Article

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Prenatal Chromosomal Microarray Analysis and Whole-Exome Sequencing in Fetuses with Thickened Nuchal Translucency

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Background: Fetal chromosomal abnormalities predispose the fetus to developmental malformations, which can reduce the quality of newborn births. This study aimed to investigate the clinical utility of chromosomal microarray analysis (CMA) and whole-exome sequencing (WES) in fetuses with thickened nuchal translucency (NT).

Methods: A total of 62 singleton pregnant women were enrolled in this study. Ultrasonography showed increased fetal NT (≥3.0 mm) with or without structural malformations. The subjects were divided into four groups based on the NT value: 3.0–3.4 mm (33 cases), 3.5–4.4 mm (21 cases), 4.5–5.4 mm (3 cases), and 5.5 mm (5 cases). Chromosomal abnormalities were initially analyzed using CMA, followed by trio familial whole-exome sequencing (Trio-WES) in 15 subjects with CMA-negative results. All the subjects were monitored for pregnancy outcomes.

Results: Out of 62 cases, CMA identified 12 cases of aneuploidy, 1 case of pathogenic copy number variation (CNV-P), and 5 cases of unknown copy number variation (CNV-VOUS). The detection rate of fetal chromosomal abnormality was 21.0% (13/62). Fifteen CMA-negative fetuses without structural deformities were analyzed by Trio-WES, which produced six VOUS results with two loci each in SOS Ras/Rac guanine nucleotide exchange factor 1 (SOS1) and collagen type II alpha 1 chain (COL2A1) and one locus each in leucine-zipper-like transcription regulator 1 (LZTR1) and B-Raf proto-oncogene, serine/threonine kinase (BRAF).

Conclusions: This study supports the application of CMA in prenatal diagnosis. It suggests that the positive detection rate of WES may be low in CMA-negative cases with increased NT without structural malformation. Therefore, appropriate genetic counseling should be provided to optimize the use of CMA and WES in prenatal diagnosis.

Keywords: nuchal translucency (NT); whole-exome sequencing (WES); genetic counseling; chromosome microarray analysis; parental diagnosis

Introduction

Fetal chromosomal abnormalities can lead to developmental malformations, fetal deaths, and stillbirths. Even if a small percentage of fetuses survive, they often experience severe physical dysfunction and other conditions, placing a significant burden on families and society. Therefore, it is clinically significant to screen fetuses for suspected chromosomal abnormalities early in pregnancy, assess their growth and development, and, if a clear diagnosis is made, consider terminating the pregnancy as soon as possible to reduce the birth of children with disabilities. Nuchal translucency (NT) is a term used to describe the maximum thickness between the skin and subcutaneous soft tissue in the horizontal sagittal section of the fetal cervical spine, which is examined by ultrasonography at 11 to 13 weeks and 6 days of gestation. An increased NT measurement (≥95th percentile) is associated with various conditions, including chromosome aneuploidy, heart and arteries deformity, skeletal dysplasia, and genetic syndromes such as Noonan syndrome, achondroplasia, and Smith Lemli Opitz syndrome [1,2]. In addition, the presence of NT thickening is linked to an elevated risk of miscarriage, intrauterine fetal death, or delayed development [3].

The frequency of chromosomal abnormality increases exponentially with NT thickness [4]. It is recommended to perform noninvasive prenatal tests or combined screening with serum markers to determine the risk of fetal chromosomal abnormality in the early and middle trimesters for NT below the 99th percentile (3.5 mm) [5]. An invasive prenatal diagnosis is advised for fetal NT larger than 3.5 mm. Chromosomes play a crucial role in the transmission of human genetic information. Abnormalities can result in genetic changes and lead to congenital fetal disabilities, imposing a heavy burden on their families. Prenatal screening is vital for detecting fetal growth abnormalities, and adopting a reasonable diagnostic approach is essential for enhancing the accuracy of prenatal screening. The detection

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rate of chromosomal abnormalities in fetuses is unsatisfactory with a single approach due to individual differences, experimental methods, and testing conditions.

Traditional chromosome karyotype analysis is the primary method for prenatal diagnosis [6]. However, with the advancement of genomic technology, chromosome microarray analysis (CMA) has become a valuable tool for detecting chromosome aneuploidy and copy number variations. A recent meta-analysis by Grande *et al*. [1] found that CMA can increase the detection rate of pathogenic copy number variants (pCNVs) in fetuses with NT thickening but normal karyotypes by 5.0%. Despite its benefits, there are still cases of ultrasonography anomalies whose etiology cannot be determined by CMA, particularly in the case of monogenic disorders. In recent years, whole-exome sequencing (WES) has been increasingly used in scientific and clinical research due to cost reduction and advancement in NGS techniques. Xue *et al*. [7] used WES analysis to identify three potential pathogenic variants in 24 samples with NT thickening, which had no structural abnormalities and negative CMA findings. These variants included two new mutations in SOS Ras/Rac guanine nucleotide exchange factor 1 (SOS1) and Homo sapiens endothelin converting enzyme 1 (ECE1), and a compound heterozygous mutation in phosphatidylinositol glycan anchor biosynthesis class N (PIGN). Studies have indicated that the diagnostic rate of whole-genome sequencing (WGS) or WES in fetuses with NT thickening ranges from 3.2% to 32% [8–12].

Consistent with the above details, this study aims to investigate the application of chromosome microarray analysis and whole-exome sequencing in fetuses with NT thickening and, more importantly, to explore the clinical utility of whole-exome sequencing in NT thickening fetuses without structural abnormalities after CMA examination.

Materials and Methods

Subjects

The retrospective research was conducted on patients who visited the obstetric clinic of the Urumqi Maternal and Child Health Hospital from June 2018 to June 2019. The study focuses on fetal NT measurements (≥3.0 mm), accompanied by or without structural malformations, as indicated by ultrasound screening during 11 to 13 weeks + 6 days gestational age. After a physician's consultation, 62 singleton pregnant women underwent amniocentesis and G-band karyotype analysis. Concurrently, CMA was used to screen the amniotic fluid for CNVs. Demographic information about the subjects can be found in Table 1. All invasive samples were kept for future WES analysis, and parental peripheral blood samples were collected during the prenatal visit. In total, 15 samples showing NT thickening without structural deformities with negative CMA results were sequenced from trio familial whole-exome sequencing (Trio-WES).

Table 1. Patients characteristics.

	n = 62
Age (years)	28.7 ± 4.53
BMI (kg/m^2)	24.29 ± 3.184
Coronary atherosclerotic cardiopathy (%)	0
Hypertension (%)	0
Type 2 DM (%)	1 (1.61)
Hyperlipemia (%)	2 (3.23)
History of uterine cavity surgery (%)	2 (3.23)

The original sample size calculation estimated that 60 patients in each group would be needed to detect a 3-point difference between groups in a 2-sided significance test with a power of 0.8 and an alpha error level of 0.05.

Inclusion Criteria

Patients were included in this study if they meet any of the following criteria: (1) ultrasound abnormalities, including single-system or multi-system structural abnormalities; (2) soft ultrasound indicators, including thickened posterior neck zona pellucida, missing or short nasal bones, bright ventricular spots, enhanced bowel echo, dilated ventricles, widened posterior cranial fossa pool, choroid plexus cyst, dilated renal pelvis, or short femur; (3) absence of non-structural abnormalities such as intrauterine growth retardation, polyhydramnios/oligohydramnios; (4) non-ultrasound abnormalities, including women with non-invasive prenatal testing (NIPT) abnormalities, a history of adverse pregnancy outcomes, and other factors for prenatal diagnosis.

Exclusive Criteria

The exclusive criteria for the current study were as follows: (1) pregnant women with severe systemic organ diseases and a family history of major genetic diseases; (2) fetal merger with significant structural or chromosomal abnormalities; (3) patients with other complications such as intrauterine fetal death; (4) patients with concomitant placental diseases.

CMA Microarray Assay Genomic DNA

Genomic DNA was extracted using the QIAGEN QIAamp DNA Mini Kit (Cat. No. 51304, Hilden, North Rhine-Westphalia, Germany). CMA was conducted on the Affymetrix platform (Version: Affymetrix GeneChip® Scanner 3000 7G, Affymetrix Inc., Santa Clara, CA, USA). High-quality genomic DNA (250 ng) was digested, ligated, amplified by PCR, labeled, and hybridized into a Cytoscan 750K array. After washing and dyeing, the microarrays were scanned using the Affymetrix GeneChip® Scanner 3000 7G Scanner. Data were analyzed using a chromosome analysis suite (Version: Chas 2.2, Affymetrix Inc., Santa Clara, CA, USA). The location of CNVs was determined by sequence alignment with the GRCH37/H19 genome reference sequence. Decipher, Clinical Genome Resource

(Clingen), DGV, and OMIM were used for data interpretation. According to the American Society of Medical Genetics and Genomics (ACMG) guidelines [13], CNVs were divided into benign, pathogenic, and unknown variations of uncertain significance (VOUS). The reporting threshold for copy number results was set at 100 KB, and quantitative polymerase chain reaction (qPCR) (Version: Biometra Tone 96, Affymetrix Inc., Santa Clara, CA, USA) was used to confirm the gene dose imbalance detected by CMA.

WES Detections

The WES assay included the following steps: 1 µg of genomic DNA was enzymatically fragmented, followed by sequencing through combined ligation and bead selection of XP fragments to recover fragments ranging from 270 to 340 bp. Subsequently, DNA was recovered to create a library, and 500 ng of the pre-library was subjected to capture by nano-1-to-1 WES probe hybridization. The resulting hybridization product was then eluted and retrieved for PCR amplification. The PCR product was recovered as a new library and confirmed using agarose gel electrophoresis. Qualified libraries were sequenced using a HiSeq 2500 Genomics Analyzer (Illumina Inc., Santiago, CA, USA) for paired-end 100-base pair reads (PE100). Sequencing data were performed to evaluate quality control through FastQC, mutation locus pathogenic rating, and data interpretation rules referring to the ACMG genetic variation classification standard and guide [14]. We ruled out one thousand ExAC gnomAD genome database mutation frequencies that were more than 1% at various sites unless they were functional variation locus (such as righteousness mutation and non-coding regions mutation, etc.). Then, we identified candidate gene mutation sites after carefully considering pathogenicity prediction (SIFT, Polyphen2, CADD, etc.), phenotypically related gene verification, related disease database query (OMIM, Clinvar, HGMD), and literature reference report (PubMed).

Steps in WES Detection

Initially, the raw sequencing data were examined for low-quality reads, PCR primers, adaptors, duplicates, and other contaminants to ensure quality control. Subsequently, the FastQC app was used to generate the raw reads and determine the trimming, filtering, and alignment, thereby improving the quality of the data. Following this, the map exome sequencing of data was performed using BWA alignment to ensure efficiency and accuracy. After mapping, the duplicated map reads were removed to eliminate PCR-introduced bias.

Statistical Analysis

A Shapiro-Wilk test was used to determine the normality of the sample. Descriptive statistical data were evaluated with exploratory analyses of the Tukey test. Quantitative mean data (PES/WES, ISQ, and B.L.) were assessed with the nonparametric Wilcoxon-Mann-Whitney U-test to analyze the inferential statistics. GraphPad Prism 9.0 (GraphPad Software, LLC, San Diego, CA, USA) was used for statistical analysis. Comparisons between groups were performed using the Chi-Square test, and $p < 0.05$ was considered statistically significant.

Results

CMA Results

The average age of the patients was 29 years old (range 22–41). The median fetal NT thickness was 3.4 mm (range 3.0–9.1 mm), and the median gestational age at diagnosis was 13 weeks and 4 days (range 11 weeks and 5 days to 14 weeks). Amniocentesis was performed between the 16th and 24th weeks of pregnancy. Out of the 62 fetuses with abnormal NT values, 12 had aneuploidy (including 8 cases of trisomy 21, 2 with trisomy 18, and 2 with Turner syndrome), 1 had pathogenic CNVs, and 5 had variants of uncertain significance (VOUS) CNVs, as identified by CMA. The detection rate of fetal chromosomal abnormality was 21.0% (13/62) (Table 2).

According to the degree of NT thickening, all the patients were divided into four groups: 3.0–3.4 mm (n = 33), 3.5–4.4 mm (n = 21), 4.5–5.4 mm (n = 3), and ≥5.5 mm (n = 5). The detection rate for the four groups was 3.0% (1/33), 23.8% (5/21),100% (3/3), and 80% (4/5), respectively (Table 3).

Clinical examination and genetic counseling were performed on both parents in cases of chromosomal abnormalities listed in Table 2 to determine that they do not have any associated characteristics. CMA was used to examine both parents for the presence of fetal pathogenic CNV. It was found that the mothers in cases A190430 and A191174 share the same CNVs as the fetuses.

Trio-WES analysis was performed in only 15 of the 44 CMA-negative fetuses, with NT 3.0–3.4 mm (10 cases), NT 3.5–4.4 mm (4 cases), and ≥5.5 mm (1 case). Trio-WES was unavailable for the remaining samples due to poor DNA quality or parental refusal.

WES Findings

Six cases of VOUS were identified through Trio-WES analysis. In cases A190762 and A190977, heterozygous mutations of *SOS1* (C. 3542C>T, p.1181V) and *SOS1* (C. 3817C BBB>, p.L1273V) were detected, respectively. The *SOS1* gene may result in Noonan syndrome type 4, which exhibits an autosomal dominant inheritance pattern. Additionally, heterozygous mutations of *COL2A1* (C. 436C>T, p.146S) and collagen type II alpha 1 chain (*COL2A1*) (C. 3700G>A, p.D1234N) were found in cases A191149 and A190632, respectively. The *COL2A1* gene is associated with chondrogenic parabiosis type 2, which exhibits an autosomal dominant inheritance pattern. In cases

Table 2. Clinical data of fetal patients with abnormal karyotype and CMA results.

Patient No.	Maternal age	Ethnicity	History of gestation	Nt (Mm)	Nt thickening fetuses with structural abnormalities	Other ultrasound defects	Results of CMA	Clinical phenotype of parents/Verification result of CNVs	Clinical outcomes
A180892	29	Kazak	G2P0	3	13^{+2}	-	Arr(21)×3	(−)	Labor Induction
A180966	27	Han	G2P0	5	18	Fetal Cardiac Anomalies, Incomplete Ossification Of One Nasal Bone	Arr(21)×3	(−)	Labor Induction
A181032	27	Han	G2P0	4.9	12^{+6}	-	Arr(21)×3	(−)	Labor Induction
A181070	24	Han	G1P0	4.1	13	-	Arr(21)×3	(−)	Labor Induction
A190023	29	Hui	G2P1	3.8	12^{+5}	-	Arr(21)×3	(−)	Labor Induction
A190245	41	Han	G3P1	4.3	13^{+3}	Marginal Umbilical Cord Entry, Separation of Bilateral Renal Pelvises	Arr(21)×3	(−)	Labor Induction
A190431	37	Han	G4P1	4.3	13^{+3}	-	Arr(21)×3	(−)	Labor Induction
A190957	22	Han	G2P0	5.9	13	Fetal Cardiac Anomalies	Arr(21)×3	(−)	Labor Induction
A190664	26	Han	G1P0	4.5	14	-	Arr(18)×3	(−)	Labor Induction
A190859	29	Han	G1P0	5.8	13	-	Arr(18)×3	(−)	Labor Induction
A190178	25	Han	G1P0	6.3	12^{+2}	-	Arr(X)×1	(−)	Labor Induction
A191075	31	Han	G3P1	9.1	13	Absence of Venous Catheters, Lymphatic Cysts	Arr(X)×1	(−)	Labor Induction
A180975	28	Han	G2P0	3.6	12	Adverse Reproductive History, Small Transparent Septum	Arr[Hg19] 16p13.3(85,880-277,314)×3,18p11.32p11.22(136,227-10,709,517)×1, CNV-P	(−)/(−)	Labor Induction
A180959	27	Han	G2P0	3.4	13^{+1}	-	Arr[Hg19] 2q13(110,876,775-111,370,025)×1, CNV-VOUS	(−)/(−)	Normal
A190136	31	Han	G3P1	4.0	13	-	Arr[Hg19] Yq11.221q11.222(19,540,569-20,752,427)×0, CNV-VOUS	(−)/(−)	Normal
A190430	27	Han	G2P0	3.2	13^{+1}	-	Arr[Hg19] 18q21.2(53,292,030-53,425,338)×1, CNV-VOUS	(−)/Mother(+)	Normal
A190603	32	Han	G1P0	3.1	12^{+4}	-	Arr[Hg19] 9p21.2(26,500,924-27,378,899)×3, CNV-VOUS	(−)/(−)	Normal
A191174	26	Han	G2P1	4.1	13	-	Arr[Hg19] 6q26(162,800,236-164,123,788)×3, CNV-VOUS	(−)/Fother(+)	Normal

CNV-P, pathogenic copy number variation; CNV-VOUS, unknown copy number variation; NT, nuchal translucency; CMA, chromosomal microarray analysis.

Table 3. Chromosome abnormalities in fetuses with increased NT.

Nt (Mm)	N	T21, n (%)	T18, n (%)	T13, n (%)	Xo (%)	CNV-P, n (%)	CNV-VOUS, n (%)	Positive cases, n (%)
3.0–3.4	33	1 (3.0%)	0	0	0	0	3 (9.1%)	1 (3.0%)
3.5–4.4	21	4 (19.0%)	0	0	0	1 (4.8%)	2 (9.5%)	5 (23.8%)
4.5–5.4	3	2 (66.7%)	1 (33.3%)	0	0	0	0	3 (100.0%)
≥5.5	5	1 (20.0%)	1 (20.0%)	0	2 (40.0%)	0	0	4 (80%)

A190928 and A190327, heterozygous mutations of leucine-zipper-like transcription regulator 1 (*LZTR1*) (C. 1496T>C, p.V499A) and B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) (C. 64G BBB>, p.D22N) were detected, respectively. The *LZTR1* gene leads to Noonan syndrome type 10, and the *BRAF* gene leads to Noonan syndrome type 7, both of which demonstrate autosomal dominant inheritance. The pregnancy outcomes and the status of live births of the 15 patients were followed up, and all the live births were healthy with expected delivery (Table 4).

Discussion

Congenital genetic defects are essential factors that can lead to adverse pregnancy outcomes, such as stillbirths, malformations, and dysplasia. These not only reduce the quality of newborn births but also impose a significant burden on society and families. Therefore, early prenatal detection and interventions are beneficial to improve the quality of the population [14]. With the popularization of prenatal ultrasound diagnostic techniques, noninvasive DNA prenatal tests, and Down serological screening in China, the detection rate of high-risk fetal diseases has improved. However, their precision is low, and they are prone to false positive results. Therefore, interventional prenatal tests are appropriate for high-risk pregnant women to help with diagnosis [15]. A karyotype can determine whether the fetus has chromosomal number and structure abnormalities by analyzing the morphological characteristics of chromosomes and is often used as the gold standard. However, its resolution is low, and it can only distinguish large chromosomal deletions and duplications, and it cannot obtain small variant fragments, which can lead to the underdiagnosis of pathogenic variants [1]. With the development of second-generation sequencing technology, chromosome microarray analysis (CMA) and whole-exome sequencing (WES) have gradually become the primary diagnostic and research tools for congenital and genetic kidney diseases [16].

In this study involving 146 proband and trio whole-exome sequencing tests on fetal samples, a diagnostic yield of 32% was observed, which is slightly higher than some more recent series with reported diagnostic rates of 20–24% [15,16]. Additionally, 62 fetuses with NT thickening with/without other structural malformations were analyzed by CMA, leading to the identification of 12 cases of aneuploidy, 1 case of pathogenic copy number variation (CNV-P), and 5 cases of unknown copy number variation (CNV-VOUS). A subset of 62 ongoing pregnancies underwent exome sequencing, resulting in a diagnosis rate of 35% [12]. The total diagnostic rate of CMA was 21.0% (excluding 5 cases of CNV-VOUS detection), which is consistent with previous reports [7]. Another research looked at 100 fetuses with high NT and normal karyotypes and discovered no detrimental CNVs using CGH [2]. In this study, 80 pregnancies resulted in live births, 20 had spontaneous fetal death or termination of pregnancy or were lost to follow-up, and 10 had ultrasound-found fetal abnormalities. The CGH they used could only detect CNVs of 3 Mb or greater, so while their findings are congruent with ours, it is conceivable that some pathogenic CNVs were missed [15].

The utility of CMA in fetuses with NT thickening was identified in 19.4% (12/62) of aneuploidy fetuses (8 cases of trisomy 21, 2 cases of trisomy 18, and 2 cases of Turner syndrome) and 9.7% (6/62) of CNVs (1 case of pathogenic CNV and 5 cases of CNV-VOUS). These results support the application of CMA in prenatal diagnosis. In a previous study, 22 of 225 fetuses (9.8%) had a normal karyotype and pathogenic CNV [17]. Pathogenic CNVs not previously identified as syndromes were found in 14 cases (63.6%) and those previously described as syndromes in 8 cases (36.4%). CNVs were found in two nonhomologous chromosomes in 9 fetuses (41%), suggesting a high possibility of balanced translocations in the parents. When the parent's karyotype was examined, balanced translocations were discovered in one case. However, in this study, no heterozygous variants were found.

In addition to chromosomal abnormality, thickening of the NT is associated with specific genetic syndromes such as Noonan syndrome and Smith-Lemli-Opitz syndrome [3]. CMA cannot detect the monogenetic defect that caused NT thickening [4], unlike WES which can cover a broader spectrum of diseases caused by single nucleotide variants (SNV) and small insertions and deletions (Indels). Lord *et al*. [12] demonstrated that genetic diagnosis was not common in fetuses with isolated NT thickening in early pregnancy, and the detection rate is only 3.2% (3/93) using WES. Trio-WES analysis was performed on 15 thickening NT fetuses with negative CMA results and no structural malformations, and a total of 6 cases of VOUS mutation were detected. Heterozygous mutations of *SOS1* (c.3542C>T, p.1181V) and *SOS1* (c.3817C BBB>G, p.L1273V) were detected, which may lead to Noonan syndrome type 4, presenting an autosomal dominant inheritance pattern. Similarly, heterozygous mutations

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Table 4. Trio-WES results of 15 fetuses with CMA-negative results and increased NT thickness without structural defects.

Patient No.	Nt (Mm)	Other Ultrasound Defects	WES Result	Heritable Variation	Disease	Delivery Type
A190365	3.5	-	Negative	-	-	Normal
A190380	3.1	-	Negative	-	-	Normal
A190434	5.9	-	Negative	-	-	Normal
A190556	3.2	-	Negative	-	-	Normal
A190563	3.2	-	Negative	-	-	Normal
A190762	3.3	-	Vous	*SOS1*: c.3542C>T (p.A1181V), Heterozygous, Paternal	Noonan Syndrome Type 4, Ad	Normal
A190777	3.2	-	Negative	-	-	Normal
A190928	3.5	-	Vous	*LZTR1*: c.1496T>C (p.V499A), Heterozygous, Paternal	Noonan Syndrome Type 10, Ad	Normal
A190977	3.2	-	Vous	*SOS1*: c.3817C>G (p.L1273V), Heterozygous, Maternal	Noonan Syndrome Type 4, Ad	Normal
A190991	4.2	-	Negative	-	-	Normal
A191149	3.0	-	Vous	*COL2A1*: c.436C>T (p.P146S), Heterozygous, Paternal	Achondroplasia Type 2, Ad	Normal
A190248	3.9	One Nasal Bone Unclear	Negative	-	-	Normal
A190280	3.1	-	Negative	-	-	Normal
A190327	3.2	-	Vous	*BRAF*: c.64G>A (p.D22N), Heterozygous, Maternal	Noonan Syndrome Type 7, Ad	Normal
A190632	3.2	Absence Of Nasal Bone, Choroid Plexus Cyst Of The Left	Vous	*COL2A1*: c.3700G>A (p.D1234N), Heterozygous, Paternal	Achondroplasia Type 2, Ad	Normal

Ad, autosomal dominant; WES, whole-exome sequencing; *SOS1*, SOS Ras/Rac guanine nucleotide exchange factor 1; *LZTR1*, leucine-zipper-like transcription regulator 1; *COL2A1*, collagen type II alpha 1 chain; *BRAF*, B-Raf proto-oncogene, serine/threonine kinase.

of *COL2A1* (c.436C>T, p.146S) and *COL2A1* (c.3700G BBB>A, p.D1234N) were found. The *COL2A1* gene leads to chondrogenic parabiosis type 2, presenting an autosomal dominant inheritance pattern. Furthermore, heterozygous mutations of *LZTR1* (c.1496T>C, p.V499A) and *BRAF* (c.64G BBB>A, p.D22N) were also identified. CMA in combination with WES diagnosis enables the detection of structural variants at microscopic and submicroscopic levels, which has positive implications for improving the efficiency and accuracy of prenatal diagnosis [5].

The *LZTR1* gene is associated with Noonan syndrome type 10, and the BRAF gene is associated with Noonan syndrome type 7 [6,7]. Both genes exhibit autosomal dominant inheritance. Previous literature has shown that *KMT2D* variants are associated with several phenotypes, including multisystem anomalies, isolated complex cardiac defects, fetal hydrops, and cystic hygroma [18]. Furthermore, KMT2D mutations cause Kabuki syndrome, which is characterized by developmental delay, epilepsy, cardiac, genitourinary, and musculoskeletal anomalies [19]. In another study, WES analysis revealed that two VOUS and three pathogenic variants, including two dominant *de novo* mutations in SOS1 and ECE1, and one recessive inherited compound heterozygous mutation in PIGN, are associated with cardiac defects [7]. Surprisingly, no autosomal recessive mutations were found, contradicting the prior study's findings. The cause of the unusual result of the study is unknown. No further structural abnormalities were discovered during the ultrasound evaluation of the 15 samples, and the pregnancy outcomes were all healthy live babies with expected delivery. As a result, the WES diagnosis rate for thickened NT fetuses with negative CMA and no anatomical abnormality may be poor. At the same time, because fetal phenotypes are non-specific, interpreting the clinical relevance of identified VOUS may be difficult. It can be seen that CMA as the preferred detection method and WES as a complementary inspection method to detect NT thickening fetuses have better advantages and promising prospects.

The maternal peripheral serologic index examination has several disadvantages, including long waiting time for results, difficulty in detecting 35–45% of fetal chromosomal abnormalities, and imprecise diagnostic results. Most pregnant women need to combine this test with other diagnostic methods in late pregnancy to confirm the diagnosis [20]. Fetal NT ultrasonography is a standard examination method for pregnant women in early pregnancy, but its value in determining chromosomal abnormalities and evaluating fetal prognosis is average. However, with the development of second-generation sequencing technology, CMA has emerged as a high-resolution, high-throughput genome-wide screening technology. It is mainly used to detect chromosomal microdeletions and microduplications, allowing for the rapid and accurate identification of unbalanced variants in genomic copy numbers [21,22]. Chro-

mosomal microarray analysis CMA enables genome-wide copy number variation analysis of DNA by high-throughput specific nucleic acid probes, allowing for the detection of microdeletions and microduplications with high throughput, resolution, and automated detection compared to traditional karyotype analysis [23,24]. CMA, in combination with WES diagnosis, can detect structural variants at microscopic and submicroscopic levels, thereby improving the efficiency and accuracy of prenatal diagnosis [25].

The study's main limitation was the small sample size. Therefore, enrolling more patients to further clarify the clinical utility of WES for isolated patients with thickening of NT is necessary. In addition, the small sample of the WES study and the failure to effectively compare the single-person-WES (preclear only) model with the parent-preclear Trio-WES model in terms of single-gene variant detection efficiency are also limitations of this study.

Conclusions

In conclusion, CMA can improve the detection rate of NT thickening in fetuses during prenatal screening. However, WES may have a low positive detection rate for fetuses with NT thickening but no morphological abnormalities and negative CMA. Once NT thickening is identified, prenatal genetic testing is required. Physicians should adequately inform patients about the advantages and disadvantages of CMA and WES tests and provide appropriate genetic counseling. In addition, invasive prenatal diagnosis of NT-thickening fetuses can be performed using a step-by-step detection and exclusion approach, with CMA as the preferred test and WES as a complementary test. The need for monogenic etiology testing should be communicated to parents to assess fetal bilateral renal echogenicity.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Author Contributions

XL and GD designed the research study. WD and HL performed the research. YL, GM and XL contributed to data collection, analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. 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

All patients provided informed consent and signed the consent forms for specimen collection. The research protocol was approved by the Ethics Committee of Urumqi Maternal and Child Health Hospital and conducted following the principles of the Declaration of Helsinki. The ethics approval code is XJFYLL2018001.

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Conflict of Interest

The authors declare no conflict of interest.

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