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Original Research

Second Trimester Screening Markers of Fetal Chromosomal Abnormalities Other than Common Trisomies: A Case-Control Study

Zhiling Wu[1,*], Min Ou[1], Xueyan Wang[1]

[1]Department of Medical Genetics and Prenatal Diagnosis, Sichuan Provincial Maternity and Child Health Care Hospital, 610045 Chengdu, Sichuan, China

*Correspondence: wudoudou0506@sina.com (Zhiling Wu)

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Abstract

Background: To enhance the efficacy of maternal serum screening (MSS), we conducted an analysis to examine the correlation between certain factors identified during second-trimester screening (STS) and fetal chromosomal abnormalities, excluding the common trisomies (trisomies 13, 18, and 21). Additionally, specific risk factor ranges were established for each category. **Methods**: A retrospective 1:3 matched case-control study was conducted. Case data were obtained from 311 STS samples of fetal chromosomal abnormalities other than common trisomies, with testing performed in the Prenatal Diagnosis Center of the Maternal and Child Health Care Hospital of Sichuan Province in China between 6 January 2013 and 12 April 2023. A total of 933 controls were matched accordingly. Univariate and multivariable conditional logistic regression analyses were implemented and sensitivity analysis was performed. **Results**: Multivariable logistic analyses revealed that the independent risk factors for fetal chromosomal abnormalities other than common trisomies were ultrasonographic structural abnormalities (odds ratio (OR) = 3.038; 95% confidence interval (CI), 1.774–5.202; $p < 0.001$); free β-human chorionic gonadotropin (free β-hCG) as multiples of the median (MoMs) of $\leq$0.34 (OR = 3.006; 95% CI, 1.803–5.013; $p < 0.001$), 2.82–3.53 (OR = 1.884; 95% CI, 1.321–2.688; $p < 0.001$), 3.54–4.67 (OR = 1.949; 95% CI, 1.300–2.923, $p = 0.001$), and $\geq$4.68 (OR = 1.730; 95% CI, 1.045–2.866; $p = 0.033$); and a trisomy 21 (T21) risk of 1/271–1/1000 (OR = 2.434; 95% CI, 1.706–3.472; $p < 0.001$), 1/101–1/270 (OR = 3.330; 95% CI, 2.300–4.821; $p < 0.001$), and $\geq$1/100 (OR = 3.441; 95% CI, 2.178–5.438; $p < 0.001$). **Conclusions**: Ultrasonographic structural abnormalities, free β-hCG MoMs, and T21 risk were identified as independent risk factors for fetal chromosomal abnormalities (with the exception of common trisomies) in STS. Our findings thus provide data to support clinical decision-making.

Keywords: second-trimester screening; fetal chromosomal abnormalities; soft markers; structural abnormalities; trisomy 21; free β-hCG

1. Introduction

Maternal serum screening (MSS) has been the most commonly applied technique for the screening of fetal aneuploidies for the last three decades [1], but it is increasingly being replaced by non-invasive prenatal testing (NIPT) [2]. Despite NIPT possessing a high detection rate and a low false-positive rate, many pregnant women in China are still likely to opt for MSS, as NIPT is not currently covered by health insurance in most areas [3]. However, MSS has the advantages of ease of operation and having a low cost, making it relatively easy to conduct in community hospitals. In addition, MSS includes both combined first-trimester screening (cFTS) and second-trimester screening (STS). STS in particular is more accessible than cFTS, as the latter requires a high-quality and time-consuming fetal nuchal thickness (NT) assessment [4]. As a result, STS remains the most widely adopted screening method at this time in many underdeveloped regions of China.

The main principle of STS is the assessment of the risk of trisomy 21 (T21), trisomy 18 (T18), and open neural tube defects (ONTDs) by combining the concentration of biomarkers with maternal age, gestational age, and weight, among other factors [5]. These biomarkers typically include alpha fetoprotein (AFP), free β-human chorionic gonadotropin (free β-hCG), and either unconjugated estriol (uE3) or inhibin-A. Generally, STS can be divided into three main strategies: double screening (AFP, free β-hCG), triple screening (AFP, free β-hCG, and uE3), and quadruple screening (AFP, free β-hCG, uE3, and inhibin-A). According to previous studies, the detection rate for T21 ranges from 60% to 80% with a 5% false-positive rate using STS [6,7], and the detection rate for T18 is approximately 60% [8]. Some authors have also emphasized the importance of this traditional technique for screening rare fetal chromosomal abnormalities other than the common trisomies (T21, T18, and T13) [9,10]. Wijngaard *et al.* [11] also reported that fetuses with low maternal free β-hCG levels were at an increased risk of pathogenic copy number variants (CNVs). Therefore, some clinicians recommend further examination for pregnant women with other abnormal STS indicators, despite reflecting low-risk T21 and T18 values. It has been demonstrated that indicators of cFTS are associated with chromosomal aberrations. In a recent study by Gadsbøll *et al.* [12], it was found that among pregnant women with

cFTS as low risk, there was a greater risk of a chromosomal aberration if the distribution of NT and biochemical indicators was extremely heterogeneous. In addition, another study by Gadsbøll *et al*. [13] found that maternal serum β-hCG MoM was decreased in both 22q11.2 aberrant fetuses relative to normal chromosome fetuses. However, few studies have addressed which abnormal indicators in STS specifically indicate fetal chromosomal abnormalities other than common trisomies. Thus, clinicians have not been able to provide reference data for pregnant women.

The aim of this study was to explore the association between the serum profiles of STS and fetal chromosomal abnormalities other than common trisomies through the STS data of our center over the past decade. We also compartmentalized the meaningful indicators into different ranges. Univariate and multivariable logistic regression analyses were then conducted to determine the correlation between each range and fetal chromosomal abnormalities. We expect that our findings will portray a significant role in the interpretation of STS results and thus inspire clinicians to provide reasonable counseling.

2. Materials and Methods

In the present investigation, we implemented a matched case-control study design, with all samples sourced from the Prenatal Diagnosis Center of the Sichuan Provincial Maternity and Child Health Care Hospital (SPMCHCH), China. Our analysis was focused on second-trimester biochemical screening samples collected at SPMCHCH over the past decade. The inclusion criteria for this study encompassed pregnant women with gestational weeks falling between 15^{+0} and 20^{+6} weeks, as well as samples containing complete risk-assessment results. Conversely, the exclusion criteria comprised twin pregnancies, individuals who smoked, patients diagnosed with type 1 diabetes, and those undergoing *in vitro* fertilization (IVF) procedures. For the case group, the inclusion criteria were the presence of fetal chromosomal abnormalities other than common trisomies. For the control group, the inclusion criteria were the presence of normal chromosomes. The case and control groups were matched at a ratio of 1:3, based on the same screening strategy, and with test dates differing by no more than 3 days. Furthermore, the matching process considered the presence or absence of ultrasound findings. If ultrasound findings were present, cases were matched to controls with ultrasound findings. If ultrasound findings were absent, cases were matched to controls without ultrasound findings.

2.1 Pregnancy Information and Sample Collection

Clinicians gathered information on pregnant women before collecting blood samples, including details such as date of birth, ultrasonographic information, number of fetuses, maternal weight, last menstrual period, and pregnancy history. Blood samples were then taken from pregnant women at 15^{+0} to 20^{+6} weeks of gestational age. Venous blood was collected in vacuum-dried tubes (2–3 mL) and left at room temperature for 30–120 minutes. The serum was then separated by centrifugation at 3000 rpm for 10 minutes.

2.2 Sample Testing and Quality Control

Two screening strategies were offered for pregnant women at our center: a dual test (AFP and free β-hCG) and a triple test (AFP, free β-hCG, and uE3). All biochemical markers were detected by a 1235 automatic fluorescence immunoassay analyzer (PerkinElmer, Inc., Waltham, MA, USA), and the reagents used in the assays were sourced from time-resolved immunofluorescence kits (Guangzhou Fenghua Bioengineering Co., Ltd., Guangzhou, China). Indoor quality control was conducted for each test batch in order to ensure the accuracy of the results.

2.3 Risk Assessment

First, the concentrations of biochemical markers were transformed into multiples of the median (MoMs) based on the median values for the same gestational age. Second, the MoM values were corrected for maternal weight, mode of pregnancy, some comorbidities, and smoking status. Third, the likelihood ratio of every biochemical marker was calculated based on the heights of Gaussian distributions in abnormal and normal pregnancies. Finally, the fetal chromosomal risks for T21 and T18 were determined using all of the likelihood ratios combined with the maternal age risk. The entire process of risk calculation was performed using PRsoft V2.0 software (Guangzhou Fenghua Bioengineering Co., Ltd., Guangzhou, China); the results of the risk assessment were categorized as follows: high-risk (T21 $\geq$1/270, T18 $\geq$1/350), intermediate-risk (T21, 1/271–1/1000; T18, 1/351–1/1000), and low-risk (T21 $<$1/1000, T18 $<$1/1000).

2.4 Prenatal Diagnosis

The methods for prenatal diagnostic testing included chromosome karyotype analysis, fluorescence *in situ* hybridization (FISH), quantitative fluorescence PCR (QF-PCR), chromosomal microarray analysis (CMA), and CNV-seq detection. Pregnant women showing high-risk STS were typically advised to undergo prenatal diagnostic testing through amniocentesis. Advanced maternal age was also a factor that prompted recommendations for prenatal diagnostic testing. However, some of those who were hesitant to undergo invasive procedures chose non-invasive prenatal testing (NIPT). The recommendation for prenatal diagnosis would still be made if NIPT indicates the presence of chromosomal abnormalities. Pregnant women at intermediate risk were generally recommended to undergo NIPT. Furthermore, pregnant women with elevated AFP MoMs ($\geq$2.5) were advised to undergo prenatal ultrasonographic diagnosis for neural tube defects.

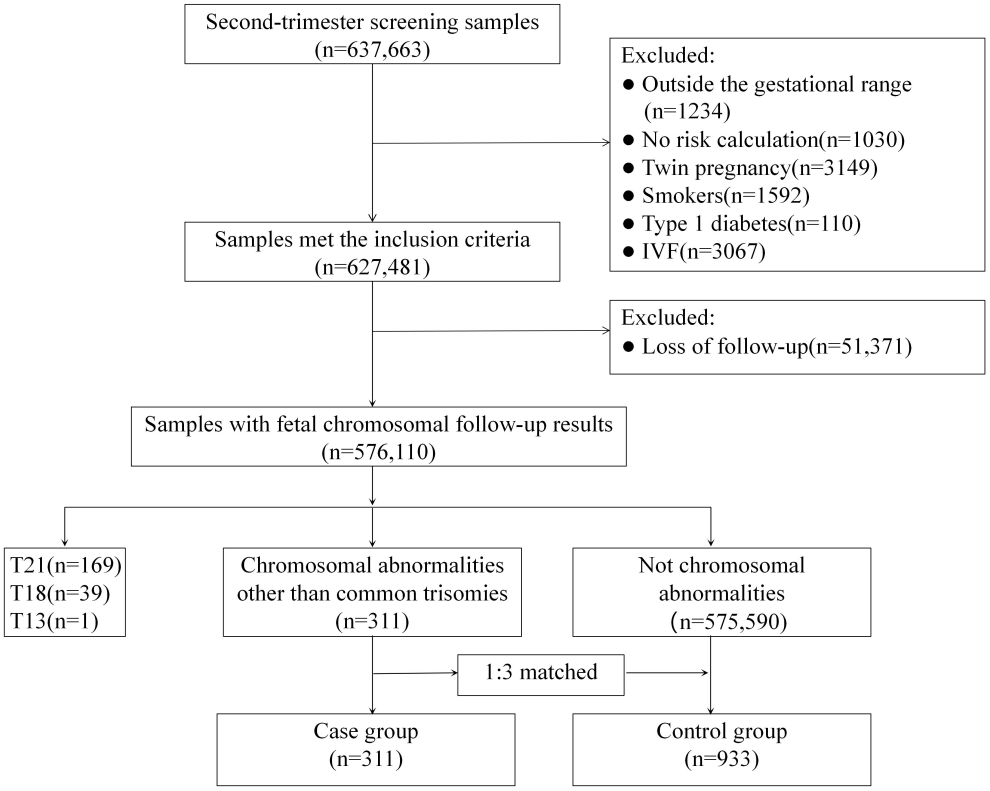

Fig. 1. Flow chart of study sample selection. The range of gestational ages was between 15^{+0} and 20^{+6} weeks; T, trisomy; IVF, *in vitro* fertilization.

2.5 Follow-up of Postpartum Outcomes

All STS data were uploaded to the prenatal diagnosis information management system (PDIMS) for pregnancy outcome follow-up, with follow-up data including the presence of fetal chromosomal abnormalities and abnormal karyotypes. We emphasized that if a chromosomal abnormality was detected in a neonate after birth, it was also reported to this system, as all prenatal screening and diagnosis institutions in Sichuan Province must upload their data to the PDIMS as required by the provincial health administration. In addition, all data were completed for follow-up within one year postpartum. Since the PDIMS is a shared online management system for prenatal screening and diagnostic institutions in the province, we could obtain the follow-up results from any pregnant woman who underwent STS at our institution but did not receive prenatal diagnosis there. Furthermore, we obtained fetal imaging results from pregnant women through the PDIMS system.

2.6 Fetal Ultrasound Findings

Fetal ultrasound findings were obtained from PDIMS for the case and control groups, and categorized into normal, soft markers, and structural anomalies. According to an expert consensus [14] published by the Society for Maternal-Fetal Medicine (SMFM) 2021, we define "soft marker" as minor abnormalities on second-trimester ultrasound that are not "structural abnormalities". Common "soft markers" are "Echogenic intracardiac focus", "Echogenic bowel", "Choroid plexus cyst", "Single umbilical artery", "Urinary tract dilation", "Shortened humerus, femur, or both", "Thickened nuchal fold" and "Absent or hypoplastic nasal bone". We define fetal structural anomalies involve morphological changes in anatomical structures compared to normal, such as "Neural malformation", "Cardiac malformation", and "urogenital malformation".

2.7 Statistical Analysis

The 2.5th, 5th, 95th, 97.5th, and 99th percentile values for AFP MoMs and free β-hCG MoMs were calculated as cutoff points in 627,481 singleton pregnancies undergoing STS between 6 January 2013 and 12 April 2023: 0.52, 0.58, 1.69, 1.90, and 2.22 for AFP MoMs, and 0.34, 0.41, 2.82, 3.54, and 4.68 for free β-hCG MoMs.

Enumeration data were expressed as frequencies and percentages. The Fisher exact-probability test was used for univariate analysis, and the odds ratio (OR) values were calculated at the same time. Variables that were $p < 0.10$ in univariate analysis were then enrolled in multivariable analysis. We conducted multivariable assessments via conditional logistic regression analysis, and the forward stepwise regression was applied to screen out the high factors. We performed data processing and statistical analysis using

Table 1. Number of different types of abnormalities in 311 samples of fetal chromosomal anomalies other than common trisomies.

Fetal chromosomal abnormalities	n (%)
Rare autosomal trisomy	2 (0.63)
Sex chromosome abnormality	50 (16.08)
Structural abnormality	64 (20.58)
Mosaicism	57 (18.33)
Pathogenic CNV	128 (41.16)
Karyotype unknown	10 (3.22)
Total	311 (100.00)

CNV, copy number variation.

SPSS version 25 (IBM Inc., Armonk, NY, USA) and R version 4.3.2 (R foundation for Statistical Computing, Vienna, Austria). The R package "forestploter" was used to construct a forest plot, and two-sided $p < 0.05$ was considered to be statistically significant.

2.8 Sensitivity Analyses

We executed sensitivity analyses on the primary indicators of STS. The Wilcoxon rank-sum test was used for comparing the differences of various types of fetal chromosomal abnormalities with the control group for various indicators. The results of sensitivity analyses are shown as a bar graph.

3. Results

Between 6 January 2013 and 12 April 2023, a total of 637,663 pregnant women underwent STS examination at our prenatal diagnosis center. Of these pregnancies, there were 627,481 singleton pregnancies that met our inclusion criteria, and we excluded 51,371 (8.2%) samples that were lost to follow-up. Reasons for loss to follow-up included incorrect telephone numbers, disconnected or unreachable numbers, or refusal to answer. Thus, 576,110 samples were obtained with fetal chromosomal follow-up results. Among these samples, there were 311 samples with chromosomal abnormalities other than common trisomies. These 311 samples were then considered as the case group. After case-control matching in a 1:3 ratio based on the identical screening strategy, with test dates differing by no more than 3 days, and the presence or absence of ultrasound findings, we identified a total number of 933 controls (Fig. 1).

The data from the case group were classified into six main categories. They were rare autosomal trisomy, sex-chromosome abnormality, structural abnormality, mosaicism, pathogenic CNV, and karyotype unknown. Of these, mosaicisms comprised seven cases of T21 mosaicism and others. The two cases of rare autosomal trisomy were one with T20 and one with T17. The "karyotype unknown" indicated that due to the erroneous data entry, several samples in the PDIMS had incorrect follow-up information or irregular karyotype descriptions (Table 1).

Adopting univariate analysis, we observed that the differences in the case group were statistically significant ($p < 0.05$) when contrasting ultrasonographic findings, AFP MoMs, free β-hCG MoMs, T21 risk, and T18 risk with those of the control group. However, the differences in the case group were not statistically significant ($p > 0.05$) when comparing the age of the expected date of confinement (EDC), gestational age, the methodology used for gestational age, the maternal weight, AFP MoMs, or T18 risk with those individuals in the control group (Table 2).

Multivariable conditional logistic regression analysis using a forward stepwise procedure was performed for factors that were $p < 0.10$ in the univariate analysis. This indicated that the markers for fetal chromosomal abnormalities, excluding common trisomies, were ultrasound structural abnormalities (OR = 3.038; 95% CI, 1.774–5.202; $p < 0.001$); free β-hCG MoMs $\leq$0.34 (OR = 3.006; 95% CI, 1.803–5.013; $p < 0.001$), 2.82–3.53 (OR = 1.884; 95% CI, 1.321–2.688; $p < 0.001$), 3.54–4.67 (OR = 1.949; 95% CI, 1.300–2.923; $p = 0.001$), and $\geq$4.68 (OR = 1.730; 95% CI, 1.045–2.866; $p = 0.033$); and a T21 risk of 1/271–1/1000 (OR = 2.434; 95% CI, 1.706–3.472; $p < 0.001$), 1/101–1/270 (OR = 3.330; 95% CI, 2.300–4.821; $p < 0.001$), and $\geq$1/100 (OR = 3.441; 95% CI, 2.178–5.438; $p < 0.001$) (Table 3 and Fig. 2).

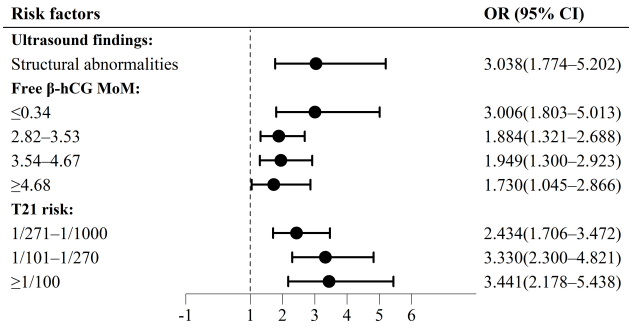

Fig. 2. Forest map of risk factors for fetal chromosomal abnormalities other than common trisomies, in second-trimester screening. T, trisomy; MoM, multiple of the median; β-hCG, β-human chorionic gonadotropin; OR, odds ratio; CI, confidence interval.

Different fetal chromosomal abnormality groups and the control group were compared with respect to AFP MoMs, free β-hCG MoMs, T21 risk, and T18 risk (Fig. 3). Regarding AFP MoMs, only rare autosomal trisomy ($p = 0.040$), mosaicism ($p = 0.034$), and structural abnormality ($p = 0.028$) were statistically different compared to the control group. In the comparison of free β-hCG MoMs, rare autosomal trisomy ($p = 0.020$), mosaicism ($p < 0.001$), sex chromosome abnormality ($p < 0.001$), structural abnormality ($p < 0.001$), pathogenic CNVs ($p < 0.001$), and karyotype unknown ($p = 0.022$) were statistically different com-

Table 2. Univariate analysis of factors associated with fetal chromosomal abnormalities other than common trisomies.

Factors	Control group (n = 933)	Case group (n = 311)	x^2	p	OR (95% CI)
Age of EDC, years, n (%)			6.926	0.063	
<30*	675 (72.35)	211 (67.85)			
30–34	247 (26.47)	90 (28.94)			1.166 (0.875–1.553)
35–39	9 (0.96)	8 (2.57)			2.844 (1.084–7.463)
≥40	2 (0.21)	2 (0.64)			3.199 (0.448–22.850)
Gestational age, days, n (%)			5.391	0.141	
105–111*	99 (10.61)	32 (10.29)			
112–118	373 (39.98)	147 (47.27)			1.219 (0.784–1.897)
119–125	257 (27.55)	76 (24.44)			0.915 (0.570–1.469)
126–146	204 (21.86)	56 (18.01)			0.849 (0.517–1.395)
Methodology for gestational age, n (%)			0.450	0.823	
Calculated with BPD*	597 (63.99)	195 (62.7)			
Calculated with CRL	304 (32.58)	107 (34.41)			1.078 (0.820–1.416)
Calculated with LMP	32 (3.43)	9 (2.89)			0.861 (0.404–1.836)
Ultrasonographic findings, n (%)			16.353	<0.001	
Normal*	298 (31.94)	84 (27.01)			
Soft markers	20 (2.14)	10 (3.22)			1.774 (0.800–3.935)
Structural abnormalities	12 (1.29)	16 (5.14)			4.730 (2.154–10.388)
No results	603 (64.63)	201 (64.63)			1.183 (0.885–1.580)
Maternal weight, kg, n (%)			1.977	0.584	
≤49.99*	206 (22.08)	61 (19.61)			
50.00–59.99	446 (47.80)	163 (52.41)			1.234 (0.881–1.729)
60.00–69.99	213 (22.83)	66 (21.22)			1.046 (0.703–1.557)
70.00–79.99	53 (5.68)	16 (5.14)			1.019 (0.544–1.910)
≥80.00	15 (1.61)	5 (1.61)			1.126 (0.393–3.222)
AFP MoM, n (%)			8.508	0.070	
0.59–1.68*	820 (87.89)	258 (82.96)			
≤0.52	38 (4.07)	24 (7.72)			2.007 (1.182–3.410)
0.53–0.58	25 (2.68)	11 (3.54)			1.398 (0.679–2.881)
1.69–1.89	24 (2.57)	6 (1.93)			0.795 (0.321–1.965)
≥1.90	26 (2.79)	12 (3.86)			1.467 (0.730–2.949)
Free β-hCG MoM, n (%)			178.182	<0.001	
0.42–2.81*	824 (88.32)	182 (58.52)			
≤0.34	25 (2.68)	17 (5.47)			3.079 (1.629–5.820)
0.35–0.41	34 (3.64)	2 (0.64)			0.266 (0.063–1.119)
2.82–3.53	28 (3.00)	49 (15.76)			7.923 (4.848–12.950)
3.54–4.67	14 (1.50)	35 (11.25)			11.319 (5.967–21.471)
≥4.68	8 (0.86)	26 (8.36)			14.714 (6.555–33.028)
T21 risk, n (%)			192.094	<0.001	
<1/3001*	554 (59.38)	95 (30.55)			
1/2001–1/3000	104 (11.15)	15 (4.82)			0.841 (0.469–1.507)
1/1001–1/2000	117 (12.54)	26 (8.36)			1.296 (0.804–2.089)
1/271–1/1000	94 (10.08)	63 (20.26)			3.908 (2.656–5.751)
1/101–1/270	45 (4.82)	67 (21.54)			8.683 (5.614–13.427)
≥1/100	19 (2.04)	45 (14.47)			13.812 (7.743–24.637)
T18 risk, n (%)			7.910	0.071	
<1/3001*	906 (97.11)	294 (94.53)			
1/2001–1/3000	3 (0.32)	2 (0.64)			2.054 (0.342–12.355)
1/1001–1/2000	11 (1.18)	3 (0.96)			0.840 (0.233–3.033)
1/351–1/1000	7 (0.75)	6 (1.93)			2.641 (0.881–7.922)
≥1/350	6 (0.64)	6 (1.93)			3.082 (0.986–9.628)

* indicates a control. Fisher's Exact Test was used to compare the differences between groups. Differences were considered statistically significant at $p < 0.05$. EDC, expected date of confinement; BPD, biparietal diameter of the fetus; CRL, head-rump length of the fetus; LMP, last menstrual period; T, trisomy; AFP, alpha fetoprotein; MoM, multiple of the median; β-hCG, β-human chorionic gonadotropin; OR, odds ratio; CI, confidence interval.

Table 3. Multivariable conditional logistic regression analysis of the risk factors associated with fetal chromosomal abnormalities other than common trisomies.

Risk factors	Beta	Standard error	Wald	p	OR (95% CI)
Ultrasound findings			20.557	<0.001	
Soft markers	0.619	0.337	3.372	0.066	1.856 (0.959–3.592)
Structural abnormalities	1.111	0.274	16.386	<0.001	3.038 (1.774–5.202)
No results	0.027	0.131	0.042	0.837	1.027 (0.795–1.328)
Free β-hCG MoM			36.579	<0.001	
≤0.34	1.101	0.261	17.794	<0.001	3.006 (1.803–5.013)
0.35–0.41	–0.862	0.713	1.460	0.227	0.422 (0.104–1.710)
2.82–3.53	0.634	0.181	12.213	<0.001	1.884 (1.321–2.688)
3.54–4.67	0.667	0.207	10.414	0.001	1.949 (1.300–2.923)
≥4.68	0.548	0.257	4.535	0.033	1.730 (1.045–2.866)
T21 risk			54.181	<0.001	
1/2001–1/3000	–0.136	0.279	0.239	0.625	0.873 (0.506–1.506)
1/1001–1/2000	0.215	0.223	0.930	0.335	1.240 (0.801–1.921)
1/271–1/1000	0.890	0.181	24.093	<0.001	2.434 (1.706–3.472)
1/101–1/270	1.203	0.189	40.603	<0.001	3.330 (2.300–4.821)
≥1/100	1.236	0.233	28.034	<0.001	3.441 (2.178–5.438)

T, trisomy; MoM, multiple of the median; β-hCG, β-human chorionic gonadotropin; OR, odds ratio; CI, confidence interval.

pared with the control group. With respect to T21 risk, mosaicism ($p < 0.001$), sex chromosome abnormality ($p < 0.001$), structural abnormality ($p < 0.001$), and pathogenic CNVs ($p < 0.001$) were statistically different compared with the control group. In the comparison of T18 risk, only structural abnormalities ($p = 0.021$) and pathogenic CNVs ($p < 0.001$) were statistically different compared with the control group. In conclusion, the differences in free β-hCG MoMs and T21 risk between the various types of fetal chromosomal abnormalities and the control group were more significant than AFP MoMs or T18 risk.

Regarding the risk of fetal chromosomal abnormalities other than common trisomies, our study found that STS samples with structural abnormalities, low free β-hCG MoM values (≤0.34), and high T21 risk values (1/101–1/270, ≥1/100) had a relatively higher risk. STS samples with high free β-hCG MoM values (2.82–3.53, 3.54–4.67, ≥4.68) had relatively lower risk. The risk of STS samples with T21 intermediate-risk values was between the first two.

4. Discussion

Although our report focused solely on the STS markers as risk indicators for T21 and T18, the presence of numerous indicators associated with other fetal chromosomal abnormalities provided suggestive evidence that the incidence rates of these chromosomal abnormalities were not low. Norton *et al.* [15] reported that among 2993 high-risk pregnant women who underwent invasive testing, 2487 (83.1%) were diagnosed with aneuploid abnormalities, while 506 (16.9%) were diagnosed with other abnormalities. Similarly, the findings of Wang *et al.* [16] showed that among the 100 high-risk pregnant women who underwent a prenatal diagnosis, the number of chromosomal aneuploidies, chromosomal structural abnormalities, and pathogenic CNVs were 55, 15, and 30, respectively. In the present study, utilizing data obtained from our prenatal diagnostic center over the past decade, we conducted a case-control study to investigate the indicators associated with chromosomal anomalies and calculated odds ratios (ORs) for each indicator. Despite potential confounding factors inherent to this analytical approach, our data provided valuable insights for clinicians based on the available dataset. Our findings revealed that ultrasound structural abnormalities, free β-hCG MoMs (≤0.34, 2.82–3.53, 3.54–4.67, and ≥4.68), and T21 risk (1/271–1/1000, 1/101–1/270, and ≥1/100) were risk indicators in STS for fetal chromosomal abnormalities other than the common trisomies.

Prenatal ultrasonographic examination remains one of the most important examinations during pregnancy, as it is a cost-effective, non-invasive, and relatively reliable method for screening fetal malformations. Fetal ultrasonographic abnormalities principally include structural abnormalities and soft-marker abnormalities. Fetal structural abnormalities primarily occur in the cardiovascular system, nervous system, maxillofacial areas, genitourinary and digestive system [17]. Soft markers, which are considered non-specific indices, include echogenic intracardiac focus, single umbilical artery, thickened nuchal translucency, absent nasal bone, pyelectasis, mild tricuspid regurgitation, and choroid plexus cyst. By analyzing single nucleotide polymorphism (SNP) data from 713 fetuses with ultrasound-detected abnormalities and normal karyotypes, Cai *et al.* [18] found that pathogenic CNVs were detected more fre-

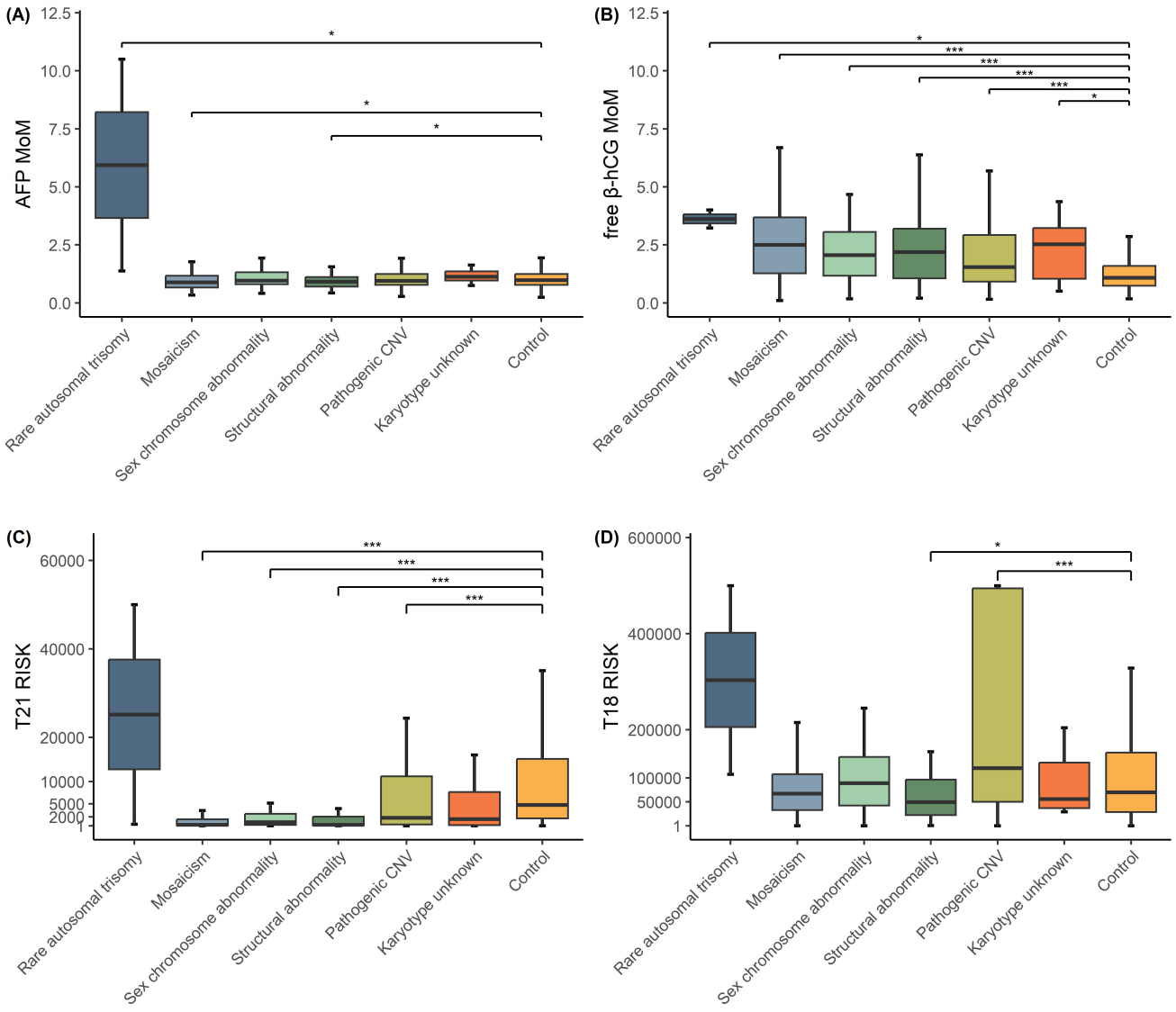

Fig. 3. Boxplot comparing AFP MoMs, free β-hCG MoMs, T21 risk, and T18 risk for second-trimester screening markers in 311 case-group samples of different types and 933 control-group samples. (A) Boxplot depicting AFP MoMs. (B) Boxplot depicting free β-hCG MoMs. (C) Boxplot depicting T21 risk. (D) Boxplot depicting T18 risk. Statistical differences between the two groups were assessed via the Wilcoxon rank-sum test. Error bars represent 95% confidence intervals. * represent $p < 0.05$; *** represent $p < 0.001$. AFP, alpha fetoprotein; MoM, multiple of the median; β-hCG, β-human chorionic gonadotropin; T, trisomy.

quently in fetuses with sonographic structural abnormalities (12.7%, 30/237) than in fetuses with non-structural abnormalities (5.7%, 27/476). In addition, some studies have shown that soft-marker abnormalities were also associated with chromosomal abnormalities, and that the higher the number of soft markers, the higher the risk of chromosomal abnormalities [19,20]. Therefore, some authors have postulated that ultrasonographic screening can complement serologic screening, especially for pregnant women with a single abnormal serological marker [21]. In our investigation, we found after multivariable analysis that ultrasound structural abnormalities (OR = 3.038; 95% CI, 1.774–5.202; $p < 0.001$) were independent risk factors for fetal chromosomal abnormalities other than common trisomies. And ultrasound soft markers (OR = 1.856; 95% CI, 0.959–3.592; $p = 0.066$) were not an independent risk factor for fetal chromosomal abnormalities other than common trisomies (Table 3 and Fig. 2). These results suggest that fetal ultrasound findings of structural abnormalities should be recommended for invasive prenatal diagnosis to rule out the possibility of chromosomal aberrations, whereas ultrasound findings of soft markers should be considered in conjunction with other indicators to determine the need for further testing.

It is acknowledged that a higher risk of fetal chromosomal abnormalities exists with pregnancies for women after 35 years of age. While chromosome mis-segregations in the oocyte constitute one of the most significant causes

of chromosomal abnormalities with advanced maternal age [22], our study revealed that maternal age was not a significant risk factor for fetal chromosomal abnormalities other than the common trisomies. This might be because of the law on Maternal and Infant Health Care in China, in which pregnant women over 35 years of age are mandated to receive a direct prenatal diagnosis. As a result, very few women of advanced maternal age undergo STS in China.

Abnormal MoM values generally indicate a range of chromosomal abnormalities and adverse pregnancies. AFP, which is a fetus-derived glycoprotein, is produced by the yolk sac as well as by fetal hepatocytes, and can be detected in maternal serum as early as six weeks of gestation, with levels gradually increasing until 32 weeks [23]. The transport of AFP to the maternal serum occurs either through the placenta or via diffusion across fetal membranes, and its increase or decrease in the maternal context is correlated with a range of pregnancy complications, including preterm labor, placental abruption, and preeclampsia [24,25]. In fetuses with Down syndrome, the presence of an AFP transport defect leads to a diminution in maternal serum levels [26,27]. hCG is a glycoprotein hormone secreted by placental trophoblast cells and comprises two primary subunits, alpha and beta. It begins to be secreted 6–8 days after fertilization and peaks at approximately 8–11 weeks. In Down-syndrome fetuses, both maternal serum hCG and free β-hCG exhibit elevated levels; however, the elevation in free β-hCG is more pronounced [28]. In addition to T21 and T18, changes in free β-hCG MoMs have been reported in the literature to correlate with a number of other fetal chromosomal abnormalities. Huang *et al*. [10] found that maternal median free β-hCG MoMs (1.86) was significantly higher in pregnant women with unbalanced chromosomal translocations relative to those with balanced chromosomal translocations (1.21) and normal karyotypes (1.29) in 97 cases of first-trimester screening with chorionic villus sampling. In a case report of an amniocentesis performed at 21 weeks of gestation, the AFP MoMs and free β-hCG MoMs of the pregnant woman were 1.026 and 8.678, respectively, and the final karyotyping result was a deletion of the distal region of chromosome 7 [29]. Another study [30] showed that some pregnant women with Beckwith-Wiedemann syndrome (BWS) which is associated with chromosome 11p15 possess abnormal free β-hCG MoMs and normal or abnormal AFP MoMs. As shown in Tables 2,3, AFP MoM value was removed after stepwise regression, although it was $p < 0.10$ in univariate analysis. As can also be seen in Fig. 3A,B, the differences between groups with respect to free β-hCG MoMs were more significant than with AFP MoMs. Another interesting finding was a significantly higher OR for low values of free β-hCG MoMs (i.e., $\leq$0.34) compared to high values ($\geq$2.82), which might offer valuable information for clinicians.

Our findings demonstrated that T21 risk was a risk factor for fetal chromosomal abnormalities other than the common trisomies. This risk was primarily determined by multiplying the age risk of Down syndrome with the likelihood ratio of the associated serum markers. The likelihood ratio was calculated based on the relative height of the Gaussian distribution curve for Down syndrome pregnancies compared to that of normal pregnancies [31], although the STS risk model for T21 and T18 was developed based on a certain number of abnormal versus normal pregnancies. Thus, we posit that these indices might confer the ability to detect other fetal chromosomal abnormalities. According to Lindquist *et al*. [32], women with a T21 risk of first-trimester screening below 1 in 300 were most likely to develop atypical abnormalities (excluding common trisomies and sex-chromosome aneuploidies). Similarly, Iwarsson and Conner [9] also noted that 55% of atypical chromosomal pregnancies had an increased risk of T21, and Mak *et al*. [33] demonstrated that the greater the number of risk factors for atypical chromosomes at cFTS, the higher the incidence of atypical chromosomes. In our study, the incidence of fetal chromosomal abnormalities other than the common trisomies was statistically significant when the T21 risk value in STS exceeded 1/1000. In addition, the higher the risk rate, the higher the incidence of abnormalities. Notably, we found that the OR for intermediate-risk (OR = 2.434) was lower than the OR for free β-hCG MoMs $\leq$0.34 (OR = 3.006), which has not been reported previously. Surprisingly, T18 risk was not an independent risk factor for fetal chromosomal abnormalities other than the common trisomies in spite of a robust significant difference ($p < 0.001$) with T18 risk between pathogenic CNVs and controls (Fig. 3B).

Our study has several strengths. First, this study provided a large amount of data, encompassing nearly a decade's worth of STS and follow-up data collected from our prenatal diagnosis center. Second, there were fewer reports of fetal chromosomal abnormalities except for common trisomies in STS, and the coverage of STS is currently still high in China. Third, to eliminate confounding factors, we exploited a matched case-control strategy. The STS samples at our center over the last decade consisted of two main screening strategies, a dual and a triple test. To ensure the inclusion of all abnormal cases in this study, we standardized the screening strategy, testing times across matched conditions and the presence or absence of ultrasound findings to minimize variations between different strategies, testing batches, and the presence or absence of ultrasound findings. Fourth, we employed sensitivity and subgroup analyses. We computed ORs for the various ranges of risk factors, providing valuable information to pregnant women with diverse STS results. The sensitivity analyses also supported the robustness of our results. However, there were some limitations to our study. Despite the existence of a province-wide networked PDIMS, fetal chromosome results were unavailable for some pregnant women. Due to the difficulty of follow-up, we may

have lost some of the fetal chromosomal abnormality data. Furthermore, some fetuses or newborns with chromosomal anomalies who were clinically asymptomatic were not subjected to relevant examination, presumably impacting our results. However, in order to include more abnormal cases, we did not analyze uE3, though it has been reported that uE3 can be used to detect a number of pathogenic CNVs, such as a Xp22.31 microdeletion [34]. In light of this, we will focus on uE3 assessments in the future.

5. Conclusions

Our research revealed that free β-hCG MoMs and T21 risk in STS—in conjunction with ultrasonographic findings—can serve as significant markers for the detection of fetal chromosomal abnormalities other than common trisomies. We also computed ORs for each subgroup of indicators for clinical referencing. We posit that our findings will assist pregnant women and clinicians in interpreting STS results so as to inform decision-making regarding the need for additional testing. For certain pregnant women at low risk for STS but with abnormalities in other indicators, our results may prompt them to opt for further testing, thereby improving the detection rate of fetuses with chromosomal abnormalities.

Availability of Data and Materials

Further inquiries could be directed to the corresponding author. The data that support the findings of this study are not publicly available due to privacy reason but are available from the corresponding author upon reasonable request.

Author Contributions

ZW contributed to the conception of the study, collected data, and drafted the manuscript. MO collected data and processed the data, participated in the writing of the corresponding results section, and plotted Table 1 and Table 2. ZW performed statistical analysis. XW designed the research study, revised the language of the first draft and checked and revised the overall content and data. Defines and categorizes ultrasound data and participates in the writing of ultrasound-related sections in the Materials and Methods section. All authors revised and approved the final version of the paper. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study protocol was approved by the Local Ethics Committee of Sichuan Provincial Maternity and Child Health Care Hospital (approval number: 20240607-219). The study protocol conforms to the ethical guidelines of the "World Medical Association (WMA) Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects" adopted by the 18th WMA General Assembly, Helsinki, Finland, in June 1964, and amended by the 59th WMA General Assembly, Seoul, South Korea, in October 2008. Patient consent has been obtained during all data collection processes.

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Conflict of Interest

The authors declare no conflict of interest.

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