Application of Chromosome Microarray Analysis and Karyotype Analysis in Diagnostic Assessment of Abnormal Down's Syndrome Screening Results

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Abstract

Background: Although screening for fetal aneuploidy with the use of cell-free DNA obtained from maternal plasma is highly effective, biomarkers screening is in extensive use in economically underdeveloped areas and poor population. This study aims to explore the application value of chromosomal microarray analysis (CMA) and karyotype analysis in prenatal diagnosis for pregnant women with abnormal Down's syndrome (DS) screening results.

Methods: The study recruited 813 pregnant women with abnormal DS screening results from Chengdu Women's and Children's Central Hospital. They underwent amniocentesis to obtain fetal amniotic fluid for CMA and G-band karyotype analysis. An Affymetrix CytoScan 750 K Array chip was used for CMA analysis according to the manufacturer's instructions.

Results: In total, CMA identified 21/813 abnormal results, which was more efficient than karyotype analysis(10/813, P<0.001.) CMA is equivalent to traditional karyotype analysis for the prenatal diagnosis of aneuploidies. However, CMA identified 1.60% more copy number variants(CNVs) than karyotype analysis. These pathogenic/likely pathogenic(P/LP) CNVs ranged from 159Kb deletion to 3616Kb deletion. 53.8% of them were recurrent pathogenic CNVs associated with risk of neurodevelopmental disorders. CMA identified 7 variants of uncertain significance (VUS) results, including 6 microduplication and 1 microdeletion, with the size ranged from 840kb-1484kb. Karyotype analysis identified 2 mosaic sex chromosome aneuploidy, 2 balanced translocation and 1 mosaic balanced translocation, which could not be identified by CMA.

Conclusions: Performing both CMA and karyotype analysis simultaneously is more beneficial to pregnant women with abnormal DS screening results.

Background

DS is the most common congenital cause of mental disability and also leads to numerous metabolic and structural problems. Since 1984 Irwin R and colleagues found an association between low maternal serum a-fetoprotein and fetal chromosomal abnormalities [1], several biomarkers have been observed in abnormally high or low concentrations in the serum of pregnant women whose fetuses are affected by DS.[2–5] Although screening for fetal aneuploidy with the use of cfDNA obtained from maternal plasma is highly effective[6], biomarkers screening is in extensive use in economically underdeveloped areas and poor population. When screening tests predict a high risk of DS, an invasive diagnostic test (amniocentesis or chorionic villus sampling) is usually needed to confirm the diagnosis.[7]

Before the era of microarray, G-banded karyotyping was the gold standard diagnostic test for pregnant women whose screening tests predict a high risk. G-banded karyotyping can identify only 5–10% deletion and duplications that are ≥ 5 Mb.[8] 1.2% congenital anomalies are caused by pathogenic CNVs, including microdeletions and duplications) while common trisomies (trisomy 21, 18, 13) accounts for 0.2%.[9]For over a decade, CNV analysis by CMA has been broadly used for detection of genomic imbalances at a much higher resolution than conventional methods such as G-banded karyotyping. It was recommended as a first-tier approach for the prenatal evaluation of fetuses with structural anomalies observed by ultrasound.[10, 11] CMA includes array comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) array. Compared with aCGH, SNP array has the advantage of detecting triploidy and regions of homozygosity which might indicate uniparental disomy (UPD).[12]

In this study we summarized the SNP array and karyotype results of 813 cases whose biomarker screening of DS was abnormal.

Methods

2.1 Patients

A total of 813 pregnant women at the Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China whose second-trimester biochemical markers screening results were abnormal were enrolled in this study from January 2018 to June 2021. Patients enrolled in the study were aged 18 to 34 years, with an average 28.48 ± 3.33 years of age. All pregnant women underwent amniocentesis to obtain fetal amniotic fluid for SNP array and G-band karyotype analysis. This study was approved by the Medical Ethics Committee of Chengdu Women's and Children's Central Hospital and all pregnant women signed informed consent forms.

2.2 Karyotype analysis and CMA methodology

Three tubes(10mL*3 tubes) of amniotic fluid was collected under ultrasound-guided localization at 18-25 gestational weeks. Two tubes were used for karyotype analysis, and one tube for CMA. For amniotic fluid samples with maternal cell contamination, the CMA test was performed after the amniotic fluid cells were cultured.

Karyotype analysis was performed independently by two individuals using two cell culture systems. After cell culture and sample preparation, a LABB M9120 instrument (Shanghai Beion Medical Technology, Shanghai, China) and matching image analysis software were used for chromosome karyotype scanning and analysis. At least three cell karyotypes were analyzed for each culture, and 20 karyotypes were counted. For the cases with chromosome mosaicism, more karyotypes were counted or analyzed. Karyotype analysis and descriptions were based on the International Human Cytogenetic Naming System(2016).

Genomic DNA from amniotic fluid was extracted using a QIAamp DNA Blood Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. An Affymetrix CytoScan 750 K Array (Affymetrix, USA) chip was used for CMA analysis according to the manufacturer's instructions. ChAS 4.2 software was used for analysis.

2.3 Results categorization

The detected results were categorized into aneuploidy and CNVs including gross deletion, gross duplication, microdeletion, microduplication and loss of heterozygosity (ROH). An arbitrary line was drawn at 5 Mb to differentiate between micro and gross deletion and duplication.

2.4 CNV interpretation and confirmation

All reported CNVs were based on the National Center for Biotechnology Information human genome build 38.The reported CNVs were classified by five-tiered system according to ACMG TECHNICAL STANDARDS[10] with the assistance of the following databases: the Database of Genomic Variants (DGV, http://dgv.tcag.ca/dgv/), the Online Mendelian Inheritance in Man database (OMIM, http://www.ncbi.nlm.nih.gov/omim), Clinical Genome Resourse(ClinGen, https://www.clinicalgenome.org/), ClinVar(https://www.ncbi.nlm.nih.gov/clinvar/),the Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER, https://decipher.sanger.ac.uk/), and PubMed (https://www.ncbi.nlm.nih.gov/pubmed/). P, LP and VUS were shown in this study, while likely benign (LB) and benign (B) were not.

2.5 Inheritance studies

Inheritance studies were performed using Fluorescence in-situ Hybridization (FISH), conventional karyotyping, or CMA when necessary. For example, unbalanced translocation would need parents' karyotype analysis or FISH depending on CNVs size.

2.6 Statistical analysis

SPSS 19.0 software was used for statistical analysis. Comparisons between groups were performed using a $\chi 2$ test, and $P < 0.05$ was considered statistically significant.

Results

3.1 Overall result

In this study, a total of 813 pregnant women were enrolled in: 660 had a high risk in DS screening, 48 borderline risk, 9 abnormal Multiple of median (MOM), and 96 abnormal DS screening result with ultrasound abnormalities. CMA and karyotype findings were summarized in Table 1. In high risk group CMA identified 16 abnormal results, karyotype analysis identified 8. In borderline risk group, CMA identified 1 abnormal results, karyotype analysis identified none. In abnormal MOM group, none abnormal results were identified by both methods. In abnormal DS screening result/ ultrasound abnormalities group, CMA identified 4 abnormal results, karyotype analysis identified 2. In total, CMA identified 21/813 abnormal results, which was more efficient than karyotype analysis(10/813, P<0.001.)

Table 1 Abnormal results of CMA and karyotype analysis in 813 pregnant women with DS screening abnormalities

Group	n	CMA results/case	Karyotype analysis results case	P value
High risk	660	16	8	<0.001
Borderline risk	48	1	0	<0.001
Abnormal MOM	9	0	0	/
Abnormal DS screening results with ultrasound abnormalities	96	4	2	<0.001
Total	813	21	10	<0.001

3.2 CMA results

CMA identified 21 abnormal results in total, with the size ranged from 159Kb deletion (microdeletion of the X chromosome, including *Duchenne muscular dystrophy (DMD)* gene) to 155 Mb whole chromosome gain/loss(XXX/X). They could be grouped into aneuploidy(0.84%, 7/813), gross duplication(0.012%, 1/813), microdeletion(0.98%, 8/813), microduplication(0.74%, 5/813).(Table 2,3) Overall, 14 P/LP CNVs were detected in 14 fetuses(Table 3): 2 case had a microdeletion in the region of azoospermia factor (AZF) locus of the Y chromosome , 2 case had microdeletion in the X chromosome involving DMD and ichthyosis respectively. 10 cases had an autosomal CNVs including 1 case of 1q21.1 recurrent microduplication syndrome, 1 case of 2p16.1p15 microduplication, 1 case of 3q29 microdeletion, 1 case of 11q22.1q23.1 duplication, 1 case of 16p13.11 recurrent microdeletion syndrome, 1 case of 16p13.11 recurrent microduplication syndrome, 1 case of recurrent 16p12.1 microdeletion syndrome, 1 case of 16p11.2 microduplication syndrome, 1 case of renal cysts and diabetes(RCAD) syndrome, and 1 case of 22q11 duplication syndrome. Among the CNVs mentioned above, only one(11q22.1q23.1 duplication,9606kb) was identified by karyotype analysis.

CMA identified 7 VUS results, including 6 microduplication and 1 microdeletion, with the size ranged from 840kb-1484kb. None of them was identified by karyotype analysis. CMA identified 1 ROH(arr[GRCh38] 4q21.22q22.3(83481723_95588274) hmz).

Table 2 The findings of CMA in 813 pregnant women with abnormal DS screening results

Categorization	n	%
Aneuploidy	7	0.84%
Gross duplication	1	0.012%
Microdeletion	8	0.98%
Microduplication	5	0.74%
VUS	7	0.84%
Total	28	3.44%

Table 3 P/LP CNVs in 14 fetuses by CMA

No	CMA results	categorization	known syndromes	dosage sensitive gene/region	OMIM gene count	size of CNVs/kb	Inheritance
1	arr[GRCh38]Yq11.223 22658726_ 26274233x0	P	AZFc	/	11	3616	NA
2	arr[GRCh38] Yq11.223 (24889425_28231736)x0	P	AZFc	/	11	3342	father
3	arr[GRCh38]Xp21.1 (31809962_31968905)x0	P	/	DMD	1	159	mother
4	arr[GRCh38]Xp22.31 6537109_8167604)x1	P	STS	STS/Xp22.31 recurrent region	4	1630	NA
5	arr[GRCh38]1q21.1q21.2(147053151_148360058)x3	P	1q21.1 recurrent microduplication	1q21.1 recurrent region	9	1812	NA
6	arr[GRCh38]2p16.1p15(60148343_61784764)x3	LP	/	/	7	1636	de novo
7	arr[GRCh38]3q29 (193373606_195885016)x1	LP	/	/	16	2511	NA
8	arr[GRCh38]11q22.1q23.1 102192300_111795977)x3	LP	/	/	50	9606	de novo
9	arr[GRCh38]16p13.11 (14799119_16364567)x1	P	16p13.11 recurrent microdeletion	16p13.11 recurrent region	14	1565	mother(learning disorder)
10	arr[GRCh38]16p13.11p12.3 (15225421_18148856)x3	P	16p13.11 recurrent microduplication	16p13.11 recurrent region	10	2923	mother
11	arr[GRCh38]16p12.1 (21728879_22430686)x1	p	Recurrent 16p12.1 microdeletion	/	5	702	father
12	arr[GRCh38]16p11.2 29401182_30178708)x3	P	16p11.2 microduplication syndrome	16p11.2 recurrent region	26	778	father
13	arr[GRCh38]17q12(36466620_37940921)x1	P	RCAD syndrome	HNF1B/17q12 recurrent (RCAD syndrome) region	14	1474	NA
14	arr[GRCh38]22q11.21(18153983_21110475)x3	P	22q11 duplication syndrome	/	45	2956	mother

DMD: Duchenne muscular dystrophy, STS: Steroid sulphatase deficiency, RCAD: renal cysts and diabetes, HNF1B: hepatocyte nuclear factor 1beta.

3.2 Karyotype results

Traditional karyotype identified 10/813 abnormal results: including 4 trisomy 21(No 1-4), 2 superfemale syndrome(No 5-6), 1 Tunner syndrome(No 7), 2 mosaic sex chromosome aneuploidy(No 8-9),1 gross duplication(No10). Besides, 2 balanced translocation(No 11-12) and 1 mosaic balanced translocation(No 13) were identified. CMA identified all of these aneuploidies. However, CMA identified neither of the two mosaic sex chromosome aneuploidy for their low proportion(<=10%). Both of the balanced translocation were inherited from healthy father, and normal results of CMA also suggested they were truly balanced. For the mosaic balanced translocation, although CMA result was normal, we couldn't discriminate it between truly balanced and unbalance.

Table 4 The findings of karyotype analysis in 813 pregnant women with abnormal DS screening results

No	karyotype results	Known syndromes	Inheritance
1-3	47,–,+21	Down's syndrome	de novo
4	46,–,rob(14;21)(q10q10),+21	Down's syndrome	NA
5-6	47,XXX	Superfemale syndrome	de novo
7	45,X	Tunner syndrome	de novo
8	mos 45,X[7]/46,XX[127]	Tunner syndrome(mosaic)	de novo
9	mos 45,X[15]/46,XY[135]	Tunner syndrome/Hermaphroditism	de novo
10	46,–,dup(11)(q22.2q23.1)	/	de novo
11	45,–,rob(14;22)(q10;q10)	/	Inherited from normal father
12	46,–,t(2;20)(p23;q13.1)	/	Inherited from normal father
13	mos 46,–,t(3;6)(q11.2;q25)[9]/46,–[43]	/	de novo

Discussion

CMA, also known as molecular karyotyping, has gradually replaced conventional G-banded karyotyping as the first-tier diagnostic test for the individual with developmental delay, mental retardation, autism spectrum disorder, and/or multiple congenital anomalies, as well as for prenatal evaluation of fetuses with structural anomalies observed by ultrasound.[10] Compared with karyotype analysis, CMA is capable of detecting clinically significant submicroscopic aberrations up to a few kb. SNP array uses high-density oligonucleotide-based arrays in which target probes are chosen from DNA locations known to vary between individuals by a single base pair. CNVs are determined by measuring the absolute fluorescence probe intensities of the patient sample compared with the intensities of multiple normal controls.[13] In this study, we used CMA(SNP array platform) and karyotype analysis for prenatal diagnosis of pregnant women with abnormal DS screening results. CMA is equivalent to traditional karyotype analysis for the prenatal diagnosis of aneuploidies. CMA provided additional clinically relevant information in 13 of pregnancies. CMA could detect 1.60% more CNVs than karyotype analysis, which is consistent with previous reports.[14] [15]However, the prevalence of P/LP CNVs in DS screening abnormal fetus(1.6%) is a bit higher than that in common population(1.2%).[9] Besides, we identified a mendelian monogenetic disease, that a male fetus had a microdeletion in DMD gene. Inheritance studies revealed the abnormalities was inherited from his mother who had no family history.

Among the P/LP CNVs identified by CMA, 53.8% were recurrent pathogenic CNVs associated with risk of neurodevelopmental disorders. The penetrance for these recurrent pathogenic CNVs vary from race to race,[16, 17] and there was no large penetrance data available in Chinese population. So it was difficult to determine the clinical significance of these recurrent pathogenic CNVs, which would cause significant stress to pregnant women and their families, in some cases even resulted in the unnecessary abortion. According to Rosenfeld JA and colleagues[17], higher penetrance is seen with CNVs that have higher de novo frequencies. It was also reported that a strong association between IQ and the probability at which CNV deletions occur de novo.[18] Therefore inheritance studies of parents would be helpful to help determining source and counseling. Among the 7 recurrent pathogenic CNVs detected in this study, 2 refused inheritance studies and chose to terminate the pregnancy, 1 were inherited from mother having learning disorder(de novo) and chose to terminate the pregnancy, and the rest 4 were inherited from healthy parents and chose to continue the pregnancy. We would keep a long-time follow-up for the 4 parents. CMA identified 7 VUS, which is a difficult problem to genetic counseling. Inheritance studies of parents should be performed to help determining source and counseling. Pregnant women and their families should be fully informed of the possible outcomes and provide consent before CMA is performed.

The American College of Obstetrics and Gynecology (ACOG) and the American Maternal-Fetal Medicine Association's 2016 guidelines clearly suggest CMA as a first-line prenatal diagnostic method in pregnant women with ultrasounds structural abnormalities.[11, 19] However, only a few reports had mentioned the effectiveness of CMA in pregnant women with DS screening abnormals. In this study, CMA idenitified 1.60% more P/LP CNVs than karyotype analysis, which is the first cause of congenital abnormies[9]. So it is necessary to perform CMA in pregnant women with DS screening abnormals.

Despite the advantages of superior sensitivity and faster turn-around time, there are also some disadvantages compared to conventional karyotyping. CMA is unable to detect balanced chromosomal aberrations and mosaic chromosome abnormalities in low proportion. In this study, karyotype analysis identified 2 mosaic sex chromosome aneuploidy(45,X[7]/46,XX[127], 45,X[15]/46,XY[135]). CMA failed to identify both of them. Although the proportions of abnormal cells were low, they might result in some symptoms of Tunner syndrome or hermaphroditism according to previous reports[20, 21] and our experience in adults with such karyotype. CMA can detect trisomy 13/21 but cannot discern whether it resulted from a non-disjunction event or due to a translocation. In such cases, karyotype analysis of the fetus and the parents is essential for determining reproductive risk for future offspring. Besides, karyotype analysis provided additional clinically relevant information in 3 of pregnancies, 2 balanced translocation and 1 mosaic balanced translocation, which would be helpful for future pregnancies.

Conclusions

In total, CMA identified 21/813 abnormal results, which was more efficient than karyotype analysis (10/813, P<0.001.) CMA identified 13 more definite CNVs than karyotype analysis. However, karyotype analysis identified 2 mosaic sex chromosome aneuploidy, 2 balanced translocation and 1 mosaic balanced translocation, which could not be identified by CMA despite its advantages of superior sensitivity and faster turn-around time.

In conclusion, performing both CMA and karyotype analysis simultaneously is more beneficial to pregnant women with abnormal DS screening results.

Abbreviations

CMA: chromosomal microarray analysis; DS: Down's syndrome; CNVs: copy number variants; P/LP: pathogenic/likely pathogenic; VUS: variants of uncertain significance; aCGH: array comparative genomic hybridization; SNP: single nucleotide polymorphism; UPD: uniparental disomy; ROH: loss of heterozygosity; DGV:Database of Genomic Variants; OMIM: the Online Mendelian Inheritance in Man database ; ClinGen:Clinical Genome Resourse; DECIPHER: the Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources; FISH: Fluorescence in-situ Hybridization; MOM: Multiple of median; DMD: Duchenne muscular dystrophy; AZF: azoospermia factor; RCAD: renal cysts and diabetes; ACOG: The American College of Obstetrics and Gynecology.

Declarations

Ethics approval and consent to participate

This study was performed in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of Chengdu Women's and Children's Central Hospital and all pregnant women signed informed consent forms.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Y.H initiated the study and drafted the manuscript; H.K designed the study and provided input into analysis design; H.K, LX.W, XY.L and YM.X performed laboratory work, CL.G and Y.H performed the clinical work. All authors contributed significantly, read and approved the final manuscript.

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