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ORIGINAL ARTICLE

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Clinical application of chromosomal microarray analysis in fetuses with increased nuchal translucency and normal karyotype

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Abstract

Background: Submicroscopic chromosomal imbalance is associated with an increased nuchal translucency (NT). Most previous research has recommended the use of chromosomal microarray analysis (CMA) for prenatal diagnosis if the NT ≥ 3.5 mm. However, there is no current global consensus on the cutoff value for CMA. In this study, we aimed to discuss the fetuses with smaller increased NT which was between cutoff value of NT for karyotype analysis (NT of 2.5 mm in China) and the recommended cutoff value for CMA (NT of 3.5 mm) whether should be excluded from CMA test.

Methods: Singleton pregnant women ($N = 192$) who had undergone invasive procedures owing to an increased NT (NT ≥ 2.5 mm) were enrolled. Fetal cells were collected and subjected to single nucleotide polymorphism array and karyotype analyses simultaneously. Cases were excluded if the karyotype analysis indicated aneuploidy and apparent structural aberrations.

Results: Fourteen cases of aneuploidy and four cases of structural abnormalities were excluded. Of the remaining 174 cases, 119 fetuses had NTs of 2.5–3.4 mm, and 55 fetuses with NT ≥ 3.5 mm. Eleven copy number variants (CNVs) were identified. In fetuses with smaller NTs, six (6/119, 5.9%) variations were detected, including two (2/119, 1.6%) clinically significant CNVs (pathogenic or likely pathogenic CNV), one likely benign CNV, two variants unknown significance, and one incidental CNV. Five (5/55, 9.1%) variations were found in fetuses with NT ≥ 3.5 mm. Among these CNVs, three (3/55, 5.5%) cases had clinically significant CNVs, and two had likely benign CNV. There were no statistically significant differences in the incidence of all CNVs and clinically significant CNVs in the two groups ($p > 0.05$).

Conclusion: CMA improved the diagnostic yield of chromosomal aberrations for fetuses with NTs of 2.5–3.4 mm and apparently normal karyotype, regardless of whether other ultrasonic abnormalities were observed.

KEYWORDS
chromosomal microarray analysis, karyotyping, nuchal translucency, prenatal diagnosis

1 | INTRODUCTION

Abnormally high nuchal translucency (NT) values indicate an increased NT thickness, which is associated with chromosomal abnormalities and fetal malformations (Souka, Von Kaisenberg, Hyett, Sonek, & Nicolaides, 2005). The incidence of chromosomal defects increases with NT thickness from approximately 7% for those with NT between the 95th percentile for crown rump length and 3.4 mm to 75% for NT of 8.5 mm or more (Kagan, Avgidou, Molina, Gajewska, & Nicolaides, 2006). Moreover, the absence of aneuploidy in fetuses with increased NT has been shown to be associated with an increased risk of other fetal defects, particularly cardiac and skeletal abnormalities (Grande et al., 2012; Westin et al., 2006).

Previous studies have shown that the general population (95%–96%), exhibiting unremarkable anatomic ultrasound results and a normal karyotype, will have uneventful outcomes (Alamillo, Fiddler, & Pergament, 2012). However, some cases still show adverse outcomes, such as structural or neurodevelopmental abnormalities and genetic syndromes identified postpartum (Alamillo et al., 2012; Bilardo et al., 2007; Lund, Christensen, Petersen, Vogel, & Vestergaard, 2015). A series of recent studies revealed that many fetuses with increased NT show submicroscopic chromosomal imbalances other than apparent chromosomal disorders (Leung et al., 2011; Maya et al., 2017), and these anomalies cannot be detect by conventional cytogenetic techniques because of the limited resolution of such approaches.

With advancements in genetic analysis technologies, chromosomal microarray analysis (CMA) has been developed to examine the chromosome aneuploidy, large fragment deletions or duplications, and submicroscopic copy number variant (CNV) abnormalities that cannot be detected by karyotyping. Moreover, single nucleotide polymorphism (SNP) array analysis also can detect triploidy, uniparental disomy (UPD), and loss of heterozygosity (LOH). Because of these advantages, CMA has been recommended as the first-line test for prenatal diagnosis of fetuses with one or more major structural abnormalities identified on ultrasonographic examination (Committee on, G., & the Society for Maternal-Fetal, M, 2016).

Most researchers have suggested that CMA should be recommended for prenatal diagnosis if the NT is 3.5 mm or more (Armour et al., 2018). However, it is still unclear whether the cutoff value for karyotyping can also be applied to CMA. Therefore, in this study, we described our experience using CMA to discuss the fetuses with smaller increased NT which is between cutoff value of NT for karyotype analysis (NT of 2.5 mm in China) and the recommended cutoff value for CMA (NT of 3.5 mm) whether should be excluded from CMA test.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

All procedures implemented in the study were in accordance with the ethical standards under the supervision of the Declaration of Helsinki and the 《Methods for Ethical Review of Biomedical Research involving people》 by the State and the Ethics Committee of Fujian Provincial Maternity and Children's Hospital (ethics approval number 2016-051) in 2016. Written informed consents were collected from all participants.

2.2 | Patients and samples

All invasive samples were collected from singleton pregnant women with NT measurement who had both G-banded karyotyping and CMA performed at Fujian Provincial Maternity and Children's Hospital, China between November 2016 and March 2019. NT measurements in these cases were performed at gestational ages of 11–13 + 6 weeks. In the pretest counseling session, all pregnant couples received genetic counseling, including information regarding the risk of miscarriage during invasive testing, the relative advantages and disadvantages of karyotype and SNP array analyses, and the potential for finding a variant of unknown significance (VOUS). According to NT values, the cohort was divided into two groups: those with NTs of 2.5–3.4 mm (Group A) and those with NTs of 3.5 mm or more(Group B). Data for maternal age, gestational age at screening, value of NT, parity, fetus gender ratio, karyotype and CMA results, and other fetal defects were collected during first-trimester screening. Cases were excluded if the karyotype analysis indicated aneuploidy and structural aberrations.

2.3 | SNP array analysis

Genomic DNA was extracted from cultured chorionic villi, direct amniocytes, cultured amniocytes, or fetal blood using a Qiagen DNA Mini kit (250; Qiagen, Valencia, CA, USA), according to the manufacturer's protocol. DNA samples were purified and concentrated using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific Inc.) and BioPhotometer plus (Eppendorf Inc.). For SNP array analysis, Genomic DNA was screened using CytoScan 750K (Affymetrix Inc., CA, USA), which contained 200,000 SNP and 550,000 CNV tags. Array images were analyzed using Affymetrix gene chip kit software ChAS 3.2.

2.4 | Data interpretation

Copy number variants were compared with our in-house database of CNVs and public CNV databases (Database of

Genomic Variants [http://projects.tcag.ca/variation/]; Decipher [http://decipher.sanger.ac.uk]; ISCA [https://www.iscaconsortium.org]; UCSC [http://genome.ucsc.edu/]; OMIM [http://www.omim.org/]) by trained analysts and were classified as pathogenic, likely pathogenic, VOUS, likely benign, or benign according to the guidelines of the American College of Medical Genetics. Benign CNVs were not reported to the parents. However, both pathogenic CNVs and VOUS were reported to the parents during posttest counseling. Parental testing was commended to confirm the aberration if a VOUS was detected.

2.5 | Statistical analysis

Maternal clinical characteristics and experimental results were analyzed using SPSS statistical software at the time of collection. Data are presented as means and standard deviations, and independent exponent *t* tests and Chi-squared tests were performed to determine the significance of differences. Results with *p* values of less than 0.05 were considered significant.

3 | RESULTS

3.1 | Findings

In total, 192 cases were subjected to both karyotype and CMA analyses, among which 14 cases of aneuploidy and four cases of chromosomal structural abnormalities were excluded. The remaining 174 cases (119 fetuses with NTs of 2.5–3.4 mm and 55 fetuses with NTs of ≥3.5 mm) with normal karyotypes were collected (Figure 1). Maternal age, gestational age at screening, parity, and fetus gender ratio

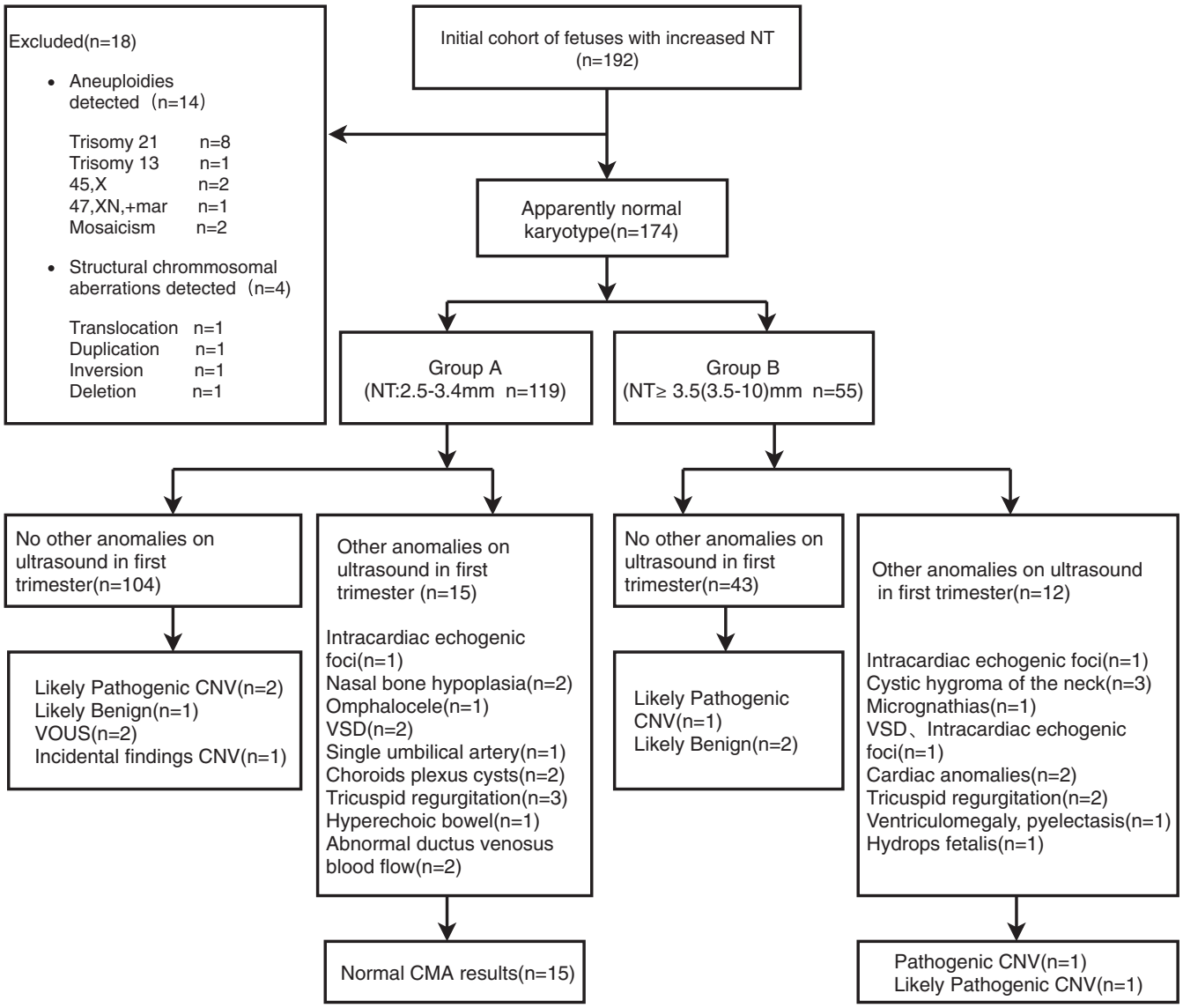

FIGURE 1 Flowchart of characteristics of pregnant women and aberrant findings from SNP array analysis of fetuses with increased NTs greater than or equal to 2.5 mm. CNV, copy number variant; NT, nuchal translucency; SNP, single nucleotide polymorphism; VOUS, variant of uncertain significance

TABLE 1 Clinical characteristics of the women between Group A compared to Group B

	Group A $N = 55$	Group B $N = 119$	*p*-Value
Maternal age (year) (Mean ± *SD*)	30.75 ± 3.86	31.35 ± 4.83	0.414[a]
GA at screening (days) (Means ± *SD*)	88.76 ± 5.15	89.92 ± 4.40	0.127[a]
NT (mm) (Means ± *SD*)	4.60 ± 1.40	2.94 ± 0.27	<0.001[a]
Parity(*n*) (Means ± *SD*)	2.33 ± 1.263	2.46 ± 1.326	0.527[a]
Female fetuses: *n* (%)	18 (32.7%)	35 (29.4%)	0.659[b]

Abbreviations: GA, gestational age; NT, nuchal translucency.
[a]*t* tests.
[b]Chi-squared tests.

did not differ between the groups according to *t* tests and Chi-squared tests. However, NT values were significantly different between the two groups. Additional details are shown in Table 1.

Among 174 cases, CNVs were detected in 11 fetuses. In Group A, six (6/119, 5.9%) fetuses with CNVs were detected, included two clinically significant CNVs (pathogenic or likely pathogenic CNV), one likely benign CNV, two VOUSs, and one incidental CNV. These cases had no other sonographic findings except increased NTs. In Group B, among five detected CNVs (5/55, 9.1%), three cases had clinically significant CNVs and two had likely benign CNV. Therefore, the incidence rates of clinically significant CNVs were 1.6% (2/119) and 5.5% (3/55) in Group A and Group B, respectively. There were no statistically significant differences in the incidence rates of all CNVs and clinically significant CNVs between the two groups ($p > 0.05$; Table 2).

3.2 | Fetuses with NTs of 2.5–3.4 mm

In Group A, 104 pregnancies had no ultrasonic indication for prenatal diagnosis other than NT in the first trimester for invasive prenatal diagnosis. The other 15 cases had other sonographic findings (Figure 1).

There were two cases (cases 2 and 6) with clinically significant CNVs (Table 3). In case 2, the deleted fragment locus was a genomic hotspot, particularly rich in low-copy repeats on 16p13.11 (Hannes et al., 2009). The incidence of this variant was less than 1% in the general population. The clingen haploid dose effect of the variant was 3. Its penetrance was 10%–20%, and the variant was associated with a wide spectrum of disorders including schizophrenia, autism, mental retardation, intellectual disability, epilepsy, and mild microcephaly (Hannes et al., 2009; Heinzen et al., 2010). Therefore, we assumed the deletion was likely pathological, regardless of the genotype of the parents. For case 6, a de novo duplication of 3.1 Mb was detected on 22q. This repetitive fragment could cause 22q11.2 microduplication syndrome. Because of the incomplete penetrance of this disorder, the phenotype of patients with microduplications is extremely diverse. Carriers range from having no clinical phenotype to having variable degrees of developmental delay, cardiac malformation, or dysfunction (Yobb et al., 2005). We classified this variant as probably pathogenic.

In case 3, CMA suggested a 5.6-Mb deletion involving parts of chromosome Yq. This deletion contained two regions of azoospermia factor (AZF; the entire AZFb region and the partial AZFc region), and three pivotal genes (*HSFY1*(*400029), *PRY*(*400019), and *DAZ1*(*400003)), which would likely cause severe oligospermia and male infertility. Because the CNVs were unrelated to the abnormal phenotype and beyond the aim of our prenatal analysis, we classified these results as incidental findings.

For specimens with uncertain clinical significance, parental investigations were performed. A 0.62-Mb duplication on 5p23 was confirmed to be inherited from the mother in case 1, and this variant was probably benign. Additionally, a 0.43-Mb duplication and a 0.37-Mb deletion were observed in cases 4 and 5. Because parental verification was not carried out, the sources of CNVs were unknown. We classified this duplication as a VOUS. Because the repeated fragment was relatively small, that is, smaller than the threshold of 500kb for deletions and 1 Mb for duplications (Buchanan et al., 2015). This variant was likely to be benign.

3.3 | Fetuses with NTs of 3.5 mm or more

There were 43 pregnancies with isolated abnormal NTs and 12 pregnancies had other sonographic findings in fetuses with NTs of 3.5 mm or more.

The duplication in case 7 and deletion in case 8 were both inherited from their father, who had a normal phenotype. Moreover, no definite pathogenic evidence had been reported. Hence, these variants were classified as likely benign. In case 9, ultrasound examination revealed increased NT accompanied by intracardiac echogenic foci at 13 weeks. Bilateral choroid plexus cysts were observed at 17 weeks. A de novo, pathogenic, 1.6-Mb deletion on 3q29 was detected by CMA, which overlapped with the region of 3q29 deletion syndrome, leading to mental retardation, autism, infantile

TABLE 2 Summary of the detection rates of microarray from pregnancies with increased NT

Group	*n*	Modality		Isolated NT (*n*)	Nonisolated NT (*n*)	All CNVs detected by CMA *n* (%)	Clinically significant CNVs *n* (%)	Likely Benign CNVs *n* (%)	VOUS *n* (%)	Incidental findings CNVs *n* (%)
		AC	CVS							
Group A (NT 2.5–3.4 mm)	119	115	4	104	15	6 (5.9%)[a]	2 (1.6%)[b]	1	2	1
Group B (NT ≥ 3.5 mm)	55	47	8	43	12	5 (9.1%)[a]	3 (5.5%)[b]	2	0	0
Total	174	162	12	148	22	11 (6.3%)	5	3	2	1

Abbreviations: AC: amniocytes; CMA, chromosomal microarray analysis; CNV, copy number variant; CVS: Chorionic Villus Sampling; NT, nuchal translucency; Path, pathogenic; VOUS, variant of unknown significance.

[a]Detectable by CMA in Group A versus Group B: $p = 0.493 > 0.05$.

[b]Clinically significant CNVs by CMA in Group A versus Group B: $p = 0.369 > 0.05$.

autism, language retardation, microcephaly, or characteristic facial abnormalities. Case 10 was found to exhibit increased NT accompanied by cystic hygroma of the neck at 13 weeks. At 17 weeks, an ultrasound examination showed normal fetal and placental anatomy. At CMA, the male fetus was shown to have a de novo, 1.0-Mb deletion of 22q11.21. This deleted fragment overlapped with the common ~3.0-Mb deletion of DiGeorge/velocardiofacial syndrome (Carlson et al., 1997). Although the deletion was inherited from normal parents, haploinsufficiency could also lead to thymic aplasia, cardiac anomalies, mental retardation, and facial dysmorphia because of incomplete penetrance (Goes & Sawa, 2017).

Notably, one specimen showed karyotyping results that were different from the results of CMA. Amniocentesis was performed at 19 weeks of gestation because of an isolated NT of 3.5 mm and revealed low-level (23%) mosaicism of trisomy 2, whereas CMA suggested that there were no CNVs in direct amniocentesis at the same time point. Subsequently, secondary karyotyping of both umbilical cord blood and amniotic fluid cells was performed at 25 weeks; neither of these tests showed abnormalities, and the infant was born healthy.

3.4 | Pregnancy outcomes

In total, for 11 cases with CNVs, three women underwent elective termination of pregnancy for chromosomal imbalance, five women continued the pregnancy and had successful live births, and three women were still pregnant at the time of writing this manuscript.

4 | DISCUSSION

To date, karyotype analysis is still the preferred method of prenatal diagnostic testing in China. However, owing to the limitations of karyotype analysis, most prenatal diagnostic facilities discuss the benefits and limitations of CMA and conventional karyotyping with all pregnant women who undergo prenatal diagnostic testing, and provide additional options for CMA, similar to Committee Opinion (Committee on, G., & the Society for Maternal-Fetal, M, 2016).

Increased NT is an indication for CMA (Armour et al., 2018). This technique has a higher resolution than conventional karyotyping, allowing for the detection of smaller, submicroscopic imbalances, even the UPD and LOH, by SNP array. For fetuses having an increased risk of submicroscopic chromosomal imbalances, such as fetuses with increased NT, the usefulness of the additional information supplied by CMA in prenatal diagnosis is obvious, and the rate of undiagnosed or potential genetic disorders is decreased. CMA could offer more precise prognostic insights

TABLE 3 Summary of genetic imbalances, identified by chromosomal microarray (CMA), in 11 fetuses with increased nuchal translucency (NT)

Case	NT (mm)	Findings on US	Karyotype	Sample	SNP array result (ISCN)	Size (Mb)	Related syndrome/gene	Conc.	Inheritance	Outcome
1	2.6	Normal	46,XY	Direct Amni	arr[hg19]5q23.1(115,690,982-116,314,598)x3 mat	+0.62	*COMMD10* (*616704), *SEMA6A* (* 605885) *and RPS17P2*	Likely benign	maternal	ongoing
2	2.6	Normal	46,XX	Direct Amni	arr[hg19] 16p13.11(14,910,158-16,508,123)x1	−1.6	45 genes (3 morbid OMIM genes)	Likely Path	—	TOP
3	2.7	Normal	46,XY	Direct Amni	arr[hg19] Yq11.222q11.223(20,252,055-25,863,576)×0 dn	−5.6	*HSFY1* (*400029), *PRY* (*400019) *and DAZ1* (*400003)	Incidental findings	De novo	LB
4	3.0	Normal	46,XX	Direct Amni	arr[hg19] Xq23(109,823,197-110,252,333)x3	+0.43	*CHRDL1* (* 300350), *PAK3* (*300142)	VOUS	—	LB
5	3.2	Normal	46,XX	Direct Amni	arr[hg19]9q31.3(113,177,821-113,544,998)x1	−0.37	*SVEP1* (*611691) *and MUSK* (*601296)	VOUS	—	ongoing
6	3.3	Normal	46,XY	Direct Amni	arr[hg19] 22q11.21(18,648,855-21,800,471) ×3 dn	+3.1	22q11.2 duplication syndrome (#608363)	Likely Path	De novo	LB
7	3.6	Normal	46,XY	Direct Amni	arr[hg19] 15q13.2q13.3 (30,386,398-32,915,723)x3 pat	+2.5	28 genes (2 morbid OMIM genes)	Likely benign	Paternal	LB
8	4.0	Normal	46,XY	Direct Amni	13q33.2(105,477,621-106,424,479)x1 pat	+0.95	*DAOA* (*607408), *DAOA-AS1* (*607415) *and LINC00343*	Likely benign	Paternal	LB
9	4.5	Intracardiac echogenic foci,	46,XY	Direct Amni	arr[hg19]3q29(195,678,474-197,340,833)x1 dn	+1.6	3q29 deletion syndrome (OMIM #609425)	Path	De novo	TOP
10	5.0	Cystic hygroma of the neck	46,XY	Direct Amni	arr[hg19]22q11.21(20,716,876-21,800,471)x1 dn	+1.0	29 genes (5 morbid OMIM genes)	Likely Path	De novo	TOP
11	5.2	Normal	46,XY	Cultured CVS	arr[hg19]22q11.21(20,730,143-21,800,471)x3	+1.0	29 genes (5 morbid OMIM genes)	Likely Path	—	TOP

Abbreviations: Amni, amniocytes; CVS, Chorionic Villus Sampling; DV, Ductus venosus; LB, live birth; Path, pathogenic; SNP, single nucleotide polymorphism; TOP, termination of pregnancy; VOUS, variants of unknown significance.

than cytogenetic analysis, influencing pregnancy management and outcomes.

The most frequent clinically significant CNVs reported were 22q11.2 microdeletions/microduplications (cases 6, 10, and 11), as in a study by Grande et al. (Grande et al., 2015). Owing to the incomplete penetrance and variable phenotype, these CNVs also heightened parental anxiety and frustration and led to confusion regarding the future health and development of their children. The limited information of likely pathogenic CNVs is not meaningless, although the effects of these potentially pathogenic abnormalities do not show up after birth or for a long time after birth. Providing such information to parents may not only help parents better understand the possible abnormalities in their children but also help families to better care for the child and seek therapeutic intervention earlier if necessary. For fetuses having a high risk of mental retardation, parents may pay more attention to their child's development.

For VOUSs, it is necessary to carry out parental testing. Three VOUSs (case 1, 7, and 8) were inherited from their parents (who all had normal phenotypes). However, the clinical significance of these variants cannot be predicted before parental verification. Additionally, CMA was unable to accurately demonstrate the clinical significance of a previously unreported CNV. Instead, parental verification is required to rule out some variants, which tend to benign. Fortunately, with the application of CMA and continuous improvements in database data, the incidence of VOUSs should decrease.

Several studies using SNP arrays have examined the ability of this method to detect mosaicism, and the results showed that mosaicism could be identified in variable cells at levels less than 5% (Conlin et al., 2010; Xiang et al., 2008). SNP arrays could have significant advantages compared with karyotype analysis for the detection of mosaicism. Because the cultivation itself could cause mutations in chromosomes, karyotyping results of amniocytes may be influenced by cell mutations obtained after adherent growth in vitro. Microarray analysis of uncultured amniocytes could eliminate the possibility of chromosomal aberrations during cultivation and could reflect the actual genome of the fetus (Biesecker & Spinner, 2013). Moreover, eliminating the need for cultivation reduces the time to diagnosis, which provides more time for appropriate prenatal counseling.

Most clinicians use a fixed cutoff value for NT(NT of 2.5 mm) as an indication for prenatal diagnosis in our country, consistent with previous studies (Hassold & Hunt, 2001; Sadlecki, Grabiec, Walentowicz, & Walentowicz-Sadlecka, 2018; Shakoor et al., 2016). In contrast to the cutoff value of NT for karyotype analysis, CMA research of fetuses with increased NT have focused on a fixed cutoff value of NT of more than 3.5 mm (Armour et al., 2018; Egloff et al., 2017; Grande et al., 2015; Lund et al., 2015; Pan et al., 2016), and few studies have reported the clinical significance of NTs of 2.5–3.4 mm. It is unclear whether the cutoff value of NT for karyotyping also can be applied to CMA and therefore whether fetuses with NTs of 2.5–3.4 mm should be excluded from CMA. Accordingly, in this prospective study, we evaluated the range of NT values for CMA tests. We examined 174 cases and found 11 cases with CNVs. All samples had normal karyotypes, and additional microdeletions/microduplications were detected by CMA. In cases with NTs of greater than or equal to 3.5 mm, three cases of CNVs with clinical significance were detected, providing a 5.5% (3/55) incremental yield of detecting CNVs, similar to that in previous reports (5.0%) (Grande et al., 2015). There was two case of clinically significant CNVs detected in fetuses with NTs of 2.5–3.4mm, revealing a 1.6% (2/119) incremental yield. The detection rates of NT values in this range had not been reported previously. In cases with NTs of 3.5 mm or more, clinically significant CNVs were mostly found in fetuses without isolated NT increases. This was consistent with the conclusions of previous studies demonstrating that the incremental yield of clinically significant CNVs in cases of increased NT combined with other ultrasonic abnormalities was higher than that in cases of isolated increased NT when the NT was greater than or equal to 3.5 mm (Grande et al., 2015). In cases with NTs of 2.5–3.4 mm, all CNVs were detected in cases of isolated increased NT. We speculated that these findings may be related to the small sample size of NTs of 2.5–3.4 mm with other ultrasound abnormalities. Alternatively, these other ultrasound abnormalities may not be closely related to fetal submicroscopic chromosomes.

We incorporated fetuses with NTs in the range of 2.5–3.4 mm to determine the superiority of CMA compared with conventional karyotyping in fetuses with increased NTs of 2.5 mm or larger and to establish a reference for application of CMA in fetuses with increased NT. Because the value of 2.5 mm was also the cutoff value for karyotype analysis, no additional invasive procedures would be required. Of course, neither karyotype analysis nor CMA can detect single gene syndromes. In consideration of the association between increased NT and single gene syndromes, such as Noonan syndrome, patients should be made fully aware of the limitations of these techniques during pre- and posttest counseling.

There were some limitations to this study. This was a small study from a single center, and we did not obtain sufficient amounts of parental material to trace the origins of the unbalanced chromosomes. Thus, further studies are required to perform multicenter surveys with larger sample sizes in order to confirm our findings.

In conclusion, based on our current data, in our population, CMA improved the diagnostic yield of chromosomal aberrations for fetuses with NTs of 2.5–3.4 mm whose

karyotypes were normal, regardless of the presence of other ultrasonic abnormalities.

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CONFLICT OF INTEREST

The author(s) declare that he has no conflict of interest.

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