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ORIGINAL RESEARCH

# Application of Copy Number Variation Sequencing Technology in 422 Foetuses with Abnormal Ultrasound Soft Markers

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**Purpose:** The application value of ultrasound soft indicators in prenatal diagnosis was evaluated by copy number variation sequencing (CNV-seq).

**Methods:** The authors conducted a retrospective analysis of 422 pregnant women who underwent CNV-seq testing at Luoyang Maternal and Child Health Hospital between January 2020 and November 2021. The women had presented with abnormal ultrasound soft markers; those identified as high-risk through non-invasive prenatal screening were excluded.

**Results:** A total of 43 abnormal cases were detected in 422 pregnant women, including 24 aneuploidy (including chimerism) and 19 pathogenic or likely pathogenic copy number variations (CNVs). Based on the characteristics of ultrasound soft indicators, pregnant women were divided into five groups: isolated nuchal translucency (NT) group, combined NT group, isolated soft indicators group, combined soft indicators group and combined non-NT group. The abnormality detection rates in the five groups were 12.38% (13/105), 36.11% (13/36), 3.74% (4/103), 3.08% (2/63) and 10.09% (11/109), respectively. Statistical tests showed that the detection rate in the NT thickening combined with other abnormalities group was significantly higher than the other four groups, while there was no statistical difference in the detection rate among the other four groups.

**Conclusion:** When NT thickening is combined with other abnormalities, it is more likely to indicate chromosome abnormalities or CNVs, so it should be regarded seriously upon finding, and pregnant women should be referred for prenatal diagnosis according to the examination results. In addition, NT thickening is an important indicator for prenatal diagnosis and should be considered regardless of whether it occurs independently. The authors recommend CNV-seq for prenatal diagnosis to prevent missing small fragments of CNVs during traditional karyotyping.

**Keywords:** ultrasound soft indicators, isolated type, combined type, amniotic fluid, CNV-seq

## Introduction

Ultrasound soft markers refer to small nonspecific variations in foetal structure found in prenatal ultrasound that are often associated with abnormal chromosome number or pathogenic copy number variations (CNVs).[1,2] Common ultrasound soft markers include nuchal translucency (NT) thickness, nuchal fold (NF) thickness, nasal bone dysplasia, choroid plexus cyst, intracardiac strong echo focus, intestinal echo enhancement, renal pelvis dilatation, single umbilical artery and short long bones.[3,4] Clinically, the application value of single or combined ultrasound soft marker abnormalities in foetal chromosomal abnormalities needs to be clarified, as these can provide an essential basis for prenatal diagnosis and genetic counselling.

CNV sequencing (CNV-seq) technology, a prenatal diagnosis method based on high-throughput sequencing developed in recent years,[5,6] can not only accurately detect chromosomal aneuploidy but also reduce the lower limit of fragment detection of CNVs to 0.1 megabases (Mb) compared to traditional karyotyping. Therefore, CNV-seq can be used to detect more rare and severe microdeletion/microduplication syndromes. What makes it even more appealing is that CNV-seq does not require cell culture to significantly shorten the detection cycle. At present, CNV-seq is included in

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the first-line prenatal diagnosis in China[7,8] and has become one of the important technical means in the secondary prevention and control system of birth defects in China. In this study, CNV-seq analysis was performed on high-risk pregnant women with abnormal ultrasound soft markers admitted to Luoyang Maternal and Child Health Hospital from January 2020 to December 2021, with a view to systematically evaluating the clinical application potential of CNV-seq in the diagnosis of foetuses with abnormal ultrasound.

## Subjects and Methods

### Subjects and Inclusion Criteria

A total of 422 pregnant women who underwent amniocentesis in Luoyang Maternal and Child Health Hospital from January 2020 to December 2021 were selected as subjects by convenience sampling. There were three inclusion criteria: (1) pregnant women aged 18–44; (2) pregnant women who had not had non-invasive prenatal screening (NIPS) or had negative NIPS results; and (3) pregnant women with at least one abnormal ultrasound soft marker. Ultrasound soft markers and their criteria for abnormal judgment were as follows (See Figure 1 for an example): (1) NT thickness ≥2.5 mm; (2) NF thickness ≥6 mm; (3) choroid plexus cyst: choroid plexus cysts of any size or number in the ventricle; (4) echogenic intracardiac focus: a spot-like echogenic intracardiac focus in any single ventricle, with echo intensity comparable to bone; (5) intestinal echo enhancement: intestinal echo intensity ≥ bone echo intensity; (6) pyelic separation: anteroposterior diameter of renal pelvis >4 mm; (7) nasal bone dysplasia; (8) ventricle enlargement; (9) short long bones: ratio of observed value to expected value <0.9; (10) single umbilical artery; and (11) tricuspid regurgitation. Exclusion criteria were as follows: (1) pregnant women with aneuploidy risk soft markers and mild hydronephrosis; (2) pregnant women with multiple pregnancies; (3) pregnant women with signs of threatened abortion in the previous 3 months; and (4) pregnant women who did not consent to participate in this study.

Pregnant women were divided into the following five groups according to the characteristics of ultrasound soft markers: (1) isolated NT group: only NT thickening was detected; (2) combined NT group: NT thickening combined with other soft markers or clinical abnormalities; (3) isolated soft marker group: only soft marker anomalies with non-NT thickening were detected; (4) combined soft marker group: two or more soft marker anomalies with non-NT thickening were detected simultaneously; and (5) combined non-NT group: soft marker anomalies with non-NT thickening were detected combined with other clinical abnormalities. All subjects signed informed consent and received full genetic counselling before and after the test.

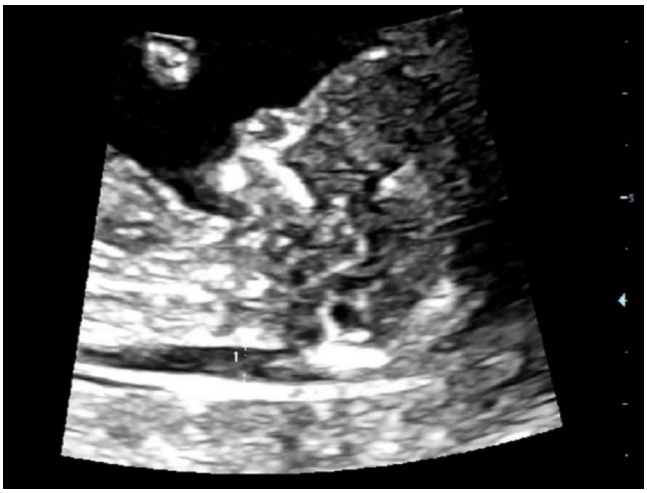

**Figure 1** The fetal nuchal translucency was thickened.

## CNV Sequencing Analysis

Genomic deoxyribonucleic acid (DNA) of amniotic fluid cells was extracted with the kit from Annaroad Gene Technology (Beijing) Co., Ltd. After passing quality inspection, short tandem repeats (STR) detection technology was used to eliminate maternal contamination in uncultured amniotic fluid cells. The CNV-seq was continued on the samples without maternal contamination; DNA samples of about 10 ng were taken for sequencing library preparation, including digestion, ligation, amplification, purification, quantification and quality control. Downstream experiments were carried out when the library concentration was >1 ng/L. Sequencing was performed on the Illumina NextSeq 550AR platform using a single-end 40 bp sequencing mode, with a sequencing data volume of 7.5 Mb. Subsequently, the sequencing data were compared with the hg19 genome sequence, and the identified CNVs were queried via public databases, including the Database of Genomic Variants, Genome Aggregation Database, Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources, Online Mendelian Inheritance in Man and Clinical Genome Resource. In terms of pathogenicity, CNVs were classified into five categories according to the guidelines of the American College of Medical Genetics and Genomics:[9] benign, likely benign, pathogenic, likely pathogenic and uncertain significance. In this study, only pathogenic and likely pathogenic CNVs (pCNVs) were statistically analysed.

## Statistical Analysis

All data were analysed by R (version 3.5.3) software for statistical analysis. Numerical data were described by the number of cases or the percentage, the chi-squared ($\chi^2$) test or Fisher's exact test were employed for the comparison between groups, and post hoc pairwise comparisons were performed with Bonferroni correction. Unless otherwise specified, $P < 0.05$ indicates a statistically significant difference.

# Results

## Numbers of Ultrasound Soft Markers

Among the 422 pregnant women, the abnormal ultrasound soft markers detected and the number of cases of each (from highest to lowest) were as follows: NT thickening (144 cases), choroid plexus cyst (80 cases), echogenic intracardiac focus (65 cases), pyelic separation (53 cases), nasal bone dysplasia (33 cases), ventricle enlargement (32 cases), tricuspid regurgitation (32 cases), single umbilical artery (20 cases), short long bones (15 cases), echogenic bowel (10 cases) and NF thickening (five cases). In general, the detection rate of the non-isolated type of abnormal soft markers was higher than that of the isolated type. Within the isolated soft markers, the top three detection rates were for NT thickening, pyelic separation and short long bones, while the top detection rates within the non-isolated soft markers were for NT thickening, single umbilical artery, nasal bone dysplasia and short long bones (Table 1).

**Table 1** Statistics of Chromosome Aneuploidy and pCNVs Detected by Ultrasound Soft Markers

| | Isolated Indication (Cases) | Aneuploidy (Cases) | pCNVs (Cases) | Detection Rate (%) | Non-Isolated Indication (Cases) | Aneuploidy (Cases) | pCNVs (Cases) | Detection Rate (%) |
|---|---|---|---|---|---|---|---|---|
| NT thickening | 105 | 9 | 4 | 12.38 | 36 | 7 | 6 | 36.11 |
| Choroid plexus cyst | 29 | 0 | 1 | 3.45 | 51 | 2 | 0 | 4.44 |
| Echogenic intracardiac focus | 10 | 0 | 0 | 0.00 | 55 | 1 | 3 | 7.27 |
| Pyelic separation | 9 | 0 | 1 | 11.11 | 44 | 0 | 2 | 4.55 |
| Nasal bone dysplasia | 21 | 1 | 0 | 4.76 | 12 | 1 | 1 | 16.67 |
| Ventricle enlargement | 13 | 0 | 0 | 0.00 | 19 | 1 | 0 | 5.26 |
| Tricuspid regurgitation | 2 | 0 | 0 | 0.00 | 30 | 2 | 0 | 6.67 |
| Single umbilical artery | 6 | 0 | 0 | 0.00 | 14 | 2 | 2 | 28.57 |
| Short long bones | 9 | 1 | 0 | 11.11 | 6 | 0 | 1 | 16.67 |
| Echogenic bowel | 4 | 0 | 0 | 0.00 | 6 | 0 | 0 | 0.00 |
| NF | 4 | 0 | 0 | 0.00 | 1 | 1 | 0 | 100.00 |

## Comparisons of Abnormality Detection Rates

As shown in Table 2, the detection rate of abnormal foetal NT thickening was 12.38% when it was detected alone and increased to 36.11% when it was combined with other indications. However, the detection rates of other ultrasonic soft marker abnormalities, whether isolated or combined, were low (3.74% and 3.08%, respectively). Only when ultrasound soft marker abnormalities were combined with other clinical abnormalities did the detection rate (10.09%) come close to that of the isolated NT group.

The $\chi^2$ test showed a statistically significant difference in the detection rates of abnormalities among the five groups ($\chi^2 = 35.44$, $P < 0.001$). Further pairwise comparison (using correction with the Bonferroni method) found that the detection rate of the combined NT group was significantly higher than that of the other four groups, with a statistically significant difference ($P < 0.01$), while there was no statistically significant difference among the other four groups.

## Detection of Chromosomal Abnormalities

According to CNV-seq analysis, a total of 43 cases of chromosomal aneuploidy and pCNVs were confirmed in the 422 pregnant women with abnormal ultrasound soft markers, of which 18 cases were common trisomy abnormalities, four were sex chromosome abnormalities, two were aneuploidy chimerism and 19 were pCNVs (Table 3). Of these cases, 33 women had an induced labour, five were lost to follow-up and five continued the pregnancy to delivery; of the five cases that continued to delivery, four of them had no abnormalities at follow-up to date and one of them had pulmonary artery stenosis detected at about 1 year of age, with a good postoperative condition (Table 3).

**Table 2** Grouping of 422 Pregnant Women and Statistics of Abnormal Detection Rate

| Group | True Positive (Cases) | False Positive (Cases) | Detection Rate (%) |
|---|---|---|---|
| Isolated NT group[a] | 13 | 92 | 12.38 |
| Combined NT group[b] | 13 | 23 | 36.11 |
| Isolated soft marker group[a] | 4 | 103 | 3.74 |
| Combined soft marker group[a] | 2 | 63 | 3.08 |
| Combined non-NT group[a] | 11 | 98 | 10.09 |
| Total | 43 | 379 | 10.19 |

**Notes**: The same superscript [a]Indicates no statistical difference between the two groups, while different superscripts [a]And [b]Indicate a statistical difference between the two groups (correction with the Bonferroni method, $P<0.05/5$).

**Table 3** Summary of Information from 43 Fetuses Diagnosed with Aneuploidy and pCNVs by CNV-Seq

| Case | Abnormal Type | Key Gene/Syndrome | Prenatal Indication | Pregnancy Outcome |
|---|---|---|---|---|
| 1 | 47,XY,+18 | Edward's syndrome | NT thickening, high risk of ES | Induced abortion |
| 2 | 47,XY,+18 | Edward's syndrome | Old age, choroid plexus cyst, single umbilical artery | Induced abortion |
| 3 | 47,XX,+18 | Edward's syndrome | NT thickening | Induced abortion |
| 4 | 47,XY,+18 | Edward's syndrome | NT thickening, neck hydrocele, fetal edema | Induced abortion |
| 5 | 47,XY,+18 | Edward's syndrome | Massive tricuspid regurgitation, large right atrium, ascites in abdominal cavity, choroid plexus cyst | Induced abortion |
| 6 | 47,XY,+21 | Down's syndrome | Old age, NT thickening, NF thickening and third ventricle enlargement | Induced abortion |

(*Continued*)

**Table 3** (Continued).

| Case | Abnormal Type | Key Gene/Syndrome | Prenatal Indication | Pregnancy Outcome |
|---|---|---|---|---|
| 7 | 47,XX,+21 | Down's syndrome | NT thickening | Induced abortion |
| 8 | 47,XX,+21 | Down's syndrome | NT thickening, high risk of DS | Induced abortion |
| 9 | 47,XY,+21 | Down's syndrome | NT thickening, old age | Induced abortion |
| 10 | 47,XY,+21 | Down's syndrome | High risk of DS, tricuspid regurgitation, duodenal atresia | Induced abortion |
| 11 | 47,XX,+21 | Down's syndrome | NT thickening | Induced abortion |
| 12 | 47,XX,+21 | Down's syndrome | NT thickening | Induced abortion |
| 13 | 47,XY,+21 | Down's syndrome | NT thickening | Induced abortion |
| 14 | 47,XY,+21 | Down's syndrome | NT thickening, old age | Induced abortion |
| 15 | 47,XX,+21 | Down's syndrome | Fetal nasal bone dysplasia and vagal right subclavian artery | Induced abortion |
| 16 | 47,XY,+21 | Down's syndrome | Fetal nasal bone dysplasia | Induced abortion |
| 17 | 47,XX,+21 | Down's syndrome | High risk of DS, left ventricular echogenic intracardiac focus | Induced abortion |
| 18 | 47,XY,+21 | Down's syndrome | NT thickening | Induced abortion |
| **Case** | **Abnormal type** | **Key gene/syndrome** | **Prenatal indication** | **Pregnancy outcome** |
| 19 | 45,X | Turner's syndrome | Single umbilical artery, persistent left superior vena cava, asymmetric left and right atrioventricular sizes, ventricular septal defect | Induced abortion |
| 20 | 45,X | Turner's syndrome | Short femur | Induced abortion |
| 21 | 47,XXY | Klinefelter's syndrome | NT thickening | Induced abortion |
| 22 | 47,XXY | Klinefelter's syndrome | NT thickening | Induced abortion |
| 23 | 47,XN,+9[11%]/46,XN[89%] | 9-trisomy syndrome | NT thickening, old age | Induced abortion |
| 24 | 46,XY[85%]/47,XXY[15%] | Klinefelter's syndrome | NT thickening | Natural labor, developed well in all aspects at present |
| 25 | chr10:g.82860005–92560004del | *CYFIP1, GOLGA6L1, GOLGA6L22, NIPA1, NIPA2, TUBGCP5*/15q11.2 deletion syndrome | NT thickening | Natural labor, developed well in all aspects at present |
| 26 | chr15:g.22676624–23226623del | *BMPR1A, PTEN, GLUD1, ACTA2, GRID1* and other 47 protein coding genes/juvenile polyposis syndrome/Cowden syndrome type 1 | NT thickening | Induced abortion |
| 27 | chr15:g.22676624–23276623del | *CYFIP1, GOLGA6L1, GOLGA6L22, NIPA1, NIPA2, TUBGCP5*/15q11.2 deletion syndrome | NT thickening, old age | Induced abortion |
| 28 | chr16:g.28710001–29010000del | *CYFIP1, GOLGA6L1, GOLGA6L22, GOLGA8IP, NIPA1, NIPA2, TUBGCP5*/15q11.2 deletion syndrome | Large gallbladder, short femur, excessive amniotic fluid | Lost to follow-up |
| 29 | chr17:g.34800001–36250000del | *ATXN2L, CD19, SH2B1* and other 11 protein coding genes/16p11.2 deletion syndrome. | Bilateral renal parenchymal echo enhancement, left ventricular echogenic intracardiac focus | Lost to follow-up |

(*Continued*)

**Table 3** (Continued).

| Case | Abnormal Type | Key Gene/Syndrome | Prenatal Indication | Pregnancy Outcome |
|---|---|---|---|---|
| 30 | chr2:g.199379852–201279851del | *HNF1B, GGNBP2, ACACA, LHX1* and other 17 protein coding genes/17q12 deletion syndrome. | Duplicate kidney, hydronephrosis, ventricular septal defect, old age | Verified by the parents, the CNV was newly mutated. Induced labor |
| 31 | chr1:g.143660001–148860000del | *C2orf69, FTCDNL1, MAIP1, SATB2, SPATS2L, TYW5*/2q33.1 deletion syndrome | Single umbilical artery, persistent left superior vena cava, permanent right umbilical vein | Verified by the parents, the CNV was inherited from the mother. Natural labor. Developed well in all aspects at present |
| 32 | chr1:g.145710001–147960000dup | *GJA5,GJA8,PIAS3,BCL9,RBM8A and* other 41 protein coding genes/1q21.1 deletion syndrome | Mild bilateral pyelic separation | Verified by the parents, the CNV was inherited from the mother. Induced abortion |
| 33 | chr22:g.18850001–20300004dup | *GJA5, GJA8, PIAS3, BCL9, RBM8A* and other 16 protein coding genes /1q21.12 duplication syndrome | NT thickening, high risk of DS | Lost to follow-up |
| 34 | chr22:g.18900001–20300004del | *TBX1\GP1BB\PRODH\TANGO2 \CDC45\TXNRD2\SLC25A1* and other 28 protein coding genes/22q11 deletion syndrome | NT thickening, old age | Induced abortion |
| **Case** | **Abnormal type** | **Key gene/syndrome** | **Prenatal indication** | **Pregnancy outcome** |
| 35 | chr22:g.18950001–21500004del | *TBX1\GP1BB\PRODH\TANGO2 \CDC45\TXNRD2\SLC25A1* and other 28 protein coding genes/22q11 deletion syndrome | NT thickening | Induced abortion |
| 36 | chr22:g.18850001–20300004del | *TBX1\GP1BB\PRODH\TANGO2 \CDC45\TXNRD2\SLC25A1*/ 22q11 deletion syndrome | NT thickening | Induced abortion |
| 37 | chr2:g.50837852–50987851del | *TBX1\GP1BB\PRODH\TANGO2 \CDC45\TXNRD2\SLC25A1* and other 28 protein coding genes/22q11 deletion syndrome | Dysplasia of the nasal bone on one side and absence of the twelfth rib | QPCR confirmed that there was no deletion in fetal exon region. Natural labor. Developed well in all aspects at present |
| 38 | chr2:g.50987852–51287851del | *NRXN1* (exon 7–9 deletion) | NT thickening, ultra-old age | Lost to follow-up |
| 39 | chr2:g.122429852–148129851del | *NRXN1* (deletion of 5'UTR region and exon 1–6) | NT thickening, high risk of DS | Induced abortion |
| 40 | chr2:g.161079852–163529851del | *ZEB2, BIN, MAP3K2, IWS1, WDR33, AMMECR1L, SAP130* and other 69 protein coding genes/Mowat-Wilson syndrome | Choroid plexus cyst | Induced abortion |
| 41 | chr7:g.72700001–74200000del | *TBR1, IFIH1, RBMS1, SLC4A10, PSMD14, TANK, DPP4, FAP, GCA, GCG, KCNH7* | Left ventricular echogenic intracardiac focus, intersecting pulmonary artery | Lost to follow-up |

(*Continued*)

**Table 3** (Continued).

| Case | Abnormal Type | Key Gene/Syndrome | Prenatal Indication | Pregnancy Outcome |
|---|---|---|---|---|
| 42 | chr8:g.8060001–11910000del | *ELN, LIMK1, GTF2I, BAZ1B, CLIP2* and other 26 protein coding genes/Williams-Beuren syndrome | NT thickening, old age | No abnormalities were found at birth, but pulmonary artery stenosis was found at the age of 1, and surgery was performed. Now it is growing well |
| 43 | chrX:g.6410001–8160000del | *GATA4, BLK, XKR6, SOX7, TNKS* and other 23 protein coding genes/8p23.1 deletion syndrome | Single umbilical artery, left ventricular echogenic intracardiac focus and high risk of ES | It was verified by parents that the CNV was inherited from his mother and induced labor because it was a male fetus |

## Discussion

Since the introduction of China's two-child policy, the birth rate in China has shown an obvious upward trend, triggering a broad market demand for accurate prenatal diagnosis. In this study, to further the improvement in the resolution and accuracy of prenatal diagnosis, the genome copy number variation sequencing technology, described as "accurate and efficient, was applied to prenatal diagnosis, with a view to providing theoretical guidance for accurate screening for clinical prenatal diagnosis".

In addition to serious congenital structural abnormalities, prenatal ultrasound can detect some minor anatomical changes; most of these small changes are transient, but some may be related to an abnormal chromosome number in the foetus, pCNVs or even monogenic diseases, which are called "ultrasound soft markers".[10] In the study by Zhao et al,[3] 513 foetuses with abnormal ultrasound soft markers but without definite structural abnormalities were analysed, and the detection rates of abnormal chromosome number and pCNVs were 10.14% and 6.43%, respectively. Zhu et al[11] also analysed 580 foetuses with abnormal ultrasound soft markers but without definite structural abnormalities, and the detection rates of abnormal chromosome number and pCNVs were 8.62% and 4.14%, respectively. Another study by Yao et al[12] analysed 542 foetuses with abnormal ultrasound soft markers in the second trimester, and the detection rates of abnormal chromosome number and pCNVs were 2.40% and 3.14%, respectively. In this study, the detection rates of abnormal chromosome number and pCNVs in 422 foetuses with abnormal ultrasound soft markers were 5.68% and 4.50%, respectively, which differ from previous studies; this may be due to factors such as the study population, soft marker type and diagnostic technology.

Several previous studies have revealed a significant increase in the probability of detecting chromosomal abnormalities or pCNVs when non-isolated ultrasound soft markers are present.[4,13] Despite the higher detection rate of combined ultrasonic soft markers than that of isolated ones in this study, the $\chi^2$ test only supported a statistically significant difference in the detection rate between the "combined NT group" and the other four groups, while the difference in detection rates between the "combined soft marker group" and the "isolated soft marker group" was not statistically significant. It was further found that the majority of cases in the "combined soft marker group" contained two ultrasound soft markers, and the cases with three or more soft markers accounted for a small proportion. It was thus concluded that non-NT thickened ultrasound soft markers may have minimal influence on the risk of genetic diseases, at least for the population in Luoyang area; therefore, prenatal diagnosis should be made carefully. For three or more non-NT thickening ultrasonic soft markers, more population data are needed to support the clinical application value.

Some studies have proved the reliability of using certain isolated ultrasound soft markers as indications for prenatal diagnosis, for example, NT thickening, which is currently one of the most clinically significant ultrasound soft markers. Other studies have revealed a clear correlation between NT thickening and chromosomal aneuploidy, which can significantly increase the risk of congenital heart disease, intellectual disability, developmental retardation and other related genetic syndromes.[14,15] Nuchal fold thickening is considered to be the most important ultrasound soft marker indicating chromosomal abnormalities in the second trimester, and its detection cannot be replaced by NT

examination.[16,17] Of the 26 positive samples with NT thickening in this study, aneuploidy accounted for 61.54% (16/26), indicating a close relationship between NT thickening and aneuploidy, while pCNVs accounted for 38.46% (10/26), so they should also be regarded seriously. It was also found that NT thickening was detected in all 22q11.2 (proximal) microdeletion or microreplication syndromes, which supported the conclusion of Cao et al[18] that NT thickening was the early clinical manifestation of these syndromes. The correlation of choroid plexus cysts, the second most common ultrasound soft marker in this study behind NT thickening, with chromosomal abnormalities is uncertain.[19,20] In this study, it was shown that in foetuses with choroid plexus cysts, the detection rate of abnormalities was low in both isolated and non-isolated indications, which may imply that there is no correlation between choroid plexus cysts and chromosomal abnormalities.[19] Despite isolated nasal dysplasia[11,21] and NF thickening[16] being reported in some studies as possible indications for prenatal diagnosis, such conclusions cannot be supported as valid by this study due to the small number of samples included. The detection rates of two other indications, pyelic separation and short long bone, were close that of NT thickening in this study; the isolated occurrence of these markers may have prenatal diagnostic significance for the population in Luoyang area, although previous studies consider that these two may not be related to chromosomal abnormalities.[16] Therefore, further verification is needed in the future.

In this study, pCNVs were confirmed by CNV-seq in 19 foetuses, 15 of which involved severe syndromes. There were 16 cases of pCNVs with a fragment size <5 Mb, indicating that 78.95% of pCNVs were likely to be missed under the current detection capability of NIPS.[22,23] Further exploration found that 86.67% of cases (13/15) were in the "isolated NT group", "combined NT group" and "combined non-NT group", suggesting that prenatal diagnosis should still be a concern in most foetuses with pCNVs <3 Mb. It can be seen that the soft markers found on prenatal ultrasound, which can minimise the risk of missed detection of pCNVs when combined with NIPS, are still critical markers. Besides routine karyotype analysis, CNV-seq is also recommended for prenatal diagnosis to detect chromosome deletion or duplication larger than 0.1 Mb and to screen pathogenic chromosome copy number variation, which cannot be resolved by karyotype analysis.

Nevertheless, there were still some shortcomings in this study. First, the small sample led to insufficient statistical power of some ultrasound soft markers. Second, the inclusion of chromosome number abnormalities and pCNVs alone makes it impossible to exclude the correlation between some ultrasound soft markers and monogenic diseases. In future research, a more in-depth exploration will be conducted.

## Conclusion

This study has shown NT thickening to be of great clinical significance in indicating chromosome aneuploidy and pCNVs in foetuses with abnormal ultrasound soft markers. The use of CNV-seq diagnosis, whether in isolated NT thickening or in combination with other abnormalities, is recommended to identify chromosomal number abnormalities and the risk of pCNVs. In summary, the CNV-seq diagnosis, once applied, boasts numerous benefits, such as providing more references for the evaluation of prenatal diagnostic technology in China, screening and diagnosing foetal abnormalities as early as possible, and reducing the psychological and economic burden on pregnant women and their families.

## Data Sharing Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

## Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Luoyang Maternal and Child Health Hospital. Written informed consent was obtained from all participants.

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## Disclosure

All of the authors had no any personal, financial, commercial, or academic conflicts of interest separately.

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