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Prenatal diagnosis of fetuses with increased nuchal translucency using an approach based on quantitative fluorescent polymerase chain reaction and genomic microarray

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ABSTRACT

Objective: To assess the clinical value of prenatal diagnosis of fetuses with increased nuchal translucency (NT) using an approach based on quantitative fluorescent polymerase chain reaction (QF-PCR) and chromosomal microarray (CMA).
Study design: From January 2013 to October 2014, we included 175 pregnancies with fetal NT ≥3.5 mm at 11–13 weeks' gestation who received chorionic villus sampling. QF-PCR was first used to rapidly detect common aneuploidies. The cases with a normal QF-PCR result were analyzed by CMA.
Results: Of the 175 cases, common aneuploidies were detected by QF-PCR in 53 (30.2%) cases (30 cases of trisomy 21, 12 cases of monosomy X, 7 cases of trisomy 18, 3 cases of trisomy 13 and 1 case of 47, XXY). Among the 122 cases with a normal QF-PCR result, microarray detected additional pathogenic copy number variants (CNVs) in 5.7% (7/122) of cases. Four cases would have expected to be detectable by conventional karyotyping because of large deletions/duplications (>10 Mb), leaving three cases (2.5%; 3/118) with pathogenic CNVs only detectable by CMA.
Conclusion: It is rational to use a diagnostic strategy in which CMA is preceded by the less expensive, rapid, QF-PCR to detect common aneuploidies. CMA allows detection of a number of pathogenic chromosomal aberrations in fetuses with a high NT.

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Introduction

A high correlation between increased nuchal translucency (NT) and trisomy 21 and other chromosomal defects has been reported. Using a value of NT ≥3.5 mm, chromosomal abnormality rate approached 48.8% [1]. The majority of chromosomal anomalies associated with enlarged NT are common aneuploidies, especially trisomies 21, 18, and 13 and monosomy X. A high NT has also been found to be associated with submicroscopic chromosomal abnormalities, which can be detected by chromosomal microarray analysis (CMA). The entire genome can be screened for DNA gains or losses, called copy number variants (CNVs), as small as 50–100 kb, which are not routinely seen on karyotyping, the standard cytogenetic analysis performed. One systematic review of seventeen publications and meta-analysis found that the use of genomic microarray provides a 5% incremental yield in fetuses with increased NT and normal karyotype [2]. Quantitative fluorescence polymerase chain reaction (QF-PCR), in which DNA polymorphic markers (microsatellites) locate at autosomes 13, 18 or 21 or sex chromosomes, is also a useful test for prenatal diagnosis, as it provides a rapid diagnosis of common aneuploidies and can detect maternal contamination.

Recent reports have highlighted that adverse outcomes are much more common with an NT that exceeds a set threshold of 3.5 mm, a measurement that essentially represents 99th percentile or more throughout the gestational age window for first trimester screening [3]. The objective of this study was to assess the performance of a prenatal diagnostic service using combined QF-PCR and CMA as an investigative strategy for fetuses with an NT above the 99th percentile (NT ≥3.5 mm) during a first-trimester prenatal screening program.

Materials and methods

We performed a retrospective cohort study of all fetuses referred because of an NT ≥3.5 mm to the Prenatal Diagnostic

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Center, Guangzhou Women and Children Medical Center during the period 2013–2014. We chose NT ≥3.5 mm, commonly considered to represent the 99th centile for normal ranges, as this is the cutoff that many studies use to define a cohort for sequential follow-up after karyotyping. Our unit serves as one of referral centers for prenatal diagnosis for the Guangzhou region of Southern China. NT was assessed according to the standards of the Fetal Medicine Foundation (FMF) of London at first trimester (fetal crown-rump length ranged between 45 and 84 mm) by FMF certified sonographers. All patients with an NT ≥3.5 mm were offered direct chorionic villus sampling (CVS) without combining maternal serum biomarkers for risk calculations.

All women received pretest counseling regarding the procedure-related risks and the scope of diagnostic possibilities from the QF-PCR and microarray. Written consent was obtained. Results of the QF-PCR tests were available to the patient within 48 h. It was told that if there were copy number abnormalities of the targeted chromosomes 21, 18, 13 and X, the results would be treated as a final report, and no further test would be offered; if the rapid karyotyping result was normal, microarray analysis would be followed. A CytoScan 750K array (Affymetrix Inc., Santa Clara, CA, USA) was used for assessing submicroscopic chromosomal abnormalities, which has greater than 99% sensitivity and can reliably detect 200 kb copy number changes across the genome at high specificity with SNP (allelic) call corroboration. The procedures for DNA digestion, amplification, segmentation, labeling, and hybridization with the arrays were performed according to the manufacturer's standard protocols (Affymetrix Inc., Santa Clara, CA, USA). The results were analyzed using Chromosome Analysis Suite software and the results were determined by using publicly available CNV databases and by investigating gene content and the scientific literature. The public databases include database of genomic variants (DGV; http://projects.tcag.ca/variation), database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER; http://decipher.sanger.ac.uk), online Mendelian Inheritance in Man (OMIM; http://www.omim.org), and University of California Santa Cruz (UCSC; http://genome.ucsc.edu/, hg19). Blood samples were collected from both parents alongside CVS and were analyzed if variants of uncertain significance (VOUS) were detected in the fetal sample by CMA. The typical turn-around time was 2 weeks. Our specialists in the fetal medicine team offered counseling for women with array results.

Results

Over the study period, there were 175 fetuses with an NT ≥3.5 mm referred for further investigation. The median maternal age was 28.8 (range, 18–45) years, the median gestational age was 12.4 (range, 11.0–13.8) weeks and median fetal NT thickness was 4.6 (range, 3.5–9.3) mm.

Of the 175 cases, common numerical chromosomal abnormalities were detected by QF-PCR in 53 (30.2%) cases (30 cases of trisomy 21, 12 cases of monosomy X, 7 cases of trisomy 18, 3 cases of trisomy 13 and 1 case of 47, XXY). All of these affected pregnancies were terminated by parents' request.

In the remaining 122 cases CMA was performed. The array detected additional pathogenic CNVs in 5.7% (7/122) of cases, and their size ranged from 1.7 Mb to 23.2 Mb (Table 1). Four cases would have expected to be detectable by conventional karyotyping because of large deletions/duplications (>10 Mb), leaving three cases (2.5%; 3/118) with pathogenic CNVs only detectable by CMA. One case of VOUS was detected. Termination of pregnancy was carried out in six of the seven cases with pathogenic CNVs (except case 6) after the genetic counseling. Fig. 1 is a flow diagram of the prenatal QF-PCR or CMA results. In Table 2 we compare our present findings with those of recent publications. Of the remaining 114 cases with normal QF-PCR and array results, 91 (79.8%) resulted in live births, one (0.9%) intrauterine fetal death, 20 (17.5%) termination of pregnancy because of second trimester structural defect, and two (1.8%) miscarriage.

Comments

Fetal NT between 11 and 14 weeks' gestation is an effective screening method for chromosomal abnormalities. The incidence of chromosomal defects increases with NT thickness. Studies have found that the incidence was approximately 7% for those with NT between the 95th centile and 3.4 mm, and 75% for NT of 8.5 mm or more [4]. In a previous study, we found a prevalence of 0.5% of fetuses with an NT ≥3.5 mm in a population performing prenatal screening for trisomy 21 in the first trimester of pregnancy [5]. The current study showed that 30% of fetuses with an NT ≥3.5 mm had common aneupoidies; of these, trisomy 21 is the most common. All of these common aneupoidies were detected by QF-PCR. This means that about one third of fetuses with an increased NT would be one of the common aneupoidies which were targeted by rapid QF-PCR, and would not need a further deep test. The clinical utility of this assay has been repeatedly confirmed, together with its high sensitivity and specificity (>99%) in detecting major chromosomal abnormalities [6–10]. In clinical practice, this approach would help patients save time and money.

An important concern over those prenatally undetectable conditions is a heavy burden for parents who have a high NT scan. Although the majority of structural anomalies are amenable to ultrasound detection, unspecified genetic syndromes involving developmental delay may only emerge after birth. Therefore we used CMA as a second-line prenatal diagnostic test without additional conventional karyotyping following a normal QF-PCR result. In 122 pregnancies with high fetal NT and normal QF-PCR results, CMA detected pathogenic CNVs in 5.7% of cases. Four cases could have been detected on karyotyping, resulting in 2.5% of cases

Table 1
Pathogenic copy number variants (CNVs) and variants of uncertain significance (VOUS) detected by chromosomal microarray (CMA).

Case	MA (years)	GA (weeks)	NT (mm)	Microarray results	Size (Mb)	Genes affected/syndromes	Conclusion
1	27	13.2	7.1	arr9q33.2-q34.3 (125,152,552–141,020,389)×3	15.87	9q subtelomeric deletion syndrome	Pathogenic
2	21	12.4	5.1	arr3p26.3-p25.3 (61,891–11,762,262)×1	11.7	81 genes (24 morbid OMIM genes)	Pathogenic
				arr5q35.3(178,478,240–180,719,789)×3	2.24	16 genes (4morbid OMIM genes)	Pathogenic
3	23	11.6	4.9	arr1q42.11-q44(224,225,265–247,517,799)×3	23.29	92 genes (23 morbid OMIM genes)	Pathogenic
				arr1q44(247,525,490–249,224,684)×1	1.7	2 genes (1 morbid OMIM gene)	Pathogenic
4	25	11.7	5.6	arr9q21.33q22.33(89,745,719–101,837,787)×1	12.09	58 genes (16morbid OMIM genes)	Pathogenic
5	31	12.0	3.6	arr22q11.21(18,631,364–21,800,471)×1	3.17	22q11.2 deletion syndrome	Pathogenic
6	32	13.1	3.6	arr22q11.21(18,970,561–21,290,591)×3	2.32	22q11.2 duplication syndrome	Pathogenic
7	30	12.4	7.6	arr17p12p11.2(15,759,453–20,547,625)×1	4.79	Smith-Magenis Syndrome	Pathogenic
8	29	11.2	6.5	arr16p13.11(14,927,371–16,458,424)×3	1.53	16p13.11 microduplication	VOUS

MA, maternal age; NT, nuchal translucency; VOUS, variants of uncertain significance.

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