Combined Application of Chromosome Karyotype and Microarray Analysis in Fetus With Increased Nuchal Translucency

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Research

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Abstract

Objective To examine the risk of chromosomal abnormalities when the thickness of the nuchal translucency (NT) is 2.5-2.9mm, to evaluate the cutoff value of NT for prenatal diagnosis, to explore the value of combined application of chromosome karyotype and microarray analysis, and to explore the relationship between NT $\geq$ 5.0mm and fetal prognosis.

Methods A total of 366 pregnant women who underwent prenatal diagnosis in Anhui Province Maternity and Child Health Hospital from January 2018 to August 2020 were collected, of which 241 cases had NT $\geq$ 2.5mm, 125 cases were elderly (35-38 years old) with NT < 2.5mm. We made grouping statistics on chromosome abnormalities, and compared the detection of chromosome abnormalities by karyotype and microarray analysis. At the same time, we followed up the fetuses with NT $\geq$ 5.0mm and analyzed their prognosis.

Results: (1) Among the 366 cases with NT thickening, the detection rates of chromosome abnormalities by karyotype analysis and microarray analysis (CMA) were 13.39% (49/366) and 13.93% (51/366), respectively, and there was no significant difference ($P > 0.05$), including 25 cases of trisomy 21, 5 cases of trisomy 18, 5 cases of Turner syndrome and 16 cases of other chromosome abnormalities. (2) We compared the effect of different NT value on the detection rate of pathogenic chromosomes, and found that the difference between NT $\geq$ 2.5mm and NT < 2.5mm was statistically significant ($P < 0.05$). The detection rates of pathogenic chromosomal abnormalities in NT < 2.5mm group, 2.5-2.9mm group, 3.0-3.4mm group, 3.5-4.4mm group, 4.5-5.4mm group and NT $\geq$ 5.5mm group were 0.8% (1/125), 11.63% (10/86), 17.81% (13/73), 20% (10/49), 47.62% (10/21) and 63.64% (7/11) respectively. (3) Our study found that different prenatal diagnostic indicators for abnormal chromosome detection rate difference was statistically significant ($P < 0.05$). The detection rates of NT thickening alone and NT thickening combined with other abnormalities were 13.17% (22/167) and 35.14% (26/74) respectively ($P < 0.05$). (4) Among 18 pregnant women with NT $\geq$ 5.0 mm, 9 fetuses were chromosomal abnormalities, and 9 fetuses survived healthily.

Conclusions When the NT value is 2.5-2.9mm, the incidence of fetal chromosome abnormality is significantly higher than that in the normal group. It is suggested that invasive prenatal diagnosis should be performed for pregnant women with NT $\geq$ 2.5mm. Chromosome karyotype analysis and CMA can complement each other, which is conducive to prenatal genetic counseling. The fetuses with NT thickening usually have good pregnancy outcomes when excluding fetal chromosome and prenatal ultrasound does not indicate any abnormalities.

Background

The nuchal translucency refers to the fetal neck subcutaneous echoless zone, namely the subcutaneous hydrops between fetal neck and skin [1]. According to statistics, about 5 % of fetal NT measurements are greater than 95 % percentile [2]. Fetal NT thickening in early pregnancy is closely related to adverse pregnancy outcomes such as chromosomal abnormalities [3], single genetic diseases [4] and fetal or perinatal death [5]. With the thickening of NT, the incidence of fetal chromosomal abnormalities also increases. When the NT value is greater than 6.5 mm, the incidence of fetal chromosomal abnormalities is as high as 64.5% [6]. CMA is a high-resolution and high-throughput molecular detection technology for detecting human genomic DNA copy number variation, which can detect chromosomal microdeletions or microduplications that cannot be detected by karyotype analysis [7–9]. Studies have shown that CMA can increase the detection rates of chromosome abnormalities by 4% and 7% compared with the traditional karyotype analysis [10, 11]. At present, most studies have set the cutoff value of abnormal NT as 3.0-3.5 mm [12], and the application data of chromosome karyotype analysis and CMA in NT value of 2.5–2.9 mm are limited. Therefore, the purpose of this study is to examine the risk of chromosomal abnormalities in fetuses with NT value of 2.5–2.9, to evaluate the cutoff value of NT thickening for prenatal diagnosis, and to explore the value of combined application of chromosome karyotype and microarray analysis, and to explore the relationship between NT $\geq$ 5.0 mm and fetal prognosis.

Methods

Subjects

Within a 35-month period (January 2018 to October 2020), 365 pregnant women (age: 17~44 years old; gestational week: 16⁺⁵~24⁺¹) were enrolled for the prenatal diagnosis using G-band karyotyping and CMA in the Anhui Maternal and Child Health Care Hospital. The cohort was stratified according to the NT measurement: $\leq$ 2.5; 2.5-2.9; 3.0-3.4; 3.5-4.4; 4.5-5.4; $\geq$ 5.5mm with/without structural malformations. Patients were stratified according to the NT measurement only. The study was carried out under the approval by the Hospital Ethics Committee of the Anhui Maternal and Child Health Care Hospital. Informed written consents were obtained from all pregnant women who received karyotyping and CMA.

Conventional G-banded cytogenetic analysis

The tests were obtained by patient agreement with the knowledge. Under the guidance of ultrasound, High-risk pregnant women underwent interventional surgery, and amniotic fluid samples were collected for prenatal diagnosis. Following steps: inoculation, culture, G-banding and Lysra GSL-120 automatic karyotype scanner were followed to identify karyotypes. According to the principle of "An International System for Human Cytogenetic Nomenclature, ISCN2016", a total of 30 dividing phases were counted per sample, using an AI chromosome image analysis system (CytoVision, Switzerland), and 5 karyotypes were analyzed and double counts were obtained in case of mosaic.

Chromosomal microarray analysis (CMA)

The amniotic fluid samples were centrifuged at 4 °C, 1,000 g for 10 minutes. Cell precipitation was retained to extract DNA samples. The absorbance of DNA samples (A260 / 280 nm) was maintained at 1.7~1.9. DNA samples (4 μ L) were taken, and SNP array was detected according to the instructions of Humancyto SNP-12 BeadChip kits (Illumina, Santiago, USA). The data were collected by iSCAN scanning system, and the results were analyzed by GenomeStudio software. SNP-array 750K containing about 750,000 SNP probes. Chromosomal analysis is performed with Affymetrix Chromosome Analysis

Suite (ChAS) software. Data was visualized and analyzed by Chromosome Analysis Suite (ChAS) software package (Affymetrix, USA). Based on NCBI human genome build 37 (hg 19) to reported the CNVs. CNVs were evaluated through a filtering procedure and identified with five categories based on the ACMG guideline. Detected CNVs were evaluated based on the following public databases: DECIPHER, OMIM, etc. The detected CNVs were classified into five categories: pathogenic CNVs, benign CNVs, variants of uncertain significance (VOUS), likely pathogenic CNVs and likely benign CNVs. In current study, we only reported the pathogenic CNVs, likely pathogenic CNVs and VOUS.

Statistical analysis

Statistical analysis was performed with SPSS version 23 (SPSS Inc., Chicago, IL, USA). Comparisons between groups were performed by using chi-squared test. A *P*-value of <0.05 was considered as statistically significant.

Results

Conventional karyotype analysis and CMA results

Among the 366 samples, there were 241 cases with NT $\geq$ 2.5mm and 125 cases with advanced age (35-38 years old) with NT < 2.5mm. The detection rates of chromosome abnormalities in karyotype analysis and CMA were 13.39% (49/366) and 13.93% (51/366), respectively, and the difference was not statistically significant ($P>0.05$). Conventional karyotype analysis and CMA detected 48 cases of chromosomal abnormalities, including 25 cases of trisomy 21, 5 cases of trisomy 18, 5 cases of Turner syndrome, and 13 cases of other chromosome abnormalities (2 cases of 47, XXX, 3 cases of 47, XXY, 1 case of 47, XYY / 46, XY, 1 case of 47, XX, + mar, 6 cases of chromosome partial trisomy and monomer). In addition, one case of balanced translocation was only detected by routine karyotype analysis, and 3 cases of pathogenic copy number variation (CNVs), 2 cases of likely pathogenic CNVs and 15 cases of unknown significance (VOUS) variation were only detected by CMA (Table 1). We classified all CNVs and followed up the pregnancy (Table 2). Among them, 5 cases had chromosome microdeletion and microduplication, 3 cases were verified by their parents, 2 cases had new variation, and 1 case was caused by balanced translocation heredity (figure 1).

Comparison of fetal chromosomal abnormalities with different NT values

In 241 samples with NT $\geq$ 2.5mm, the detection rate of chromosomal abnormalities was 20.75% (50 / 241). The NT values of 86 cases were 2.5-2.9mm, the chromosome abnormality rate was 11.63% (10/ 86); the NT values of 73 cases were 3.0-3.4mm, the chromosome abnormality rate was 17.81% (13/ 73); the NT values of 50 cases were 3.5-4.4mm, the chromosome abnormality rate was 20% (10/ 50); the NT values of 21 cases were 4.5-5.4mm, the chromosome abnormality rate was 47.62% (10/ 21); and the NT of 11 cases was $\geq$ 5.5mm, the rate of chromosome abnormality was 63.64%. In 125 cases of normal group with NT < 2.5mm, the rate of pathogenic chromosome abnormality was 0.8% (1/ 125). The chromosome abnormality rate of each group with NT $\geq$ 2.5 mm was different from that of the normal group, and the difference was statistically significant, and the fetal chromosome abnormality rate increased gradually with the thickening of NT (Table 1).

Comparison NT thickening alone with NT thickening combined with other abnormalities

Among the 167 cases of simple NT thickening, the rate of fetal chromosome abnormality was 13.17% (22/ 167). In 74 cases of NT thickening combined with other abnormalities (fetal nasal bone dysplasia, cervical lymphocystoma, subcutaneous tissue thickening, edema, choroid plexus cyst, single umbilical artery, ventricular hyperintense spot, elderly, adverse fertility history, etc.), the fetal chromosome abnormality rate was 35.14% (26/ 74), and the difference between the two groups was statistically significant (Table 3).

Follow-up prenatal and postnatal examination of fetuses with NT $\geq$ 5.0 mm

In 18 samples with NT $\geq$ 5.0mm, the abnormal rate of fetal chromosome was 50% (9/ 18). We also followed up the subsequent pregnancy and fetal delivery of all samples (Table 4).

Discussion

The NT value measured by 11–13 + 6W ultrasound is an important indicator for evaluating fetal chromosomal abnormalities in the early pregnancy [13]. Studies have shown that [14], when the NT value is 3.4mm, the fetal chromosome abnormality rate is only 3.7% when NT $\geq$ 3.5mm, the fetal chromosome abnormality rate is as high as 21.1%. In this study, among 240 samples with NT $\geq$ 2.5mm, the fetal chromosome abnormality rate was 20.75% (50/241). Among them, trisomy 21 syndrome accounted for 50% (25/ 50), 18 trisomy syndrome accounted for 10% (5/ 50), Turner syndrome accounted for 10% (5/ 50), and other chromosome abnormalities accounted for 30% (15/ 50). The results suggest that NT thickening is closely related to chromosome abnormalities, especially trisomy 21 syndrome. Souka et al showed that when NT $\geq$ 3.5mm, the risk of chromosome abnormality increased exponentially with the thickening of NT [14]. Maya et al reported that the fetal chromosome abnormality rates in NT $\leq$ 2.9 mm, 3.0-3.4mm and $\geq$ 3.5mm groups were 1.7%, 6.5% and 13.8% respectively [15]. In this study, the fetal chromosome abnormality rates of the six groups with NT values < 2.5mm, 2.5-2.9mm, 3.0-3.4mm, 3.5-4.4mm, 4.5-5.4mm and $\geq$ 5.5mm were 0.8%, 11.63%, 17.81%, 20%, 47.62% and 63.64% respectively. The above results showed that the thicker the NT was, the higher the abnormal rate of fetal chromosome was, which is consistent with the previous literature. At present, most studies [16–18] set the cutoff value for prenatal diagnosis of NT thickening as $\geq$ 3.5mm. Maya and Lena [15, 19] suggest that fetuses with NT $\geq$ 3.0mm should be evaluated for prenatal diagnosis. However, the chromosome abnormality rate of fetuses with NT value of 2.5-2.9mm was 11.63% in this study, which was significantly higher than 0.8% of NT value < 2.5mm, and the difference was statistically significant. Therefore, more experimental data are needed to support the criteria for determining the critical value of NT thickening for prenatal diagnosis. Our results support the evaluation of invasive prenatal diagnosis in pregnant women with NT $\geq$ 2.5mm.

The indications of interventional prenatal diagnosis include not only increased NT value, but also the elderly, adverse fertility history, abnormal ultrasound, high risk of serological screening, and chromosomal abnormalities in both husband and wife, etc [20]. In the study of XueShuya [21], the rate of chromosomal abnormalities in fetuses with simple NT thickening was 22.6 %, while the rate of chromosomal abnormalities combined with other ultrasound abnormalities such as lymphocystoma, nasal bone deficiency or fetal edema was 60.9 %. In this study, according to the different indications of prenatal diagnosis, 241 cases of NT thickening were divided into NT thickening alone and NT thickening combined with other abnormalities (fetal nasal bone dysplasia, cervical lymphocystoma, subcutaneous tissue thickening, edema, choroid plexus cyst, single umbilical artery, ventricular hyperintense spot, elderly ,adverse fertility history,etc.). The abnormal rates of fetal chromosomes were 13.17% and 35.14%, respectively. The rate of fetal chromosomal abnormalities is higher when NT thickening combined with other abnormalities, so when NT thickening combined with other ultrasonic abnormalities in early pregnancy, it is more necessary to exclude chromosomal abnormalities in time for prenatal diagnosis.

At present, CMA detection is more and more used in prenatal diagnosis, and many studies have confirmed the correlation between NT thickening and chromosome microdeletion or microrepetitive variation [22, 23]. Grande et al conducted a meta-analysis of 17 studies and concluded that about 4% more pathogenic chromosome microdeletions or microrepetitive variations could be detected by CMA technology in fetuses with normal karyotype analysis [12]. Egloff et al reported that in fetuses with NT thickening in early pregnancy, CMA could detect about 2.7 % of pathogenic chromosome variations that cannot be detected by conventional karyotype analysis [22]. In this study, the karyotype analysis and CMA of chromosome abnormality detection rate in 366 samples were 13.39 % (49 / 366) and 13.93 % (51 / 366), respectively, with no significant difference. However, CMA can bring us more data information, which can correlate genotype with phenotype, and complement each other with karyotype analysis to facilitate clinical consultation. In this study, karyotype analysis showed that 5 samples had complex chromosomal rearrangements and 1 sample had additional marker chromosomes, CMA results not only supplemented the karyotype results, suggesting that five samples had chromosome microdeletions and duplications at the same time, clarifying the source of one marker chromosome (Fig. 2), Fig. 2. was the CMA result and Fig. 2. was the karyotype result. CMA results but also supplemented the genotype was associated with phenotype, giving us more information. In view of the complex chromosome structure rearrangement of five samples, we verified the peripheral blood chromosomes of three samples of parents and found that two cases were new mutations and one case was caused by maternal inheritance (Fig. 1), Fig. 1. and were the CMA result of fetus, Fig. 1. was the karyotype result of fetus, Fig. 1. was the karyotype result of mother. We also classified all CNVs and followed up the pregnancy: 10 cases of pathogenic CNVs (1), 7 cases were detected by karyotype analysis and CMA, and pregnant women chose induced labor after informed choice. (2) 3 cases were detected by CMA but not detected by karyotype analysis. 1 case with a 5.37Mb deletion of 9q22.32q31.1, the related phenotypes included epileptic encephalopathy, language development disorder, macrosomia, learning disability and nephroblastoma. Ultrasound also revealed multiple fetal abnormalities: cervical fold thickening, bilateral cleft lip and palate, posterior fossa cystic structure, and bilateral renal separation. The pregnant woman decided to induce labor after informed choice. 1 case with a 1.4 Mb deletion of 17p12, whose pathogenicity was related to hereditary pressure susceptible peripheral neuropathy. The CMA of parents confirmed that it was caused by the father, and no abnormality was found in ultrasound. After careful consideration, the pregnant woman chose to continue pregnancy, and has delivered a healthy baby. 1 case with a 2.49Mb duplication of 22q11.21, the repetition in this region involves 22q11.2 microrepetition syndrome, but it is an incomplete explicit syndrome, with a penetrance rate of about 21.9%. The related abnormal phenotypes include developmental retardation and mental retardation, hypodystonia, autistic behavior, congenital heart abnormalities, and so on. Considering the normal ultrasound examination and the existence of explicit insufficiency, the pregnant woman carefully chose to continue the pregnancy, and now she has given birth to a normal fetus, about 4 months old, and the cardiac ultrasound examination is normal. 2 cases of probable pathogenic CNVs (1) 1 case with a 394Kb deletion of 1q21.1, this region is the susceptible site of thrombocytopenia-radial deficiency syndrome, the main phenotypes are thrombocytopenia, bilateral radius loss, cardiac and urinary system abnormalities and so on. The syndrome follows the autosomal recessive genetic model. In addition to the single copy deletion in the region, which is a possible pathogenic CNV, gene mutations can also be found in most patients. However, ultrasound examination is normal, the pregnant women carefully choose to continue pregnancy, and now she has given birth to a normal fetus, about 2 months old, heart, urinary and other ultrasound examination is normal. (2) 1 case with a 5.09Mb deletion of 8q22.3q23.1, involving 17 OMIM genes such as ZFPM2. The mutation of ZFPM2 gene is related to cardiac abnormalities of autosomal dominant inheritance such as tetralogy of Fallot and double outlet of right ventricle. and heterozygous mutations of ZFPM2 gene can also be found in patients with partial reversal syndrome. The deletion of ZFPM2 gene can be derived from parents with normal phenotype, and the deletion is a possible pathogenic CNV. Fetal ultrasound and other tests are normal, pregnant women carefully choose to continue pregnancy, has now given birth to a normal fetus, more than 20 days, cardiac ultrasound examination is normal. 15 cases of VOUS: 1 case lost follow-up information, 14 cases of ultrasound and other examinations were normal, It is suggested that the source of VOUS should be confirmed by fetal parents, but only 2 cases were verified by their parents, and the results were all caused by heredity. Pregnant women chose to continue pregnancy carefully and have given birth to healthy fetuses. Most fetuses with VOUS have better pregnancy outcome, but we still need to combine ultrasound and other comprehensive assessment, and carry out long-term and detailed clinical follow-up of born babies to accumulate more clinical information.

Domenico et al showed that [24] when the NT value was 3.5-4.4mm, about 70% of the fetuses had no obvious abnormality until delivery; when the NT value was 4.5-5.4mm, only 50% had no obvious abnormality; when the NT value was 5.5-6.4mm, only 30% had no obvious abnormality; when the NT value was $\geq$ 6.5mm, only 15% had no obvious abnormality. Souka et al pointed out that [14] NT thickening is not only related to chromosome abnormalities, but also with adverse perinatal outcomes caused by various fetal malformations, dysplasia, rupture and genetic syndrome. However, when chromosome problems were excluded and the fetus survived to metaphase without any abnormality in ultrasound, the risk of poor perinatal outcome and postnatal growth retardation did not increase statistically. In this paper, 18 fetuses with $\geq$ 5.0mm were followed up. (1) 9 cases of chromosome abnormalities were detected by karyotype analysis and CMA. Pregnant women chose induced labor after informed choice. (2) in 5 cases, only early ultrasound showed thickening of NT, normal ultrasound such as anaphase aberration, normal karyotype analysis and CMA. Pregnant women chose to continue pregnancy carefully and all gave birth to normal fetuses. (3) 1 case with a 524Kb accication of 3q29, the meaning of repetition in this area was unknown, the parents verified that the repetition was inherited from the father with normal phenotype, and the ultrasound examination was normal, the pregnant woman carefully chose to continue the pregnancy, and now she has given birth to a normal fetus, 18 months old. (4) 1 case of early ultrasound showed that the cervical fold thickened by NT 6.1mm, system ultrasound showed that the thickness of cervical fold is about 9mm and the other was normal, chromosome karyotype analysis and CMA were normal, and the pregnant woman chose to continue the pregnancy cautiously, and now she has given birth to a normal fetus, more than 2 years old. (5) 1 case showed

NT8.0mm and cervical and dorsal lymphoid cystic tumor in the early stage, normal ultrasound in the middle and later stage, normal chromosome karyotype analysis and CMA. The pregnant woman carefully chose to continue the pregnancy, and now she has given birth to a normal fetus for more than 7 months. (6) 1 case of early ultrasound revealed NT 5.0mm and local skin edema of the chest and abdominal wall of the fetus. In the second trimester, the width of bilateral ventricles of the fetus was about 9mm, chromosome karyotype analysis and CMA detection were normal, and the width of the left ventricle of the fetus in the late pregnancy was about 9mm. Noonan and other syndromes were ruled out by gene detection in the whole exon group. The pregnant woman chose carefully to continue the pregnancy, and now she has given birth to a normal fetus for more than 3 months. Among the 18 fetuses, 9 fetuses had normal chromosomes and no obvious abnormalities in ultrasound, and all of them had a good pregnancy outcome. Therefore, even if the NT is thickened, the fetus usually has a better pregnancy outcome when there are no obvious abnormalities in chromosome, whole exon gene detection and middle and late ultrasound, which should be treated with caution.

Conclusions

In summary, with the thickening of NT, especially when combined with other abnormalities, the rate of fetal chromosome abnormalities gradually increased. Therefore, invasive prenatal diagnosis should be performed for pregnant women with NT ≥ 2.5 mm, and chromosome karyotype analysis and CMA detection should be combined to supplement each other for prenatal genetic counseling. However, even if the NT is thickened, we need to be cautious. After excluding chromosome, whole exon gene detection and ultrasound abnormalities, the fetus usually has a better pregnancy outcome.

Declarations

Ethics approval and consent to participate

The study was approved by the Hospital Ethics Committee of Anhui Maternity and Child Health Care Hospital. Each patient signed informed consent prior to study enrollment.

Consent for publication

Not applicable.

Availability of data and materials

The data supporting the conclusions of this article is included within the article.

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Authors' contributions

All authors have participated in the study and manuscript preparation.

Conflict of interest

The authors have declared no conflicts of interest.

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Tables

Table 1. Distribution of chromosomal abnormalities detected by karyotype and chromosomal microarray analysis(CMA).

NT(mm)	N(%)	karyotype						CMA					isolation rate
		Trisomy 21	Trisomy 18	Trisomy 13	45,X	Others	Total	pathogenic CNVs	likely pathogenic CNVs	VOUS	aneuploid	Total	
<2.5	125	0	0	0	0	2	2	0	0	4	1	5	0.8(1/125)
2.5-2.9	86	4	0	0	0	6	10	3	1	6	7	17	11.63(10/86)
3.0-3.4	73	5	2	0	1	3	11	4	0	3	9	16	17.81(13/73)
3.5-4.4	50	4	2	0	3	0	9	1	1	1	9	12	20(10/50)
4.5-5.4	21	8	0	0	0	2	10	1	0	0	9	10	47.62(10/21)
≥5.5	11	4	1	0	1	1	7	1	0	1	6	8	63.64(7/11)
Total	366	25	5	0	5	14	49	10	2	15	41	68	

Table 2. Classification of CNV and pregnancy outcome

Case	NT(mm)	CMA result	Size and type of CNV	Genes affected /syndromes	Categorization	Pregnancy outcome
1	3.0	arr 4q35.1q35.2(186,167,916-187,842,570)x3	1.67Mb dup	10 OMIM genes	VOUS	Unknown
2	3.3	arr 7q31.31q36.3(120,072,971-159,119,707)x3, 18p11.32p11.31(136,227-3,251,461)x1	39.03Mb dup, 3.11Mb del	7q31.31-qter,10 OMIM genes	Pathogenic, VOUS	Give up, induce labor
3	6.4	arr 18p11.32p11.23(136,227-7,216,195)x1, 18q11.2q23(24,858,577-78,013,728)x3	7.08Mb del, 53.15Mb dup	18p,18q	Pathogenic, Pathogenic,	Give up, induce labor
4	2.9	arr 10p15.3(100,047-1,745,277)x4, 10p12.1p11.1(24,914,898-39,030,508)x2-3	1.64Mb dup, 14.11Mb dup(mosaic)	10 OMIM genes, 43 OMIM genes	VOUS, Pathogenic	Give up, induce labor
5	3.1	arr 2q37.3(239,198,046-242,782,258)x1, 18q21.1q23(44,353,417-78,013,728)x3	3.58Mb del, 33.66Mb dup	2q37.3,89 OMIM genes	Pathogenic, Pathogenic	Give up, induce labor
6	5.5	arr 3q29(196,862,001-197,386,180)x3	524Kb dup	2 OMIM genes	VOUS	Delivery, normal
7	2.8	arr 15q13.2q13.3(31,098,690-32,915,723)x3	1.81Mb dup	7 OMIM genes	VOUS	Delivery, normal
8	3.0	arr 17p12(14,083,054-15,482,833)x1	1.4Mb del	5 OMIM genes	Pathogenic	
9	2.7	arr 9p23(9,914,588-10,133,062)x1	218Kb del	1 OMIM genes	VOUS	Delivery, normal
10	3.1	arr 11q22.3(104,708,299-105,459,967)x3	752Kb dup	7 OMIM genes	VOUS	Delivery, normal
11	2.8	arr 1p13.2(112,802,599-113,868,278)x3	1.06Mb dup	8 OMIM genes	VOUS	Delivery, normal
12	2.5	arr 11p14.2p12(26,154,097-40,951,082)x1	14.79Mb del	WAGR	Pathogenic	Give up, induce labor
13	3.2	arr 8p22(15,258,183-15,968,982)x3	711Kb dup	2 OMIM genes	VOUS	Delivery, normal
14	2.9	arr 2q37.3(241,490,065-242,782,258)x1	1.29Mb del	25 OMIM genes	VOUS	Delivery, normal
15	3.9	arr 6q12(65,196,218-65,743,530)x1	547Kb del	1 OMIM genes	VOUS	Delivery, normal
16	3.3	arr 22q11.21(18,970,561-21,461,017)x3	2.49Mb dup	22q11.2	Pathogenic (nonpenetrance)	Delivery, normal
17	5.2	arr 5p15.33p15.31(113,576-9,149,369)x1, 5p15.31q11.1(9,153,500-49,475,697)x3	9.03Mb del, 40.32Mb dup	,5p	Pathogenic, Pathogenic	Give up, induce labor
18	2.5	arr 9p24.3p23(208,454-9,085,530)x1, 13q31.1q34(87,397,574_115,107,733)x3	8.87Mb del, 27.71Mb dup	34 OMIM genes, 78 OMIM genes	Pathogenic, Pathogenic	Give up, induce labor
19	2.8	arr 12p11.21p11.1(32,407,341-34,897,417)x3, 12q11q12(37,856,237-42,720,825)x3	2.49Mb dup, 4.86Mb dup	7 OMIM genes, 11 OMIM genes	VOUS, VOUS	Delivery, normal
20	2.7	arr 1q21.1(145,382,123-145,775,966)x1	394Kb del	14 OMIM genes	likely pathogenic	Delivery, normal
21	4.4	arr 8q22.3q23.1(103,348,066-108,445,788)x1	5.09Mb del	17 OMIM genes	likely pathogenic	Delivery, normal
22	2.9	arr 22q11.21(20,716,876-21,464,764)x3	748Kb dup	12 OMIM genes	VOUS	Delivery, normal
23	2.3	arr 7q36.1(151,839,233-151,956,096)x3	117Kb dup	1 OMIM genes	VOUS	Delivery, normal
24	1.2	arr 4q21.3(87,065,802-87,601,097)x4	535Kb dup	2 OMIM genes	VOUS	Delivery, normal
25	0.9	arr 6p25.2(2,662,677-3,266,323)x3	604Kb dup	10 OMIM genes	VOUS	Delivery,

						normal
26	1.4	arr 18q12.3(39,280,178-40,567,434)x1	1.28Mb del	2 OMIM genes	VOUS	Delivery, normal
27	4.4	Arr9q22.32q31.1(97,430,110-102,801,381)x1	5.37Mb del	32 OMIM genes	Pathogenic	Give up, induce labor

Table 3. The percentages of abnormal karyotype in each groups with different indications

Indication	Number	karyotypic abnormality		X value	P value
		Number	Percentage		
Nt thickening	167	22	13.17%		
Nt thickening +other	74	26	35.14%	15.506	0.001

Table 4. Karyotype, CMA results and pregnancy outcome of fetuses with NT $\geq$ 5.0mm

Case	NT(mm)	CMA	Karyotype	Pregnancy outcome
1	9.4	arr Xp22.33q28(168,551-155,233,098)x1	45,X	Give up, induce labor
2	6.1	arr(1-22)x2,(XN)x1	46,XY	Delivery, normal
3	6.4	arr 18p11.32p11.23(136,227-7,216,195)x1, 18q11.2q23(24,858,577-78,013,728)x3	46,XX,add(18)(p11.2)	Give up, induce labor
4	5.5	arr 21q11.2q22.3(15016486-48093361)x3	47,XX,+21	Give up, induce labor
5	6.6	arr 18p11.32q23(136227_78013728)x3	47,XY,+18	Give up, induce labor
6	7	arr (1-22)*2,(XN)*1	46,XX	Delivery, normal
7	5.5	arr 3q29(196,862,001-197,386,180)x3	46,XY	Delivery, normal
8	8.0	arr 21q11.2q22.3(15,016,486-48,093,361)x3	47,XY,+21	Give up, induce labor
9	9.2	arr 21q11.2q22.3(15,016,486-48,093,361)x3	47,XX,+21	Give up, induce labor
10	8.0	arr (1-22)*2,(XN)*1	46,XX	Delivery, normal
11	5.4	arr (1-22)*2,(XN)*1	46,XY	Delivery, normal
12	5.4	arr (1-22)*2,(XN)*1	46,XY	Delivery, normal
13	7.8	arr 21q11.2q22.3(15,016,486-48,093,361)x3	47,XY,+21	Give up, induce labor
14	5.4	arr 5p15.33p15.31(113576_9149369)x1; 5p15.31q11.1(9153500_49475697)x3	46,XY,add(5)(p15.3)	Give up, induce labor
15	5.2	arr 21q11.2q22.3(15,016,486-48,093,361)x3	47,XY,+21	Give up, induce labor
16	5.2	arr (1-22)*2,(XN)*1	46,XY	Delivery, normal
17	5.1	arr(1-22)*2,(XN)*1	46,XX	Delivery, normal
18	5.0	arr(1-22)*2,(XN)*1	46,XY	Delivery, normal

Figures

Figure 1

A case with chromosome microdeletion and microduplication

Figure 2

A case with marker chromosome