Outcomes included live birth (further classified by delivery mode, gestational age at delivery, neonatal sex, and birth weight), termination of pregnancy (TOP), spontaneous miscarriage, and intrauterine fetal demise. Neonatal developmental outcomes were assessed through pediatric follow-up records when available. All participants were followed up until three months postpartum. Cases for which no pregnancy outcome could be obtained after at least three telephone attempts were classified as lost to follow-up.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on the normality of the distribution. Categorical variables were expressed as frequencies and percentages. Between-group comparisons for continuous variables employed the independent samples t-test or Mann-Whitney U test. Categorical variables were compared using the chi-square test or Fisher exact test as appropriate. The PPV for each SCA subtype was calculated with 95% confidence intervals using the Wilson score method. All statistical analyses were performed using SPSS version 25.0. P value < 0.05 was considered statistically significant.

RESULTS

Study population and baseline characteristics

A total of 68,290 pregnant women with complete clinical data underwent NIPT at the Prenatal Diagnosis Center of Guangdong Women and Children Hospital. Of these, 399 (0.58%) received a high-risk result for fetal SCA and constituted the study cohort. Excluding pregnancies with high-risk NIPT results for any chromosomal abnormality, 66,944 women with low-risk NIPT results served as the non-SCA control group for comparison of baseline characteristics and fetal fraction. The study flow from NIPT screening through confirmatory testing to pregnancy outcomes is summarized in Fig. 1.

The mean maternal age of the SCA cohort was 31.1 ± 4.8 years (range: 18–46), with 96 women (24.1%) classified as advanced maternal age (≥ 35 years). The SCA and non-SCA groups did not differ significantly in maternal age (31.1 ± 4.8 vs. 31.1 ± 4.7 years, $P = 1.000$), the proportion of AMA (24.1% vs. 25.6%, $P = 0.477$), or BMI (22.3 ± 3.2 vs. 22.5 ± 3.4 kg/m², $P = 0.214$). However, the mean fetal fraction was significantly higher in the SCA group (17.21 ± 8.13% vs. 15.48 ± 5.32%, $P < 0.001$). Among the 399 SCA cases, the majority were singleton pregnancies (n = 379, 95.0%), with 17 twin pregnancies (4.3%) and 3 classified as unknown. The baseline characteristics of the SCA screen-positive and non-SCA populations were compared in Table 1.

Table 1
The baseline characteristics of the SCA screen-positive and comparison populations.

Characteristic	**SCA group (n = 399)**	**comparison group (n = 66944)**	***P*-value**
Maternal age (years), mean ± SD	31.1 ± 4.8	31.1 ± 4.7	1.000
Maternal age ≧ 35 years, n (%)	96 (24.1%)	17150 (25.6%)	0.477
Fetal fraction (%), mean ± SD	17.21 ± 8.13	15.48 ± 5.32	< 0.001
Fetal fraction (%), median (IQR)	15.84 (12.87–19.36)	15.07 (11.61%–18.82%)	—
BMI (kg/m²), mean ± SD	22.3 ± 3.2	22.5 ± 3.4	0.214

Note: Data are expressed as n (%), mean ± SD (standard deviation) or IQR (interquartile range, 25th–75th percentile) where appropriate. *P*-value < 0.05 indicates a significant difference.

Overall performance of NIPT for SCA

Of the 399 screen-positive cases, 276 (69.2%) underwent prenatal diagnosis and were included in the PPV analysis; 123 (30.8%) had no record of invasive diagnostic testing, comprising 111 pregnant women who refused further prenatal diagnosis and 12 cases completely lost to follow-up (Fig. 1). The overall PPV for SCAs was 38.4% (106/276; 95% CI 32.6%-44.4%). 47, XYY exhibited the highest PPV at 80.0% (8/10, 95% CI 44.4%-97.5%), followed by 47, XXY at 62.9% (22/35, 44.9%-78.5%), 47, XXX at 57.7% (15/26, 36.9%-76.6%), and 45, X at 18.9% (25/132, 12.6%-26.7%). Of the 24 NIPT-positive cases with discordant confirmatory diagnoses, 17 involved a 45, X NIPT result were confirmed as a different SCA subtype. The PPV of NIPT for each SCA subtype is presented in Table 2.

Table 2
Positive predictive value of NIPT for each SCA subtype after confirmatory invasive prenatal diagnosis (n = 276).

SCA Subtype	NIPT+ (n)	Confirmed (n)	TP (n)	FP (n)	NIPT+/Dx-discordant (n)	PPV (95% CI)
45, X	209	132	25	90	17	18.9% (12.6%-26.7%)
47,XXX	34	26	15	8	3	57.7% (36.9%-76.6%)
47,XXY	48	35	22	9	4	62.9% (44.9%-78.5%)
47,XYY	14	10	8	2	0	80.0% (44.4%-97.5%)
SCA-Unspec.	72	59	28	31	0	47.5% (34.3%-60.9%)
X-CNVs	22	14	8	6	0	57.1% (28.9%-82.3%)
Total	399	276	106	146	24	38.4% (32.6%-44.4%)

Note: TP, true positive (confirmatory result matches NIPT-predicted subtype). FP, false positive with normal confirmatory karyotype. NIPT+, NIPT-positive. NIPT+/Dx-discordant, NIPT-positive with a discordant confirmatory diagnosis (confirmatory result differs from the NIPT-reported SCA subtype, including subtype mismatch and normal karyotype). SCA-Unspec., NIPT reported "SCA" without specifying subtype. X-CNVs, X-chromosome structural variants (deletions/duplications).

Impact of fetal fraction on PPV

Among 276 confirmed SCA cases (106 true-positive, 170 false-positive), the overall PPV was 38.4% (95% CI: 32.9%-44.3%). A key finding of this study was the significant association between fetal fraction (FF) and the likelihood of a true-positive result. The mean FF among true-positive cases was 20.04 ± 10.57%, compared with 16.19 ± 6.99% among false-positive cases, yielding a mean difference of 3.85 percentage points (Welch's t = 3.325, df = 163, *P* = 0.001). Table 3 shows the positive predictive value (PPV) for each SCA subtype stratified by FF categories, along with the mean FF in each stratum. Mean FF rose progressively across categories from 3.28 ± 0.36% (< 4%) to 6.71 ± 1.18% (4–8%), 10.41 ± 1.08% (8–12%), and 19.81 ± 8.46% (≥ 12%). A significant dose-response relationship was observed: PPV increased from 50.0% (1/2) at FF < 4% to 14.3% (2/14) at FF 4–8%, 25.0% (10/40) at FF 8–12%, and 42.3% (93/220) at FF ≥ 12% (Cochran-Armitage trend test: $\chi^2 = 5.86$, P = 0.015). Notably, the < 4% category contained only 2 cases, and its PPV estimate of 50.0% (95% CI: 9.5%-90.5%) should be interpreted with caution owing to the extremely wide confidence interval. The FF cutoff threshold of ≥ 12% conferred statistically significant discriminatory performance ($\chi^2 = 6.86$, P = 0.009, OR = 2.42, 95% CI: 1.23–4.76).

Table 3
Positive predictive value for SCA stratified by fetal fraction category among 276 women who underwent confirmatory invasive prenatal diagnosis.

FF Category	Confirmed (n)	TP (n)	FP (n)	PPV (95% CI)	FF Mean ± SD (%)
< 4%	2	1	1	50.0% (9.5%–90.5%)	3.28 ± 0.36
4%–8%	14	2	12	14.3% (4.0%–39.9%)	6.71 ± 1.18
8%-12%	40	10	30	25.0% (14.2%–40.2%)	10.41 ± 1.08
≥ 12%	220	93	127	42.3% (35.9%–48.9%)	19.81 ± 8.46
Total	276	106	170	38.4% (32.9%-44.3%)	17.67 ± 8.73
TP cases (all)	—	106	—	—	20.04 ± 10.57
FP cases (all)	—	—	170	—	16.19 ± 6.99

Note: FF categories: <4%; 4%–8% (inclusive of boundaries); 8%-12% (open interval, 8% < FF < 12%); ≥12%. FF, fetal fraction. PPV, positive predictive value; CI, confidence interval; TP, true-positive; FP, false-positive; SD, standard deviation. Cochran-Armitage trend test across ordered FF categories: $\chi^2 = 5.86$, $P = 0.015$. Comparison of PPV at FF ≥ 12% vs. FF < 12%: $\chi^2 = 6.86$, $P = 0.009$, OR = 2.42, 95% CI: 1.23–4.76.

Impact of maternal age on PPV

Table 4 presents the PPV values for confirmed cases stratified by maternal age group and SCA subtype. No significant overall trend in PPV was observed across age strata for all SCAs combined ($\chi^2 = 0.69$, df = 3, $P = 0.876$). The overall PPV was 38.3% (41/107) in women aged < 30 years, 36.9% (38/103) at 30–34 years, 39.3% (22/56) at 35–39 years, and 50.0% (5/10) at ≥ 40 years.

Subtype-specific analysis using the Fisher-Freeman-Halton exact test revealed heterogeneous age effects. For 45, X, PPV showed a non-significant declining trend with advancing age: 25.4% (< 30 years), 17.0% (30–34 years), 4.5% (35–39 years), and 25.0% (≥ 40 years, $P = 0.178$), likely reflecting the increasing contribution of age-related maternal somatic X chromosome loss to false-positive results. For 47, XXX, PPV increased significantly with age: 25.0% (< 30 years), 61.5% (30–34 years), 100.0% (35–39 years), and 100.0% (≥ 40 years, $P = 0.048$), consistent with the known association between meiotic nondisjunction and advanced maternal age. For 47, XXY, PPV was broadly comparable across age groups: 63.6% (< 30 years), 55.6% (30–34 years), 66.7% (35–39 years), and 66.7% (≥ 40 years, $P = 0.954$). For 47, XYY, no clear age-related pattern emerged ($P = 0.268$), although the small number of cases limited statistical power. Similarly, for SCA of unspecified subtype (SCA-Unspec.), PPV remained stable across age groups: 47.8% (< 30 years), 47.6% (30–34 years), 46.2% (35–39 years), and 50.0% (≥ 40 years, $P = 1.000$). For X chromosome copy number variants (X-CNVs), PPV varied across age groups ($P = 0.125$), but the extremely limited sample size (n = 14) precluded meaningful interpretation.

Table 4

Positive predictive value for each SCA subtype stratified by maternal age among 276 women who underwent confirmatory invasive prenatal diagnosis.

SCA Subtype	< 30y			30-34y			35-39y			≧ 40y			*P*-value
	N	TP	PPV (%)	N	TP	PPV (%)	N	TP	PPV (%)	N	TP	PPV (%)	
45,X	59	15	25.4%	47	8	17.0%	22	1	4.5%	4	1	25.5%	0.178
47,XXX	8	2	25.0%	13	8	61.5%	4	4	100.0%	1	1	100.0%	0.048
47,XXY	11	7	63.6%	9	5	55.6%	12	8	66.7%	3	2	66.7%	0.954
47,XYY	3	3	100.0%	3	3	66.7%	4	2	50%	0	0	—	0.268
SCA-Unspec.	23	11	47.8%	21	10	47.6%	13	6	46.2%	2	1	50%	1.000
X-CNVs	3	3	100.0%	10	4	40.0%	1	1	100.0%	0	0	—	0.125
All SCA	107	41	38.3%	103	38	36.9%	56	22	39.3%	10	5	50.0%	0.876

Note: *P*-values were calculated using the Fisher-Freeman-Halton exact test (Monte Carlo simulation, 100,000 replicates) for all SCA subtypes due to small, expected cell frequencies; the chi-square test was used for "All SCA" (*P* = 0.876).

Twin pregnancy analysis

Among the 399 SCA-positive cases, 17 (4.3%) were twin pregnancies. Of the twin cases receiving confirmatory testing (n = 8), only 1 was a true positive (47, XXY), yielding a PPV of 12.5% (1/8). This value was lower than the PPV of 39.2% (105/268) in singleton pregnancies, though the difference was not statistically significant (Fisher's exact test, *P* = 0.159). The mean FF in twin pregnancies (14.69 ± 7.31%) was lower than that in singleton pregnancies (17.35 ± 8.19%), but this difference was also not statistically significant (Welch's t = -1.458, df = 17.9, *P* = 0.163), probably due to the limited sample size of twin cases.

Pregnancy outcomes

Pregnancy outcome data were available for 379 of 399 cases (95.0%): 311 (77.9%) resulted in delivery, 66 (16.5%) in termination of pregnancy (TOP), and 2 (0.5%) in stillbirth; 22 cases (5.5%) were lost to follow-up for pregnancy outcomes. Among the 106 confirmed true-positive SCA cases, pregnancy outcome data were complete for 103 cases: 54 (50.9%) resulted in delivery, 49 (46.2%) resulted in TOP, and 3 (2.8%) were lost to follow-up. The TOP rate was highest for 47, XXY (18/22, 81.8%) and 45, X (15/25, 60.0%), while lower termination rates were showed for 47, XXX (2/15, 13.3%) and 47, XYY (1/8, 12.5%). Among the 170 false-positive cases (FP and FP-Disc combined), 154 (90.6%) proceeded to delivery, 10 (5.9%) underwent TOP due to other clinical indications including ultrasound abnormalities or maternal request, 5 (2.9%) were lost to follow-up, and 1 (0.6%) resulted in stillbirth. Among false-positive cases with available developmental follow-up records, 153 of 154 neonates (99.4%) exhibited normal development; one infant had documented developmental abnormalities, the specific nature of which was not recorded.

DISCUSSION

Although NIPT has become a cornerstone of prenatal screening, its accuracy for sex chromosome abnormalities lags behind that for common autosomal trisomies, primarily due to the low positive predictive values of SCA screening—a limitation caused by biological confounders including confined placental mosaicism, maternal X-chromosome mosaicism (constitutional and age-related), maternal copy number variation, and vanishing twin syndrome[10–15]. The false-positive rates are disproportionately high, complicating post-test counseling and clinical decision-making. The mild and variable phenotypes of most SCAs further raise ethical considerations regarding prenatal counseling and informed consent[18, 19]. In this context, identifying readily available clinical parameters such as fetal fraction to refine the post-test probability of a true-positive result carries direct translational value. This study was therefore designed to quantify the clinical PPV of NIPT for each SCA subtype in an unselected population and to evaluate the independent associations of fetal fraction and maternal characteristics with true-positive SCA results.

Substantial heterogeneity in PPV across SCA subtypes

The substantial inter-subtype PPV heterogeneity observed in the cohort aligns with published literature. The PPVs for 47, XXY (62.9%) and 47, XYY (80.0%) are broadly consistent with the pooled estimates of 67.6% and 70.9% reported by Bussolaro et al. (2023)[8], and the 47, XXX PPV (57.7%) is comparable to the pooled 57.5%[8]. However, the 45, X PPV (18.9%) is substantially lower than the pooled 32.0%[8] and higher than the 14.5% reported by Shear et al. (2023)[9], likely reflecting the well-characterized biological confounders driving false-positive monosomy X results.

This discrepancy can be explained by several converging factors. First, a substantial proportion of screen-positive monosomy X results originate from maternal rather than fetal chromosomes: age-dependent attrition of the X chromosome in maternal leukocytes generates a 45, X cell line that is detected by NIPT but does not reflect the fetal karyotype [14, 15, 20]. In the present cohort, only 21 of 107 cases with monosomy X on NIPT and confirmatory testing proved to be true fetal Turner syndrome, underscoring the dominant contribution of maternal X loss to the observed false-positive rate. Second, because the circulating cell-free DNA analyzed by NIPT is shed predominantly from the trophoblast layer, chromosomal abnormalities restricted to the placenta will generate a positive screening signal even when the fetus itself is euploid [21, 22]. Third, by favoring amniocentesis over chorionic villus sampling for diagnostic confirmation, the institutional protocol minimizes the risk of erroneously classifying CPM as a true fetal aneuploidy, given that amniocytes directly reflect the fetal karyotype as opposed to the placental karyotype.

Evidence from multiple systematic reviews has consistently identified monosomy X as the cfDNA-screened condition with the lowest positive predictive value. This finding has been attributed to the disproportionate influence of placental mosaicism and maternal somatic X-chromosome loss compared with other aneuploidies[23, 24, 25].

Significant association between fetal fraction and PPV for SCAs

A statistically significant difference in mean FF was observed between true-positive (20.04%) and false-positive (16.19%) cases ($P < 0.001$). A mechanistic rationale supports this observation. When the SCA is of true fetal origin, the supernumerary or missing sex chromosome alters the total cfDNA contribution from the fetoplacental unit, thereby influencing the measured fetal fraction. Conversely, when the abnormal NIPT result is attributable to maternal X-chromosome mosaicism, which constitutes the predominant cause of false-positive monosomy X

findings, the aberrant DNA signal originates from the maternal hematopoietic compartment and exerts minimal influence on the fetal fraction[25]. This hypothesis is supported by Zhang et al. (2020), who proposed the cffDNA (ChrX)/cffDNA ratio to distinguish fetal from maternal origin of X chromosome abnormalities[13], and by emerging algorithms that integrate FF directly into aneuploidy risk estimation[26]. The FF difference was most pronounced for 47, XXY and 47, YY, whereas monosomy X and 47, XXX exhibited negligible or inverse differences. This pattern is consistent with the smaller net change in chromosomal material associated with single-X abnormalities compared with supernumerary trisomies involving the Y chromosome.

FF-stratified analysis indicates that fetal fraction may provide clinically relevant information for post-test counseling. A high-risk NIPT result with FF ≥ 12% is associated with a substantially greater likelihood of a true positive outcome (PPV 42.3%) compared with low FF (P = 0.009). Nevertheless, even at the highest FF levels, the overall PPV for SCAs remains well below 50%, and confirmatory invasive testing remains essential. Current guidelines recommend that FF be reported and incorporated into NIPT interpretation, reinforcing its utility in SCA counseling[27]. This recommendation is reinforced by evidence that low FF is independently associated with an increased risk of fetal chromosomal aberrations, highlighting the value of FF as both a quality metric and a risk stratification factor[28].

Maternal age does not significantly modify overall SCA PPV

The absence of a significant overall association between maternal age and PPV (P = 0.876) reflects the opposing influences of maternal age on different SCA subtypes. The 47, XXX and 47, XXY aneuploidies both originate from errors in meiotic chromosome segregation, a process whose fidelity declines with advancing reproductive age[29, 30]. Consistent with this mechanism, these subtypes demonstrated higher PPVs among women ≥ 35 years. In contrast, the PPV for 45, X, which is predominantly of postzygotic origin, decreased with maternal age (23.8% in < 35 years vs. 8.0% in ≥ 35 years), a pattern attributable to the competing influence of age-related maternal somatic X-chromosome loss producing false-positive results[14]. These divergent age-PPV relationships offset one another when all SCAs are aggregated, highlighting the necessity of subtype-specific analysis. This observation is consistent with large-scale clinical data indicating that maternal age does not reliably distinguish true-positive from false-positive SCA results[24]. This pattern stands in marked contrast to the well-characterized age-PPV correlation for common autosomal trisomies, which is driven by the maternal age-dependence of meiotic nondisjunction and carries important implications for subtype-specific post-test counseling.

NIPT for SCA in twin pregnancies

In the cohort, 17 (4.3%) were twin pregnancies. Only one of eight twin pregnancies with confirmatory testing was a true positive (PPV of 12.5%), nominally lower than the singleton PPV of 39.2% (P = 0.159). The lower mean FF in twins (14.69 ± 7.31% vs. 17.35 ± 8.19%, P = 0.163) suggests that reduced per-fetus FF in dichorionic twins may impair aneuploidy detection[17]. NIPT performance for SCA in twins remains poorly characterized, with data indicating lower PPV versus trisomy 21[31, 32], and heterokaryotypic monochorionic twins or CPM restricted to a single placental mass further complicate interpretation[21]. These findings underscore the need for cautious SCA result interpretation in twin pregnancies and confirmatory invasive testing informed by chorionicity.

Clinical implications

These findings have several clinically actionable implications. First, the low PPV for monosomy X necessitates rigorous pretest counseling that explicitly conveys the high false-positive rate and the underlying biological basis

of this phenomenon, which principally involves confined placental mosaicism and maternal somatic X-chromosome loss[21, 33]. When confirmatory testing is pursued following a high-risk monosomy X of NIPT result, amniocentesis should be prioritized over chorionic villus sampling, given that amniocytes directly reflect the fetal karyotype and thereby reduce the risk of diagnostic misclassification attributable to placental mosaicism. Second, these findings support the incorporation of fetal fraction as a quantitative parameter within post-test counseling frameworks, as FF showed an independent, dose-dependent association with SCA PPV. Future studies with larger, multicenter cohorts are warranted to establish and externally validate an FF-integrated PPV prediction model for clinical application. Third, when the fetal karyotype is discordant with NIPT results, maternal karyotyping should be offered. Maternal sex chromosome mosaicism is a well-recognized incidental finding with potential implications for maternal health, including occult premalignant states and premature ovarian insufficiency[34, 35].

CONCLUSIONS

In this analysis of 399 screen-positive cases from 68,290 consecutive NIPT examinations, subtype-stratified PPV estimates were generated to inform individualized post-test counseling for SCA. Fetal fraction was independently associated with true-positive status, whereas maternal age and twin pregnancies did not significantly modify PPV. However, a subset of screen-positive cases lacked invasive confirmation, and placental cytogenetic analysis was not routinely performed, precluding precise quantification of CPM-attributable discordance. Prospective multicenter studies with larger cohorts are needed to establish an FF-based PPV prediction model and validate it externally.

Strengths and Limitations

Strengths of this study include: (1) a relatively large sample of 399 SCA screen-positive cases derived from a base population of 68,290 NIPT examinations; (2) the majority of screen-positive women underwent invasive prenatal diagnosis with confirmation performed across multiple complementary platforms, enabling cross-platform validation and reliable discrimination of true-positive from false-positive results; (3) comprehensive clinical data including systematically recorded fetal fraction, maternal age, BMI, and pregnancy outcomes with 95.0% follow-up completeness.

Several limitations warrant consideration: (1) a minority of screen-positive women declined invasive diagnosis, introducing potential verification bias in PPV estimation. (2) the relatively modest number of women of advanced maternal age (≥ 35 years) limited the statistical power of age-stratified analyses, particularly for older age strata, and may have introduced bias in age-related PPV estimates. (3) further expansion of the sample size—particularly for rarer SCA subtypes and twin pregnancies—is warranted to refine PPV estimates and enable more granular subgroup analyses.

Abbreviations

AMA
advanced maternal age
BMI
body mass index
cffDNA

cell-free fetal DNA
cfDNA
cell-free DNA
CI
confidence interval
CMA
chromosomal microarray analysis
CNV
copy number variation
CNV-seq
copy number variation sequencing
CPM
confined placental mosaicism
EDTA
ethylenediaminetetraacetic acid
FF
fetal fraction
FISH
fluorescence in situ hybridization
FP
false positive
IQR
interquartile range
NIPS
noninvasive prenatal screening
NIPT
noninvasive prenatal testing
OR
odds ratio
PPV
positive predictive value
SCA
sex chromosome aneuploidy
SD
standard deviation
STR
short tandem repeat
TOP
termination of pregnancy
TP
true positive
X-CNVs
X-chromosome copy number variants.

Declarations

Ethics approval and consent to participate:

This retrospective study was approved by the Institutional Review Board of Guangdong Women and Children Hospital (Approval No. 202301327). All procedures followed the Declaration of Helsinki. The requirement for informed consent was waived due to retrospective design. Patient data were anonymized prior to analysis.

Consent for publication:

Not applicable.

Competing interests:

The authors have no competing interests to declare.

Funding:

This work was supported in part by Guangdong Basic and Applied Basic Research Foundation (2019A1515111000), Medical Scientific Research Foundation of Guangdong Province, China (A2020062) and Maternal and Child Health Care Research Team Innovation and Excellence Program of Guangdong Province (YNKY1413).

Author Contribution

Z.Z. collected the clinical data, performed the data analysis and drafted the manuscript; J.Y. and X.S. contributed to data curation, analysis and follow-up; H.P., F.G., D.W. and T.H. performed the molecular genetic analyses; Y.H. conceived the study and revised the manuscript. All authors reviewed and approved the final manuscript.

Acknowledgement

We thank all the authors for their contributions and support. We are grateful to all the pregnant women in the study who provided blood samples. We would also like to thank the hospital staff who contributed to the data collection for this study.

Availability of data and materials:

The datasets used and/or analyzed during the current study are not publicly available due to privacy and ethical restrictions but are available from the corresponding author on reasonable request and with appropriate approval.

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Figures

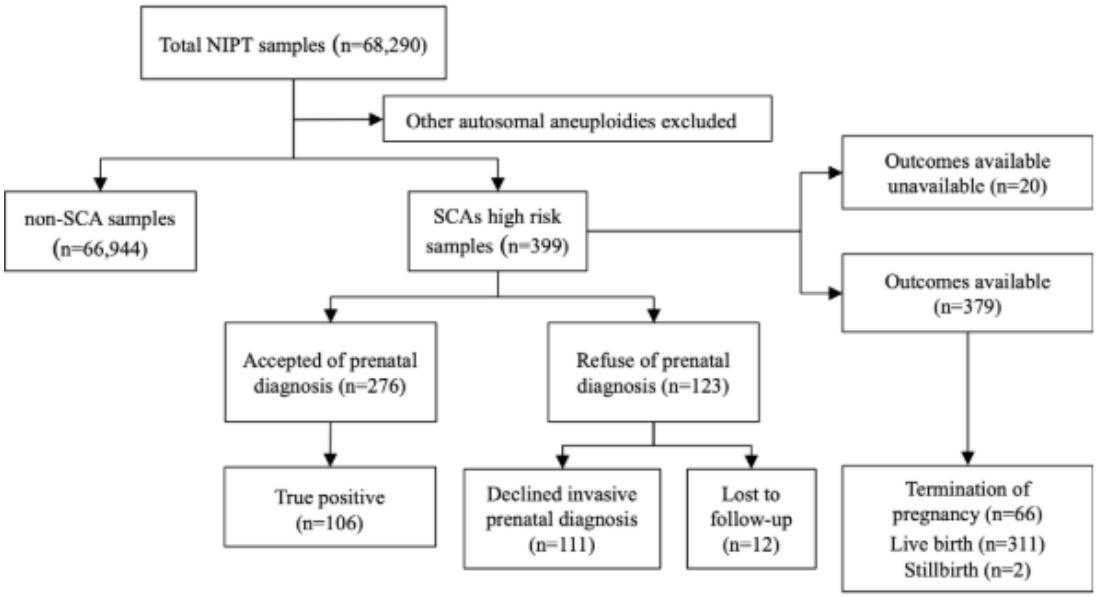


Figure 1

Study flow diagram illustrating NIPT results and pregnancy outcomes.