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Analysis results of 579 cases of genomic copy number variation sequencing of pregnant women in prenatal diagnosis

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Abstract. – OBJECTIVE: This study explored the usefulness of genomic copy number variation sequencing (CNV-Seq) in the prenatal diagnosis of pregnant women.

PATIENTS AND METHODS: Based on prenatal diagnostic indications, CNV-Seq analysis was done in the samples from the 579 pregnant women of the 7 subgroups that included advanced maternal age (group A), high risk noninvasive prenatal test (NIPT) (group B), high risk Down's (Group C), abnormal ultrasound findings (Group D), adverse pregnancy history (Group E), chromosome abnormalities in couples (Group F), and the mixed group (Group G).

RESULTS: A total of 57 (9.84%) cases have abnormal CNV-Seq results. Among them, 21 cases were aneuploid chromosomal number abnormalities (3.63%, 21/579), and 36 cases were CNV abnormalities (6.22%, 36/579), including 7 cases of pathogenic copy number alteration (pCNA) (1.21%, 7/579) and 29 cases variants of uncertain significance (VUS) (5.01%, 29/579). The total detection rates of abnormal CNV-Seq in Group G and Group B were 20.27% (15/74) and 15.91% (14/88), which were significantly higher than those in other groups ($p < 0.05$). Among 36 cases of abnormal CNV-Seq, 7 cases were chromosome fragment deletion or duplication, which were pathogenic CNV, and some rare chromosomal diseases were detected.

CONCLUSIONS: Patients with a high risk of NIPT or multiple indications of prenatal diagnosis are highly suspected of chromosomal diseases. CNV-Seq is a useful tool for detecting chromosome abnormalities for prenatal diagnosis of pregnant women more accurately and provides more comprehensive information for prenatal diagnosis to reduce birth defects.

Key Words:

Aneuploid, Chromosomes, Prenatal diagnosis, Reproductive history, Sequencing.

Introduction

Prenatal diagnosis is an effective way to prevent the birth of a child with a genetic disease. Karyotype analysis has been the "gold standard" for the diagnosis of chromosome aberration and the first-line method for prenatal diagnosis of chromosomal diseases. However, due to its long detection cycle and low resolution, it cannot detect gene copy number variations (CNV) below 5 Mb. CNV is the deletion or duplication of DNA fragments larger than 1 Kb on chromosomes. The disease caused by pathogenic CNV is an important genetic cause of fetal birth defects[1]. Copy number variation sequencing (CNV-Seq) based on next-generation sequencing provides a new method for prenatal diagnosis to detect the type of chromosomal disease, which are covered by the chromosome microarray analysis chip platform, with the aim to find the microdeletions and microduplications of chromosomes in the areas which are not covered by the chip probe. Moreover, its advantages include low cost, good repeatability, and low requirement of DNA sample size, which can make up for the deficiency of chromosome karyotype analysis[2]. As a result, CNV-Seq technology can be used as a first-line prenatal diagnosis method for fetuses with a high risk of chromosomal diseases[3,4]. However, more studies should be conducted to further analyze the efficiency of CNV-Seq in prenatal diagnosis. In this study, the CNV-Seq results of 579 cases of pregnant women with singleton pregnancies who underwent amniocentesis were retrospectively analyzed, the pathogenicity of the detected CNVs was interpreted according to the guidelines, and the pathogenic CNVs were analyzed to improve the prenatal counseling and provide a basis for fetal prognosis evaluation.

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Patients and Methods

Study Population and Study Site

A total of 579 pregnant women in the Second Affiliated Hospital of Guangxi Medical University from July 2020 to January 2022 were selected. The inclusion criteria included the following: singleton pregnancy, indication for amniotic fluid extraction for CNV-Seq due to high risk factors, such as old age, high risk of Down's syndrome after maternal serum screening, high risk of noninvasive prenatal testing (NIPT), abnormal ultrasonic detection, history of adverse pregnancy outcomes (gave birth to children with chromosome abnormalities or neural tube malformations or experienced unexplained spontaneous abortion or stillbirth), and chromosome abnormality in couples. The age of the patients ranged from 17 to 49 years, with an average age of 33.41 ± 5.42 years. The gestational age of the patients ranged from 14 to 35 weeks, with an average gestational age of 19.01 ± 2.81 weeks. This study was approved by the Hospital Medical Ethics Committee, and signed informed consent was obtained from all patients.

Methods

Amniocentesis

Under the guidance of B-mode ultrasound, qualified well-trained doctors for prenatal diagnosis performed amniocentesis to collect 10 ml of amniotic fluid on the pregnant women for the CNV-Seq detection.

CNV-Seq Detection

DNeasy Blood & Tissue Kit (Qiagen) was used to isolate the genomic DNA from amniotic fluid cells. The template DNA was used for library parathion using the PCR-free database building method. Sequencing was then done using an Illumina NextSeq CN500 sequencing platform. The sequences were analyzed by searching the public databases, such as the database of genetic variation, the database of chromosomal imbalance, and phenotype in humans using ensemble resources, and Online Frontal Analysis Mendelian Inheritance in Man.

Analysis by Group

The pregnant women were divided into seven groups according to the prenatal diagnosis indications. These groups included advanced maternal age, including pregnant women with delivery aged ≥35 years (Group A), high risk NIPT (Group B), high risk Down's (Group C), abnormal ultrasound findings (Group D), adverse pregnancy history (Group E), chromosome abnormality in couples (Group F), and mixed group with two or more prenatal diagnostic indications (Group G). The rate of abnormal chromosome and CNV detection in each group were then analyzed. Based on clinical significance, the CNV larger than 100 kb was classified into five groups[5], i.e., pathogenic CNV (pCNV), likely pathogenic CNV (lpCNV), variants of uncertain significance (VUS) CNV, likely benign CNV (lbCNV), and benign CNV (bCNV).

Statistical Analysis

The frequency of chromosome abnormalities was counted to calculate its proportion. The Chi-square test was used to analyze the enumeration data using Statistical Package for the Social Sciences version 25.0 software (SPSS Corp., Armonk, NY, USA). p-values of <0.05 were considered statistically significant.

Results

The Result of CNV-Seq in Amniotic Fluid

CNV-Seq was performed in 579 cases of amniotic fluid samples. Among them, 57 cases (9.84%) were abnormal, comprising 21 cases of aneuploid chromosomal numerical abnormalities (3.63%, 21/579) and 36 cases of CNV abnormalities (6.22%, 36/579). Among the aneuploid chromosomal numerical abnormalities, 11 cases were trisomy 21 (3.63%, 21/579), 3 were trisomy 18 (0.52%, 3/579), 1 was trisomy 13 (0.17%, 1/579), another 1 was trisomy 15 (0.17%, 1/579), 4 were monosomy X (Turner syndrome) (0.69%, 4/579), and 1 was XYY syndrome (0.17%, 1/579). Of the 36 CNV abnormalities cases, 7 were pathogenic copy number alteration (pCNA) (1.21%, 10/579), and 29 were VUS (5.01%, 29/579).

Prenatal Diagnostic Indications and Distribution of CNV Abnormalities

The top three of the seven prenatal diagnostic indications involved pregnant women with old age (35.92%), fetal abnormality in ultrasonic examination (19.17%), and high risk of NIPT (15.20%). The detection rate of abnormal CNV was 20.27% (15/74) for Group G, followed by

15.91% (14/88) for Group B, which were significantly higher than those in other groups ($p < 0.05$). Group A involved one case of trisomy 15, one case of trisomy 18, two cases of trisomy 21, and ten cases of VUS. Group B involved one case of trisomy 13, one case of trisomy 18, one case of trisomy 21, three cases of monosomy X (Turner syndrome), one case of XYY syndrome, three cases of pCNV, and four cases of VUS. Group C involved one case of trisomy 21 and one case of VUS. Group D involved one case of pCNV and eight cases of VUS. Group E involved one case of monosomy X and two cases of VUS. Group F had no abnormal CNV detected. Group G involved six cases of trisomy 21, one case of trisomy 18, three cases of pCNV, and four cases of VUS (Table I).

Analysis of Microdeletion/ Microduplication of the pCNV

Among the 36 cases of CNV abnormality, 7 cases were deletion or duplication of chromosome segments, which were pathogenic CNV, and the other 29 cases were CNV with unclear clinical significance. Of the seven pCNV, three cases were high risk chromosome abnormalities suggested by NIPT, which involved DeSanto-Shinawi syndrome, X-linked ichthyosis disease, and CHILD syndrome; one case was old maternal age, which included 16p11.2 microdeletion syndrome; one case was a fetal abnormality in ultrasound examination showing left subclavian artery vagus, suspected ventricular septal defect, and a strong spot of the left ventricular, which involved DiGeorge/velocardiofacial (DGS/VCFS) syndrome; one case was high risk NIPT combined with a strong spot in the fetal left ventricle suggested by ultrasound, which involved 1q21.1 microdeletion syndrome; and one case was old age combined with the disappearance of the fetal nasal bone suggested by ultrasound, which involved Pallister-Killian syndrome (Table II).

Discussion

The Advantages of CNV-Seq

Chromosomal diseases are the main cause of genetic diseases leading to birth defects. One of the main causes of fetal birth defects is related to the pCNV[1] that causes fetal malformation, abortion, stillbirth, and neonatal death. However, it is difficult to find the submicroscopic structure changes of the chromosome by using traditional cytogenetic analysis. The main advantages of CNV-Seq include a wide detection range, high throughput, short cycle, high resolution, simple operation, and low DNA template requirement, which can effectively improve the detection of abnormal chromosomes[3,4]. CNV-Seq was successfully amplified in all cases in this study. Among them, 57 cases (9.84%) were abnormal, including 21 cases of aneuploid chromosomal numerical abnormalities and 36 cases of CNV abnormalities. There were 21 cases of pathogenic chromosomal aneuploidy, comprising 11 cases of trisomy 21, 3 cases of trisomy 18, 1 case of trisomy 13, 1 case of trisomy 15, 4 cases of monosomy X (Turner syndrome), and 1 case of XYY syndrome. The pathogenic chromosomal numerical abnormality is still the most common cause of birth defects. Apart from the pathogenic chromosomal aneuploidy, seven cases of pCNA were detected. Therefore, CNV-Seq not only can make up for the deficiency of chromosome karyotype analysis but also can detect the pathogenic microdeletions and microduplications and plays an important role in the prevention of genetic diseases of birth defects. More recently, CNV-Seq has also been applied in preimplantation genetic diagnosis/screening (PGD/PGS), which is being offered to woman undergoing in vitro fertilization[6]. PGD/PGS can test for and prevent the transfer of embryos with genetic disorders before freezing them by vitrification technique, of which the closed vitrification system might be safer and more effective[7,8].

The Analysis of CNV-Seq Results on Different Prenatal Diagnostic Indications

Among the seven different prenatal diagnosis indications of old maternal age, high risk of NIPT, high risk of Down's syndrome, abnormal ultrasound, adverse pregnancy, childbirth history, chromosome abnormality in couples, and the mixed group with two or more prenatal diagnosis indications, the detection rate of chromosome abnormalities was the highest in the mixed group (Group G), suggesting that the pregnant women with multiple prenatal diagnosis indications had more vigilant chromosome abnormalities. In the single prenatal diagnosis indication, the detection rate of chromosome abnormalities in the NIPT high risk group (Group B) was significantly higher than that in other groups. In addition to chromosome aneuploidy abnormalities, three cases of pCNV were found due to the high risk of NIPT in this group, indicating that NIPT does

Table I. The distribution of CNV abnormalities in different prenatal diagnostic indications.

Groups	Number of cases	Total detection rate	Chromosomal numerical abnormality						CNV abnormality	
			Trisomy 13	Trisomy 15	Trisomy 18	Trisomy 21	Monosomy X	XYY syndrome	pCNV	VUS
Group A	208 (35.92)	14 (6.73)	0 (0.00)	1 (0.48)	1 (0.48)	2 (0.96)	0 (0.00)	0 (0.00)	0 (0.00)	10 (4.81)
Group B	88 (15.20)	14 (15.91)	1 (1.14)	0 (0.00)	1 (1.14)	1 (1.14)	3 (3.41)	1 (1.14)	3 (3.41)	4 (4.54
Group C	43 (7.43)	2 (4.65)	0 (0.00)	0 (0.00)	0 (0.00)	1 (2.32)	0 (0.00)	0 (0.00)	0 (0.00)	1 (2.32)
Group D	111 (19.17)	9 (8.11)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.90)	8 (7.21)
Group E	46 (7.94)	3 (6.52)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (2.17)	0 (0.00)	0 (0.00)	2 (4.35)
Group F	9 (1.55)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Group G	74 (12.78)	15 (20.27)	0 (0.00)	0 (0.00)	1 (1.35)	7 (8.11)	0 (0.00)	0 (0.00)	3 (4.05)	4 (5.40)
Total	579 (100.00)	57 (9.84)	1 (0.17)	1 (0.17)	3 (0.52)	11 (1.90)	4 (0.69)	1 (0.17)	7 (1.21)	29 (5.01)

Table II. Analysis of seven cases of chromosome abnormalities in microdeletion/microduplication pCNV.

Groups	Clinical manifestation	Number of cases	CNV abnormalities	Size (Mb)	Syndrome involved
Group A	Advanced maternal age with adverse pregnancy history	1	del(16)(p11.2p11.2): 28840000~29040000	0.2	16p11.2 deletion syndrome
Group B	NIPT suspected the deletion of chromosome 10	1	del(10)(p12.31p11.23): 21640000~30160000	8.52	DeSanto-Shinawi syndrome
Group B	NIPT suggested the high risk of trisomy 18	1	del(X)(p22.31p22.31): 6460000~8140000	1.68	X-linked ichthyosis disease
Group B	NIPT suggested the abnormal high risk of sex chromosome	1	del(X)(q24q28): 118200000~154940000	143.12	CHILD syndrome
Group D	Ultrasound suggested the fetus with right aortic arch, left subclavian artery vagus, suspected ventricular septal defect, a strong spot of the left ventricular	1	del(22)(q11.21q11.21): 18880000~21480000	2.6	DGS/VCFS syndrome
Group G	NIPT suggested the repetition of chromosome 1 and ultrasound suggested the fetus with right aortic arch	1	dup(1)(q21.1q21.2): 146500000~147840000	1.34	1q21.1 microdeletion syndrome
Group G	Advanced maternal age and ultrasound suggested the disappearance of the fetal nasal bone	1	dup(12)(p13.33p11.1): 160000~34860000	34.7	Pallister-Killian syndrome

not have an accuracy of 100%[9,10], although it still has important reference value to detection of fetal chromosome abnormalities in time.

The group with abnormal ultrasounds (Group D) had a high proportion of prenatal diagnosis indications. However, the possibility of pathogenic chromosome abnormalities was low. Besides, pCNV was found in multiple ultrasound soft indexes or combined with old maternal age and a high risk of NIPT. Previous findings indicated that the detection rate of pathogenic CNVs is lower than 1.5% when the fetus had isolated ultrasound soft indexes, and the risk of detecting pathogenic CNVs is similar to that of low risk pregnant women[11]. Therefore, there is a high possibility of pCNV when ultrasound examination of multiple soft indexes is abnormal or combined with other prenatal diagnostic indications.

Analysis of Microdeletion/ Microduplication pCNV

In this study, seven cases of pathogenic CNV were detected by CNV-Seq, which included 16p11.2 deletion syndrome, DeSanto–Shinawi syndrome, X-linked ichthyosis disease, CHILD syndrome, DiGeorge/velocardiofacial (DGS/VCFS) syndrome, 1q21.1 microdeletion syndrome, and Pallister-Killian syndrome. Due to advanced maternal age combined with schizophrenia, the patient with 16p11.2 deletion syndrome in this study had a prenatal diagnosis, and 0.2-Mb deletion on the p11.2 area of chromosome 16 was detected, which is considered as a 16p11.2 microdeletion syndrome. According to reports, clinical manifestations, such as growth retardation, mental retardation, autism spectrum disorder, giant malformation, schizophrenia, and adolescent obesity, are most commonly associated with this syndrome[12-14]. However, there are few reports[15] on prenatal diagnosis, and only a few reported the abnormal ultrasonic signs of heart malformation, unilateral polycystic kidney disease, loss of nasal bone, single umbilical artery, and intrauterine growth retardation. The pCNV of this case was inherited from her mother, but her family members did not agree with pedigree certification. NIPT results suggested that the pathogeny of the case

with DeSanto-Shinawi syndrome is chromosome 10 deletion. Therefore, 8.52-Mb deletion in p12.31-p11.23 area of chromosome 10 was finally detected by CNV-Seq. WAC mutation in this region can cause an autosomal dominant rare neurodevelopmental disorder, i.e., DeSanto-Shinawi syndrome. It is characterized by global developmental delay in infancy, accompanied by characteristic craniofacial malformations, such as a big forehead, saddle nose, bulbous nasal tip, and deep sunken eyes. Most patients also have other phenotypes, such as gastrointestinal abnormalities and mild eye abnormalities and behavioral problems[16,17].

In this study, we observed one case of ichthyosis disease that was X-linked recessive associated with the STS gene. NIPT results suggested making a prenatal diagnosis for the high risk of trisomy 18. Chromosome 18 abnormality was not detected at last, while 1.68-Mb deletion was shown in the p22.31 area of chromosome X, which includes the STS gene. It is characterized by widely and symmetrically distributed adhesion causing dry and polygonal scales on the skin[18,19].

Moreover, one case of CHILD syndrome was observed in this study. The NIPT results suggested CNV-Seq for the high risk of sex chromosome abnormality, in which 36.74-Mb deletion on q24-q28 area was detected by CNV-Seq and covered NSDHL gene, which can cause CHILD syndrome of X-linked dominant inheritance when the gene haploid dose is insufficient. Its main manifestations are mild prenatal growth retardation, hearing loss, cleft lip, single ventricle, unilateral hypoplasia of the lung, rib, and ovary, and mild intellectual impairment, among others[20,21].

The DGS/VCFS syndrome was also observed in one case. Its ultrasound showed multiple fetal malformations (fetus with right aortic arch showed, left subclavian artery vagus, suspected ventricular septal defect, and a strong spot of the left ventricular). Therefore, 2.6-Mb deletion on q11.21-q11.21 (chr22:g.18880000_21480000) area of chromosome 22 was detected. Its phenotypes include congenital heart disease (especially cone tube malformation), palatal abnormalities, special facial features, growth retardation, intellectual impairment, and behavior problems, among others[22,23].

There was a case of 1q21.1 microdeletion syndrome in this study. The NIPT results suggested repetition of chromosome 1, and ultrasound showed a strong spot on the fetal left ventricle. Therefore, 1.34-Mb repetition on q21.1-q21.2 area of chromosome 1 was detected by CNV-Seq. The main phenotypes are dysplasia of the corpus callosum and cerebellar vermis, learning difficulties, autism, schizophrenia, and mild special face. Some patients show tetralogy of Fallot, congenital heart disease, intellectual impairment, hyperactivity disorder, schizophrenia, epilepsy, spinal curvature, giant malformation, and mild genital malformation, among others[24,25].

The Pallister-Killian syndrome case was observed in advanced maternal age. The ultrasound findings revealed that fetal nasal bones disappeared. The 34.7-Mb repetition on the p13.3-p11.1 area of chromosome 12 was detected. It is also called 12p tetrad syndrome or 12p isochromosome syndrome, which is a rare chromosomal disease. It can involve multiple systems and cause many phenotypes, including craniofacial abnormalities, abnormal skin pigment, ophthalmic complications, abnormal development of the nervous system, deafness, hypotonia, short limbs, and various degrees of malformations in visceral development[26,27]. These rare chromosomal diseases have serious impacts on the fetus, but the causes of deletion or duplication of small segments of CNV are hard to detect by traditional karyotype analysis. The application of CNV-Seq can improve the detection rate of pathogenic chromosome abnormalities in prenatal diagnosis of pregnant women.

Conclusions

Chromosomal diseases are still the main cause of birth defects. Pregnant women with a high risk result after NIPT or multiple prenatal diagnosis indications should be suspected of chromosomal diseases. CNV-Seq can accurately detect chromosomal aneuploidy with a higher sensitivity in detecting chromosomal microdeletions and microduplications. Moreover, it improves the detection rate of chromosome abnormalities in prenatal diagnosis of pregnant women, providing more comprehensive information for appropriate and timely management to reduce neonatal birth defects. Therefore, CNV-Seq is useful in the first-line examination of prenatal diagnosis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution
Li Deng and Junyou Su contributed to the conception and design of the study; Shi Liao, Lilin Wei, and Yan Chen contributed to the acquisition of data; Yan Huang, Jing Luo, and Junru Tong contributed to the analysis of data; Lingling Huang and Hongfei Chen drafted the article; Yanni Wei and Hongfei Chen made critical revisions related to relevant intellectual content of the manuscript.

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Data Availability Statement
The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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