

# A 10-year retrospective study on prenatal cytogenetic analyses

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## Summary

**Objective:** To analyze the indications and results of prenatal cytogenetic screening in patients from a specialized center in Xiamen City, China, to provide a reference database for prenatal diagnosis. **Materials and Methods:** This retrospective, observational study included 7,400 pregnant women who underwent chorionic villous sampling (CVS), amniocentesis or cordocentesis at the Women and Children's Hospital, School of Medicine, Xiamen University, China, over a 10-year period (2008–2018). Clinical data and the results of the cytogenetic analysis were assessed. **Results:** Fetal chromosomal aberrations were observed in 335 of 7,400 (4.5%) cases, with trisomy 21 the most common aberration (87/335, 26%). A high risk on maternal serum screening was the indication for cytogenetic analysis in 36 of the 87 fetuses with trisomy 21 (41.4%). Abnormal fetal ultrasonographic findings were the clinical indication for cytogenetic testing in more than half of fetuses with abnormal karyotypes (181/335, 54%). **Conclusions:** Prenatal cytogenetic analysis is useful for the prenatal diagnosis of birth defects in Xiamen City, and may prevent termination of potentially healthy fetuses.

**Key words:** Aneuploidy; Cytogenetics; Karyotype analysis; Prenatal diagnosis.

## Introduction

Cytogenetic analysis was introduced in 1976 as a safe and reliable method of prenatal diagnosis [1]. The usefulness of this technique in detecting birth defects and in routine obstetrical work-up has been demonstrated in many countries [2, 3]. In mainland China, there are ~15 million births each year, with ~500,000 in the Fujian province. Presently, there are around 60 laboratories in mainland China that conduct ~60,000 prenatal cytogenetic tests each year. Although several papers on this technique have been published, these mainly relate to the evaluation of prenatal screening results. Meanwhile, reports on the use of prenatal screening and cytogenetic tests for prenatal diagnosis in a large population sample are rare in mainland China.

In 2002, we began conducting cytogenetic analysis in our prenatal diagnostic center in the Women and Children's Hospital, School of Medicine, Xiamen University, China, which is a regional referral center for fetuses with suspected anomalies and/or genetic syndromes. It is one of only two prenatal diagnosis centers in the Fujian province, and services at least five surrounding areas. Here we summarize the results of cytogenetic tests conducted in our center for the diagnosis of fetal chromosomal aberrations over a period of 10 years.

These results provide a reference database for future research on prenatal cytogenetic diagnosis in mainland China.

## Materials and Methods

After genetic counseling and explanation of the risks related to the procedure, all pregnant women included in this study signed informed consent forms. The study was approved by the ethics in research committee of the institution. From 2008 to 2018, 7,400 pregnant women who underwent invasive prenatal diagnostic procedures at this center at 11–28 weeks of gestation were included in the study. All pregnant women estimated to be aged >35 years at the time of delivery underwent routine prenatal diagnostic work-up.

Pregnant women in whom serum screening tests showed a high fetal risk for trisomies 21 and 18 (risk cut-off levels, 1/270 and 1/350, respectively) underwent prenatal testing using time-resolved fluoroimmunoassay (TRFIA). Maternal serum screening consisted of tests for the free beta-human chorionic gonadotrophin (free  $\beta$ -hCG) and pregnancy-associated plasma protein A (PAPP-A) in the first trimester, and the triple test for alpha-fetoprotein (AFP),  $\beta$ -hCG, and unconjugated estriol (uE3) in the second trimester. We used the Wallac LifeCycle software for prenatal screening and calculated the relative multiples of median (MoM) and risk values.

Abnormal ultrasonographic findings in fetuses with Down syndrome include both structural and non-structural abnormalities (or soft markers) related to the possibility of chromosomal aberrations. The fetal malformations detected in this study included cong-

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Table 1. — Summary of prenatal diagnostic tests performed between 2008 and 2018 at the Women and Children's Hospital, School of Medicine, Xiamen University, and the fetal abnormality detection rates.

Indication	Amniocentesis	Cordocentesis	CVS	Total	Abnormal result	
	n (%)	n (%)	n (%)	n (%)	n	n (%)
High risk for trisomy 21 on maternal serum screening	2407 (51%)	346 (13.2%)	8 (18.2%)	2761 (37%) <sup>#</sup>	74	2761 (2.7%)
High risk for trisomy 18 on maternal serum screening	64 (1.3%)	23 (0.9%)	1 (2.3%)	88 (1.2%)	21	88 (2.4%)
Advanced maternal age ( $\geq 35$ years)	1407 (30%)	237 (9.1%)	9 (20.5%)	1653 (22%)	38	1653 (2.3%)
Abnormal findings on fetal ultrasound	246 (5.2%)	1878 (71.9%)	12 (27.2%)	2136 (29%)	181	2136 (8.5%)
Poor obstetrical history	547 (11.5%)	116 (4.4%)	11 (25%)	674 (9.1%)	7	674 (1.0%)
Parental chromosomal abnormality	73 (1.5%)	12 (0.5%)	3 (6.8%)	88 (1.2%)	14	88* (15.9%)
<b>Total</b>	<b>4744</b>	<b>2612</b>	<b>44</b>	<b>7400</b>	<b>335</b>	<b>7400 (4.5%)</b>

<sup>#</sup>Highest frequency of prenatal diagnostic analysis. \*Highest rate of fetal abnormality detection.

Table 2. — Diagnosis of chromosomal abnormalities according to clinical indications for prenatal screening.

Indication	Trisomy 21	Trisomy 18	Trisomy 13	Sex chromosome	Structural chromosome	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
High risk for trisomy 21 on maternal serum screening	34 (39.1) <sup>#</sup>	8 (9.8)	0	11 (17.7)	21 (25.3)	74 (22.1%)
High risk for trisomy 18 on maternal serum screening	2 (2.3)	13 (15.9)	2 (9.5)	2 (3.2)	2 (2.4)	21 (6.3%)
Advanced maternal age ( $\geq 35$ years)	15 (17.2)	10 (12.2)	1 (4.8)	6 (9.7)	6 (7.2)	38 (11.3%)
Abnormal findings on fetal ultrasound	32 (36.8)	49 (59.8)*	17 (81.0)*	35 (56.5)*	48 (57.9)*	181 (54%)
Poor obstetrical history	2 (2.3)	2 (2.4)	1 (4.8)	2 (3.2)	0	7 (2.1%)
Parental chromosomal abnormality	2 (2.3)	0	0	6 (9.7)	6 (7.2)	14 (4.2%)
<b>Total</b>	<b>87</b>	<b>82</b>	<b>21</b>	<b>62</b>	<b>83</b>	<b>335 (100%)</b>

<sup>#</sup>Highest detection frequency based on clinical indication was a high risk for trisomy 21 based on maternal serum screening. \*Abnormal fetal ultrasound findings play an important role in the diagnosis of fetal chromosome abnormalities.

enital heart disease, nuchal cystic hygroma, cleft lip/cleft palate, omphalocele, diaphragmatic hernia, gastroschisis, duodenal atresia, fetal effusion/hydrops, clubfoot, renal agenesis, and skeletal dysplasia. The most commonly observed soft markers included increased nuchal translucency ( $\geq 3$  mm), thickened nuchal fold ( $\geq 6$  mm), absent nasal bone [4], pyelectasis ( $\geq 4$  mm and  $< 10$  mm), echogenic intracardiac focus [5], hyperechoic bowel [6], short femur and humerus, and mild cerebral ventriculomegaly in the second trimester [7].

Prenatal cytogenetic analysis was indicated if the parents had a family history of numerical or structural chromosomal aberrations, such as full aneuploidy, polyploidy, mosaicism, deletion, duplication, insertion, inversion, balanced and unbalanced translocation, ring chromosome, and marker chromosome. However, variant chromosomes, such as pericentric inversion of chromosomes 9 and Y, double satellites or marked satellite on acrocentric chromosomes, and hyperchromatin on various chromosomes (e.g., 1, 9, and 16), were considered normal. This included a history of a fetus/child with chromosomal abnormalities or congenital anomalies, and a history of missed or recurrent abortions unexplained death *in utero*.

## Results

Between 2008 and 2018, we performed 7,400 invasive procedures for prenatal cytogenetic analysis in our center (Table 1).

The majority were amniocentesis (4,744) and cordocentesis (2,612), with only 44 cases of chorionic villous sampling (CVS) performed. The most common indication for prenatal diagnostic testing was a high risk on maternal serum screening (38.5%, 2,849/7,400), followed by abnormal findings on fetal ultrasonography (28.9%, 2,136/7,400), and advanced maternal age (22.3%, 1,653/7,400), history of chromosomal aberrations in the parents or their families (88/7400, 1.2%), and history of adverse obstetric outcomes (674/7,400, 9.1%). The main clinical indication for amniocentesis was a high risk on maternal serum screening (52.1%, 2,471/4,744), while abnormal fetal ultrasonographic findings were the main indication for cordocentesis (71.9%, 1,878/2,612).

We also analyzed the fetal abnormality detection rates according to the various indications for prenatal screening (Table 1). Among the clinical indications for the prenatal tests, parental chromosomal abnormality was associated with the highest rate of detection of fetal chromosomal abnormality (15.9%, 14/88), followed by abnormal fetal ultrasonographic findings (8.5%, 181/2136), high risk on maternal serum screening (3.3%, 95/2,849), advanced maternal age (2.3%, 38/1,653), and poor obstetric history (1.0%, 7/674). Indeed, the detection rates associated with parental chromosomal abnormality and abnormal fetal ultrasonographic findings were higher than the rates associated with the other indications. The results of

Table 3. — *Diagnosis of chromosomal abnormalities according to ultrasound indications.*

Ultrasound indication	Trisomy21 n (%)	Trisomy 18 n (%)	Trisomy 13 n (%)	Sex chromosomal aberrations			Structural Aberrations n (%)	Total n (%)
				45,X n (%)	47,XXY n (%)	47,XYY n (%)		
Fetal malformations								
Cardiac abnormality	12 (37.5) <sup>#</sup>	4 (8.2)	3 (17.6)				15 (31.2)	34 (18.8)
Nuchal cystic hygroma		4 (8.2)		21(65.6) <sup>#</sup>			25 (13.8)	
Cleft lip/cleft palate			1 (5.9)				1 (2.1)	2 (0.5)
Fetal effusion/hydrops	1 (3.1)			4 (12.5)			1	6 (3.3)
Multiple malformations		21 (42.9) <sup>#</sup>	13 (76.5) <sup>#</sup>	1 (3.1)			1	36 (19.9)
Skeletal dysplasia	1 (3.1)					3(6.3)	4 (2.2)	
								107(59.1%)
Sonographic soft markers								
Increased NT/NF*	6 (18.8)	1 (2.0)		1 (3.1)		1	2 (4.2)	11 (6.1)
Pyelectasis					1		3 (6.3)	4 (2.2)
EIF <sup>a</sup>	1 (3.1)			2 (6.3)			4 (8.3)	7 (3.9)
Hyperechoic bowel	3 (9.4)						4 (8.3)	7 (3.9)
CPC <sup>b</sup>		13 (26.5) <sup>#</sup>						13 (7.2)
Ventriculomegaly (≥10 mm)	2	2 (4.1)		1 (3.1)		1	9 (18.8)	15 (8.3)
Absent nasal bone	1(3.1)							1 (0.6)
SUA <sup>c</sup>		3 (6.1)		1 (3.1)			5 (10.4)	9 (5.0)
Short femur and humerus	3 (9.4)							3 (1.7)
								70 (38.7%)
Fetal malformations and/or two sonographic soft markers								
Nuchal cystic hygroma+ pyelectasis			1 (3.1)			1 (0.6)		
Absent nasal bone + EIF	1 (3.1)							1 (0.6)
CPC + heart abnormality		1 (2.0)						1 (0.6)
Fetal hydrops + hyperechoic bowel	1 (3.1)							1 (0.6)
								4 (2.2%)
Total	32	49	17	32	1	2	48	181

\* NT: nuchal translucency (11–14 week gestation/CRL: 45–84 mm); NF: nuchal fold (15–23 week gestation); <sup>a</sup> echogenic intracardiac focus; <sup>b</sup> choroid plexus cyst; <sup>c</sup> single umbilical artery.

chromosomal analyses were normal in 7,068 of the 7,400 (95.5%) tests. Abnormal fetal karyotypes were identified in 335 (4.5%) tests (Table 2). The most common chromosomal abnormality detected was trisomy 21 (26%, 87/335), followed by structural abnormalities (24.8%, 83/335), trisomy 18 (24.5%, 82/335), abnormalities in sex chromosomes (18.5%, 62/335), and trisomy 13 (6.3%, 21/335).

Among the fetuses with trisomy 21, the most common clinical indications for cytogenetic testing were a high risk on maternal serum screening (41.4%, 36/87) and abnormal fetal ultrasonographic findings (36.8%, 32/87). In the case of other fetal chromosomal abnormalities, abnormal fetal ultrasonography was the most common indication for cytogenetic testing. The detection rates of trisomy 18, trisomy 13, sex chromosome abnormalities, and structural chromosomal rearrangements in women with abnormal fetal ultrasonographic findings were 59.8% (49/82), 81.0% (17/21), 56.5% (35/62), and 57.9% (48/83), respectively. Abnormal fetal ultrasonographic findings accounted for more than half of the abnormal fetal karyotypes detected (54%, 181/335, Table 2).

The 181 fetuses with chromosomal aberrations and abnormal ultrasonographic findings showed the following anomalies: malformations (107 fetuses), soft markers (70 fetuses), and multiple abnormalities (four fetuses,

including three with one malformation plus one soft marker and one fetus with two soft markers).

The most frequent fetal malformations were multiple malformations (19.9%, 36/181), cardiac anomaly (18.8%, 34/181), and nuchal cystic hygroma (13.8%, 25/181). The main sonographic soft markers were ventriculomegaly ≥ 10 mm (8.3%, 15/181), choroid plexus cyst (7.2%, 13/181), and increased nuchal translucency or thick nuchal fold (6.1%, 11/181, Table 3).

## Discussion

The diagnosis of fetal chromosomal abnormalities is one of the most important challenges in modern perinatology. Various methods have been used to identify women who are at risk of carrying a fetus with aneuploidies, including CVS, amniocentesis, and cordocentesis. CVS is the procedure of choice in the first trimester (11–14 weeks of gestation), but it is associated with a 2.3%–3.7% risk of fetal loss [8]. In addition, a major disadvantage of CVS is confined placental mosaicism, which occurs in approximately 1% of cases and necessitates further amniocentesis or cordocentesis. In this study, there were only 44 cases of CVS, which may be attributable to the fact that in our local area, screening tests, such as maternal serum biochemical testing and/or fetal nuchal translucency

measurement, are not commonly performed in the first trimester. Amniocentesis and cordocentesis can reliably determine fetal karyotype (and be performed from 16 and 22 weeks of gestation, respectively); however, these procedures are associated fetal mortality rates of 0.5%–1.0% and 1.4% [9], respectively. In our center, the rate of cordocentesis was higher than that in some previous reports, probably because prenatal diagnosis was performed at a higher gestational age. Moreover, 71.9% of the cordocentesis procedures in this study were indicated because of fetal ultrasonographic anomalies, which may have been incorrectly detected as a result of inexperience or a lack of medical technology, etc. Indeed, many fetal abnormalities were detected at less specialized medical institutions prior to a referral to our medical center, resulting in a delay in prenatal diagnostic testing. Based on our findings, we recommend maternal screening for high risk of fetal abnormalities be performed in the first trimester if possible, as this would decrease the number of cordocentesis procedures and increase the frequency of amniocentesis.

We found the most common indications for prenatal cytogenetic analysis were a high risk on maternal serum screening (38.5%), abnormal fetal ultrasonographic findings (28.9%), and advanced maternal age (22.3%). Among these indications of cytogenetic analysis, parental chromosomal abnormality (15.9%) and abnormal fetal ultrasonographic findings (8.5%) were associated with the highest frequencies of fetal anomaly detection.

Fetal chromosomal aberrations were observed in 4.5% (335/7400) of cases, which is consistent with the rates reported in previous studies on prenatal diagnosis (1.0%–6.7%) [10]. Several studies have shown trisomy 21 is the most common and clinically significant cytogenetic abnormality detected using prenatal diagnostic tests [11]. Similarly, trisomy 21 was the most common chromosomal abnormality detected (26%) in this study, followed by structural chromosomal abnormalities (24.8%), trisomy 18 (24.5%), sex chromosomal abnormalities (18.5%), and trisomy 13 (6.3%). The rate of trisomy 21 in this study is similar to those of previous reports [12]. Among the fetuses with trisomy 21, the highest detection frequency (41.4%) was associated with the clinical indication of a high risk on maternal serum screening. Therefore, we recommend the rate of fetal cytogenetic analysis for high-risk results on maternal serum screening be increased as much as possible.

We found abnormal fetal ultrasonographic findings was the clinical indication for cytogenetic analysis in more than half of the fetuses with chromosomal abnormalities (54%). In fetuses with trisomy 21, the most frequent fetal malformation and sonographic soft marker was cardiac abnormality (37.5%) and increased nuchal translucency

(18.8%), respectively, while the most common clinical indication was a high risk on maternal serum screening (41.4%), followed closely by abnormal fetal ultrasonography (36.8%). However, in fetuses with trisomies 18 and 13, multi-deformation was the most common abnormality (42.9% and 76.5%, respectively) and abnormal ultrasonographic findings was the most common clinical indication (59.8% and 81.0%, respectively). We also observed choroid plexus cyst and nuchal cystic hygroma were especially associated with fetal trisomy 18 (26.5%) and 45, X (65.6%), respectively. Therefore, prenatal diagnosis is necessary in the case of high-risk results on maternal serum screening and advanced maternal age ( $\geq 35$  years), especially if accompanied by abnormal ultrasonographic soft markers. If cytogenetic analysis is performed in patients with high-risk results on maternal serum screening and abnormal fetal ultrasonographic findings, trisomy 18 can be easily detected in a large number of patients. Trisomy 21 was the most common abnormal karyotype in this study. One-third of all pregnancies were associated with abnormal ultrasonographic findings. Maternal serum screening and fetal ultrasonography should be meticulously performed to avoid missing any diagnoses. The detection of several indications for prenatal cytogenetic analysis can improve the rate of detection of fetal chromosomal abnormalities.

In summary, we have identified the most common indications for invasive prenatal cytogenetic tests at a single institution. We reviewed the most common ultrasonographic soft markers used to screen aneuploidy and the clinical relevance of soft markers to detect chromosomal abnormalities. Our findings demonstrate the benefits of prenatal cytogenetic analysis in high-risk groups and provide valuable information for improving prenatal care, including the difficult decision of pregnancy termination in cases of lethal disorders. Finally, our retrospective analysis included data from a large cohort of samples, as well as follow-up data, and thus could be used as a database for genetic counseling.

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