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Original Research

Application of Chromosome Microarray in Diagnosis of Amniotic Fluid in Older Pregnant Women

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Abstract

Background: To improve the detection rate of chromosome abnormalities in fetuses and to reduce the birth defects rate in elderly pregnant women using chromosome karyotype analysis combined with the chromosome microarray analysis (CMA) technique. **Methods**: Overall, 210 elderly pregnant women with singleton pregnancies aged between 16 and 30 weeks (mean gestational age, 19.19 weeks) and 35 and 47 years (mean age, 38.08 years) were selected from January 1, 2020 to June 1, 2021 in the Eugenics Genetics Department of Yulin Maternal and Child Health Hospital. Chromosome G banding karyotype analysis and CMA detection were performed simultaneously. **Results**: Among the 210 elderly pregnant women with singleton pregnancies, 26 (12.38%) and 52 (24.76%) cases were detected as abnormal using chromosome karyotype analysis and CMA technology, respectively. The abnormal CMA chromosomes' total detection rate was 12.38% higher than that using chromosome karyotype analysis ($p < 0.001$). CMA detected 22 pathogenic copy number variants (CNVs), 1 probable CNV, and 7 CNVs of unknown clinical significance in patients with normal karyotype analysis. Among the patients with abnormal karyotype analysis, CMA missed detection in 5 cases. Overall, 57 abnormal cases were detected when the two methods were combined, with a detection rate of 27.14% (57/210) higher than that of CMA or karyotype analysis alone. **Conclusions**: For the prenatal diagnosis of fetal amniotic fluid in elderly pregnant women, the combined application of chromosome karyotype analysis and CMA detection technology can further improve the detection rate of abnormal chromosomes and reduce missed diagnosis rates.

Keywords: prenatal diagnosis; chromosome karyotype analysis; chromosome microarray; elderly pregnancy; fetal chromosomal abnormalities

1. Introduction

The proportion of elderly pregnant women (the expected age of delivery ≥35 years) has increased significantly with the opening of the second- and third-child fertility policies, and older pregnant women account for more than half of the total number of those with prenatal diagnosis. With the increase in gestational age, the probability of giving birth to fetuses with abnormal chromosomes in elderly pregnant women is significantly higher than that in those of appropriate age [1,2]. Currently, the technique of chromosome karyotype analysis is the "gold standard" of prenatal diagnosis. It can detect various chromosome abnormalities, such as aneuploidy and unbalanced rearrangement, although limited by its low resolution, time-consuming and tedious nature [3]. Fluorescence in situ hybridization (FISH) can quickly detect common fetal chromosome aneuploidy abnormalities without cell culture, with high specificity and ease of performing on a large scale. It can also detect small fragments of chromosome imbalance, but these require specific probes, and the type and number of probes are relatively limited. Therefore, known fragments belonging to specific regions are required for detecting some chromosome microdeletions and microrepeats [4].

With molecular biology and gene sequencing technology advances, chromosome microarray analysis (CMA), as a novel prenatal diagnosis method in molecular genetics, can detect and obtain numerous genome sequences at once within the entire genome and detect DNA copy number imbalance, that is, copy number variants (CNVs). It can detect submicroscopic chromosome deletions or duplications >50–100 kb that cannot be detected using conventional karyotype analysis without relying on cell culture and has various specimen types. It is an excellent complement to traditional karyotype analysis because of its rapidity, accuracy, high throughput, and the ability to detect small structural abnormalities that cannot be identified using "karyotype analysis" [5]. Therefore, we investigated the chromosome karyotype analysis combined with the CMA technique for the chromosomal examination of amniotic fluid specimens in elderly pregnant women to improve the detection rate of chromosomally abnormal fetuses and reduce the birth defect rate in elderly pregnancies.

2. Materials and Methods

2.1 Case Selection

Overall, 210 elderly pregnant women with gestational ages of 16–30 weeks (mean gestational age, 19.19 weeks) and 35–47 years (mean age, 38.08 years) with singleton pregnancies attending the Department of Eugenics and Genetics of Yulin Maternal and Child Health Hospital from 1 January 2020 to 1 June 2021 were selected for both chromosome G ribbon karyotyping and CMA testing. All those who underwent invasive prenatal diagnosis signed an informed consent form before the procedure and were approved by the ethics committee of the unit. All data in this study involving personally identifiable information have been excluded. The inclusion criteria were: (1) Single pregnancy pregnant woman; (2) Age of pregnant woman and expected age of delivery up to 35 years old. Exclusion criteria were: (1) Twins pregnant women; (2) Postoperative reduction of fetus, including surgical reduction of fetus and one of the twins spontaneous abortive.

2.2 Amniocentesis

Briefly, 25 mL of amniotic fluid (20 mL and 5 mL for karyotyping and CMA analysis, respectively) were transabdominally extracted with a 21 GPTC-B puncture needle under ultrasound guidance in advanced maternal age at 16–30 gestational weeks and 35–47 years.

2.3 Chromosome G-Banding Karyotype Analysis

The extracted amniotic fluid was loaded into sterile centrifuge tubes; the amniotic fluid cells were collected by centrifugation and incubated for 10–14 d in applanation until the cells were in good growth condition. Colchicine was added, and cells were collected for routine filming. G bands (320–400 bands) were revealed for karyotype analysis and automatically scanned using the American Leica fully automated scanning microscope and image analysis system. They were analyzed on the GSL-120 fully automated workstation analysis software, counting 20 mid-phase divisions and analyzing 5 karyotypes, and doubling the analysis and counting of divisions for abnormalities. Karyotype descriptions were referenced using the International System of Nomenclature for Human Cytogenomics (ISCN 2016).

2.4 Chromosome Microarray Analysis Assay

The extracted amniotic fluid DNA was assayed for CNV using Cy5-labeled samples and Cy3-labeled controls; equal amounts of different fluorescent-labeled DNA and reference DNA to be tested were hybridized simultaneously to microarrays comprising OligoDNA probes after being fixed with non-specific-repetitive sequences using human Cot-1DNA and hybridized for 24 hours. Subsequently, the whole genome scan was detected using a chromosome microarray chip produced by Agilent (Palo Alto, CA, USA), and the results were analyzed by data analysis using Agilent Genomic Workbench software (Palo Alto, CA, USA) to calculate the genotype or relative intensity of the signal generated for each locus. The results were queried in the Online Mendelian Inheritance in Man (OMIM), University of California Santa Cruz (UCSC), International Standards for Cytogenomic Arrays (ISCA), Database of Genomic Variants (DGV), DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER) databases and other databases. DNA CNVs of the genome were classified into the following four categories according to the American College of Medical Genetics and Genomics guidelines issued in 2015: (a) clinically pathogenic CNVs; (b) probable clinically pathogenic CNVs; (c) CNVs of unknown clinical significance; and (d) benign CNVs.

2.5 Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA) software was used for statistical analysis, and the count data were expressed as rate and frequency, and the χ^2 test was used for comparison between groups. Statistical significance was considered at $p < 0.05$.

3. Results

3.1 Karyotype Test Results

Among 210 pregnant women of advanced maternal age, 26 karyotypic abnormalities were detected, with an overall detection rate of 12.38% (26/210). Of these, 16 cases of chromosomal aneuploidy were detected, with a detection rate of 7.62% (16/210), including 6 cases of trisomy 21, 6 of chimerism, 2 cases of 47,XXY, 47,XYY, and 47,XXX,inv(9)(p12q13) each; 10 cases of chromosomal structural abnormalities, with a detection rate of 4.76% (10/210), including 5 cases of inversions, 3 of translocations, 1 of duplications and deletions each; and 14 cases of chromosomal polymorphisms (6.67%), excluded from the karyotyping of chromosomal abnormalities in this study (See Table 1).

3.2 CMA Results

Among 210 pregnant women with advanced maternal age, 52 cases of chromosomal abnormalities were detected, with a total chromosomal abnormality detection rate of 24.76% (52/210), including 17 cases of chromosomal aneuploidy and 35 cases of copy number variation. Among the 17 cases of aneuploidy abnormalities, 6 and 4 cases of trisomy 21 and sex chromosome aneuploidy abnormalities, respectively, were found as follows: 1 case of 47,XXX, but the karyotype analysis result was 47,XXX,inv(9)(p12q13); 2 of 47,XXY; 1 of 47,XYY; and 7 of chimerism. Of the 35 CNVs, 27 were clinically pathogenic CNVs (25 microdeletions and 2 microduplications); 1 was a probable clinically pathogenic CNV (microdeletion); 7 were CNVs of unknown clinical significance (microduplications) (See Table 2).

Table 1. Karyotype analysis results.

	Classification	Number	Incidence (%)
Number abnormalities		16	7.62
	47,Xn,+21	6	2.85
	47,XXY	2	0.95
	47,XXX,inv(9)(p12q13)	1	0.48
	47,XYY	1	0.48
	Chimera	6	2.86
Structural abnormalities		10	4.76
	inv	5	2.38
	dup	1	0.48
	t	3	1.43
	del	1	0.48
Polymorphism		14	6.67

Note: N in the table represents chromosome X or Y. Inv, inversion; T, translocation; Del, deletion; Dup, duplication.

Table 2. Chromosome microarray detection results.

	Classification	Number	Incidence (%)
Number abnormalities		17	8.09
	Trisomy 21	6	2.85
	Sex chromosome aneuploidy	4	1.90
	Chimera	7	3.33
Copy number variations		35	16.67
	Pathogenic	27	12.86
	Like pathogenic	1	0.48
	Clinical significance unknown	7	3.33

3.3 Comparison of CMA and Karyotype Analysis Test Results

3.3.1 Comparison of Chromosome Aneuploid Number Abnormalities

Among the 17 cases of aneuploidy abnormalities detected using CMA, 6 and 4 cases of abnormal autosomal number and sex chromosome number were detected. These were consistent with the karyotype analysis. Seven cases of chimerism were detected, six of which were consistent with the karyotype analysis, and one additional case of chimerism was detected using CMA, whose karyotype result was 46,Xn,1qh+. The result of CMA was 46,XX/46. Furthermore, the chimerism of 46,XY (~15% of 46,XX) was detected using CMA, and the two results were inconsistent (See Tables 2,3).

3.3.2 Clinically Pathogenic CNVs

The CMA detected 27 pathogenic CNVs, with an abnormality rate of 12.86% (27/210), of which 22 had no chromosomal abnormalities, whereas pathogenic CNVs were detected using CMA, i.e., in addition to the abnormal cases detected using the karyotype analysis, the CMA detected 22 additional pathogenic CNVs, accounting for 10.48%. The karyotype results of the other 5 pathogenic CNVs were 2 inversions: 46,Xn,inv(3)(p11.2q25.3) mat and 46,Xn,inv(9)(p12q13); 2 translocations: 46,Xn,t(10;14)(p14;q12) and 46,Xn,t(7;11)(q11.2;p11.2); and 1 case is a chimera: 45,X[18]/46,X,del(X)(p21.1) (See Tables 2,3).

3.3.3 Probable Clinically Pathogenic CNVs

One case was detected without abnormal karyotype and a CMA test result of 22q11.21, ~409 kb deletion that may be pathogenic, at a 0.48% (1/210) detection rate (see Tables 2,3).

3.3.4 CNVs of Unknown Clinical Significance

The CMA detected 7 CNVs of unknown clinical significance, accounting for 3.33% (7/210); 6 of the 7 cases of unknown clinical significance had no abnormal karyotype results, and 1 karyotype was 46,Xn,dup(14)(q24.3q32.1); the CMA result was 14q24.3q32.12 with a duplication of ~16.8 Mb, which was of unknown clinical significance. However, the clinical significance was unclear (See Tables 2,3).

3.3.5 Missing Chromosome Microarray Detection

Five cases with normal CMA and abnormal karyotype results were found, which were three with chromosomal inversions, one with chromosomal translocation, and one with chromosomal deletion (Table 3).

Table 3. Comparison of CMA and karyotype analysis.

Number	Karyotype results	CMA result	Outcome	Classification	Pregnancy outcomes
A202246	No abnormality	arr[GRCh37]22q11.21(18919528_20943564)x3	22q11.21 has about 2.02 Mb of duplication	pCNVs	BH/LB
A202677	No abnormality	arr[GRCh37]17q12(34438350_36207539)x1	The 17q12 region has a deletion of approximately 1.77 Mb, a pathogenic copy number variant. 17q12 deletion syndrome, also known as Renal Cysts and Diabetes Syndrome (RCS)	pCNVs	BH/LB
A204280	No abnormality	arr[GRCh37]Xq28(152419166_153330005)x2	Xq28 has about 910.84 kb repeats	pCNVs	TOP
A210791	46,Xn,15pstk+	arr[GRCh37]5p15.33p15.31(55550-8129512)x1,10p15.3p15.(138878-4817952)x3	5p15.33p15.31 has a deletion of approximately 8.07 Mb, which overlaps most of the region with cri-du-chat syndrome (also known as catcalling syndrome).	pCNVs	TOP
A203673	No abnormality	arr[GRCh37]22q11.21(18919528_21417548)x1	22q11.21 has about 2.5 Mb deletion	pCNVs	TOP
A204246	No abnormality	arr[GRCh37] 22q11.21(21007827_21417548)x1	22q11.21 approximately 409 kb deletion, possibly pathogenic.	LpCNVs	TOP
A204001	No abnormality	arr[GRCh37] 16p13.11(15125829_16287899)x3	16p13.11 has about 1.16 Mb of duplicates	VOUS	BH/LB
A203157	No abnormality	arr[GRCh37] 5q11.1q11.2(49986122_51806250)x3	5q11.1q11.2 has about 1.82 Mb of duplicates	VOUS	BH/LB
A200911	No abnormality	arr[GRCh37] 8q22.2(99556452_100587077)x3	8q22.2 has about 1.03 Mb of duplication	VOUS	BH/LB
A201435	No abnormality	arr[GRCh37]16p13.11p12.3(15512480_18128488)x3	16p13.11p12.3 approx. 2.62 Mb repeat	VOUS	BH/LB
A200982	No abnormality	arr[GRCh37] 12q21.31(81698253_83380025)x3, 16p12.2(21950360_22428364)x1	A duplication of approximately 1.68 Mb was present in 12q21.31, a copy number variant of unknown clinical significance, a deletion of approximately 478 kb was present in 16p12.2, involving the 16p12.2 deletion region of unknown significance.	VOUS	BH/LB
A210631	No abnormality	arr[GRCh37]16p13.11p12.3(15512480-1812488)x3	16p13.11p12.3 has about 2.62 Mb of repeats.	VOUS	BH/LB
A210950	No abnormality	arr[GRh37]16p13.3(215499-232685x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A201005	No abnormality	arr[GRCh37]16p13.3(215499_232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A201110	No abnormality	arr[GRCh37]16p13.3(215499_232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A211238	No abnormality	arr[GRh37]16p13.3(215499-232685x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A200068	No abnormality	arr[GRCh37]16p13.3(215499-232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	LB/hallux varus of the feet
A200073	No abnormality	arr[GRCh37]16p13.3(215499-232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A200111	No abnormality	arr[GRCh37]16p13.3(215499-232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A200980	No abnormality	arr[GRCh37]16p13.3(215499_232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A210281	No abnormality	arr[GRCh37]16p13.3(215499-232685)*1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A210304	No abnormality	arr[GRCh37]16p13.3(215499-232685)*1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A201504	No abnormality	arr[GRCh37]16p13.3(215499_232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A201979	No abnormality	arr[GRCh37]16p13.3(215499_232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A202357	No abnormality	arr[GRCh37]16p13.3(215499_232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB

Table 3. Continued.

Number	Karyotype results	CMA result	Outcome	Classification	Pregnancy outcomes
A202573	No abnormality	arr[GRCh37]16p13.3(215499_232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A203094	No abnormality	arr[GRCh37]16p13.3(215499_232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A203268	No abnormality	arr[GRCh37]16p13.3(215499_232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	TOP
A203884	No abnormality	arr[GRCh37]16p13.3(215499_232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A210247	46,Xn,inv(3)(p11.2q25.3)mat	arr[GRCh37]16p13.3(215499-232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A210331	46,Xn,t(10;14)(p14;q12)	arr[GRCh37]16p13.3(215499-232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A202837	46,Xn,inv(9)(p12q13)	arr[GRCh37]16p13.3(215499_232685)x1	16p13.3 has a heterozygous deletion of about 17.19 kb	pCNVs	BH/LB
A204091	46,Xn,t(7;11)(q11.2;p11.2)	arr[GRCh37]3q29(195769570_197127658)x1	3q29 has a deletion of about 1.36 Mb	pCNVs	TOP
A200428	45,X[18]/46,X,del(X)(p21.1)	arr[GRCh37]16p13.3(215499_232685)x1,Xp22.33p22.13(1_18240143)x1	Xp22.33p22.13 has a heterozygous deletion of about 18.24 Mb, which is a pathogenic copy number variant.	pCNVs	TOP
A202255	46,Xn,1qh+	arr(X)x1 2,(Y)x0 1	Suggests an abnormal sex chromosome ratio, presumably there may be a 46,XX/46,XY chimerism in this sample (the proportion of 46,XX is about 15%)	Chimera	BH/LB
A202545	46,Xn,dup(14)(q24.3q32.1)	arr[GRCh37]14q24.3q32.12(77521398_94319728)x3	14q24.3q32.12 There is a duplication of approximately 16.8 Mb, the clinical significance of which is unknown.	VOUS	BH/LB
A200826	46,Xn,t(5;22)(p10;q10)pat	arr(1-22)x2,(X,N)x1	No abnormality	normal	BH/LB
A201648	46,Xn,del(22)(p10)	arr(1-22)x2,(X,N)x1	No abnormality	normal	BH/LB
A211168	46,Xn,inv(3)(p24p26)	arr(1-22)x2,(X,N)x1	No abnormality	normal	BH/LB
A211267	46,Xn,inv(9)(p12q13)	arr(1-22)x2,(X,N)x1	No abnormality	normal	BH/LB
A211329	46,Xn,inv(12)(p13q15)mat	arr(1-22)x2,(X,N)x1	No abnormality	normal	BH/LB

Note: CMA, chromosome microarray analysis; pCNVs, pathogenic CNVs; LpCNVs, likely pathogenic CNVs; VOUS, variants of uncertain significance; TOP, termination of pregnancy; LB, live birth; BH, born healthy.

Table 4. Comparison of results detected by CMA and karyotype analysis.

CMA	Karyotype analysis		Total
	Abnormality	Normal	
Abnormality	21	31	52
Normal	5	153	158
Total	26	184	210

CMA, chromosome microarray.

3.4 Comparison of Chromosomal Abnormality Rates between CMA and Karyotype Analysis

Overall, 52 (24.76%) and 26 (12.38%) cases of abnormalities were detected using CMA alone and karyotype analysis alone, respectively. Among them, there were 21 cases with abnormal chromosomes that could be detected by both methods, but 31 cases with abnormal chromosomes detected by CMA could not be detected by karyotype analysis, and 5 cases with normal karyotype results but abnormal CMA results. Kappa consistency test was used for analysis, and the result was Kappa = 0.447, $p < 0.001$; 0.4 < Kappa = 0.447 < 0.75, indicating that the two methods have moderate consistency in detection results (Table 4).

Moreover, the rates of fetal chromosomal abnormalities detected by chromosome karyotype analysis alone, CMA alone, and chromosome karyotype analysis combined with CMA were 12.38%, 24.76%, and 27.14%, respectively. The difference among the three groups was statistically significant ($\chi^2 = 15.669$, $p < 0.001$). The rate of fetal chromosomal abnormalities detected by chromosome karyotype analysis combined with CMA detection was statistically significant compared with that by chromosome karyotype analysis alone ($\chi^2 = 14.430$, $p < 0.001$). There was no significant difference in the rate of fetal chromosome abnormality detected by chromosome karyotype analysis combined with CMA detection compared with CMA detection alone ($\chi^2 = 0.310$, $p = 0.578$). But there was a statistically significant difference in the rate of fetal chromosome abnormality detected by CMA alone compared with chromosome karyotype analysis alone ($\chi^2 = 10.643$, $p < 0.001$) (Table 5).

3.5 Pregnancy Outcome

Among the 210 pregnant women with detected fetal chromosomal abnormalities, genetic counseling was performed. Among the pregnant women with abnormal chromosome numbers, 3 chimeric cases chose to continue delivery, and the fetuses were born with no significant abnormalities in appearance; Labor induction was performed in 6 cases of trisomy 21, 2 cases of 47,XXY, 1 case of 47,XYY, 1 case of 47,XXX,inv(9)(p12q13) and 4 cases of chimeric fetus.

The results of 28 pregnancies with pathogenic or probable pathogenic CNVs were as follows: 1 case of 5p15.33p15.31 with ~8.07 Mb deletion, 1 case of CMA suggesting 3q29 with ~1.36 Mb deletion and karyotype 46,Xn,t(7;11)(q11.2;p11.2), and 1 case of 22q11.21 with ~409 kb deletion where 1 case of 22q11.21 had a deletion of ~2.5 Mb, and was induced after a lineage analysis suggesting a new mutation. One case of 16p13.3 had a heterozygous deletion of ~17.19 kb and was selected to continue delivery after a lineage analysis suggesting maternal inheritance, and the fetus was born without abnormalities; one case of Xq28 had a duplication of ~910.84 kb and was induced after a lineage analysis suggesting maternal inheritance. One case suggested severe thalassemia fetus and induced labor.

Seven CNVs of unknown clinical significance were followed up: one case with a duplication of ~1.03 Mb at 8q22.2, and the family chose to continue the pregnancy after a lineage analysis suggesting a new mutation; one with a duplication of ~2.62 Mb at 16p13.11p12.3, and the family chose to continue the pregnancy after a lineage analysis suggesting maternal inheritance; and one case with a duplication of ~16.8 Mb at 14q24.3q32.12 In one case, duplication of 14q24.3q32.12 was found with ~16.8 Mb. All seven fetuses with CNVs of unknown clinical significance were delivered alive at follow-up, and no significant abnormalities were observed at birth.

4. Discussion

The proportion of elderly pregnant women has increased with the opening of the second- and third-child policies. The older the pregnant woman is, the higher the risk of birth defects. Some studies show that the incidence of fetal aneuploidy and birth defects in elderly pregnant women are significantly higher than those in young women, and the rate of chromosome abnormality increases gradually with the increasing age [6]. Advanced age is an important indicator of prenatal diagnosis and also a high-risk factor for fetal chromosome abnormalities [7]. This study uses chromosome karyotype analysis combined with the CMA technique to examine the chromosomes of amniotic fluid samples of elderly pregnant women. Currently, chromosome karyotype analysis is the gold standard in the clinic for detecting fetal chromosome abnormalities, which can detect chromosome number and structural abnormalities >10 Mb. CMA currently has two detection techniques as follows: comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP). CGH is the main clinical CMA technology. Most CGH chips used in prenatal diagnosis are targeted microarrays designed for chromosome aneuploidy, typical microdeletions or microrepeats, and subtelomere or other chromosome structural rearrangements of obvious clinical significance. Compared with CGH, SNP uses arrays based on high-density oligonucleotides and can extract other clinically useful information from the genotype map. This includes single parent diploid, chimera, maternal cell contamination, and blood relationship, and can also identify triploids CGH cannot

Table 5. Chromosome karyotype analysis, CMA detection, chromosome karyotype analysis combined with CMA detection results.

Group	Normal cases	Abnormal cases	Detection rate
Karyotype analysis	184	26	12.38%
CMA	158	52	24.76%
Karyotype analysis combined with CMA	153	57	27.14%

CMA, chromosome microarray.

detect [8]. Here, amniocentesis, chromosome karyotype analysis, and CMA detection were performed in 210 elderly pregnant women. Overall, 26 cases of abnormal karyotypes were detected using chromosome karyotype analysis, with a detection rate of 12.38% (26/210). The total detection rate of CMA chromosome abnormalities was 24.76% (52/210), which was 12.38% higher than that of karyotype analysis. It is significantly higher than that reported by Shaffer *et al*. [9]. that CMA can increase the abnormal detection rate by 2.9% compared with chromosome karyotype analysis. However, 3.6% more chromosomal abnormalities can be detected than in traditional karyotype techniques; this is higher than that in the CMA technique reported by Hillman *et al*. [10]. The reason for the analysis may be that the cases in this study are elderly pregnant women and some elderly pregnant women also combine some high-risk factors, and old age will also increase the rate of chromosome abnormality. Second, there are more thalassemia gene carriers because Guangxi belongs to the high incidence area of thalassemia, resulting in a higher detection rate of pathogenic CNV. For chromosome number abnormalities, 16 cases of pathogenic chromosome aneuploidy were detected using CMA and karyotype analysis, including 6, 4, and 6 cases of trisomy 21, sex chromosome aneuploidy, and chimerism, respectively. CMA also detected an additional case of chimerism. The karyotype analysis result was 46 Magi Xnjue 1qhmage, whereas that of the CMA test was 46 Magi XXB and 4Q XY chimerism (the proportion of 46 Magi XX was ~15%); however, the two results were inconsistent. Based on the karyotype analysis, 22 additional cases of pathogenic CNV were detected using CMA, with an additional detection rate for pathogenic CNV of 10.48%, similar to the results of Srebniak *et al*. [11] and Van den Veyver *et al*. [12]. An Indian study indicated [13] that compared with karyotype analysis, Cmas detected an additional 3.78% copy number variation in pathogenicity and detected 3 pCNVs (13.04%) among 23 maternal age high schools. It is lower than the result of our study, which may be attributed to the fact that our study mainly targeted at older pregnant women and did not subdivide other indicators of prenatal diagnosis, leading to bias. In pregnant women who received amniotic fluid puncture with no ultrasound abnormalities, simple old age or positive aneuploidy screening, chromosome microarray analysis detected pathogenic CNVs in only 1.7% of cases [8,14]. Wapner *et al*. [15] also reported 20 cases of genetic abnormalities that were not found using karyotype analysis. Of the 20 cases, 9.3% and 11.3% of pregnant women were found with chromosome abnormalities of clinical significance and uncertain clinical significance, respectively. However, CMA can not detect some balanced chromosome structural abnormalities such as balanced translocation, inversion, insertion, and gene point mutation [16]. Some studies have shown that the traditional G-banding karyotype analysis can detect abnormalities undetected using 3% CMA. Here, 5 cases were found with normal CMA results but abnormal using chromosome karyotype, including 1, 1, and 3 cases of chromosome translocation, deletion, and inversion, respectively, accounting for 2.4%, which was close to the foreign-related studies [17,18]. Studies have shown that balanced translocation and inversion of chromosomes are important causes of reproductive abnormalities, and the rate of chromosomal abnormalities in full-term fetuses of couples carrying balanced translocation is about 10%, higher than that of the general population [19]. Balanced translocation is also associated with repeated abortion [20]. In our study, the abnormal rate of karyotype analysis combined with CMA detection was 27.14% (57/210), which was higher than that of CMA or chromosome karyotype analysis alone. The results of chromosome karyotype analysis combined with CMA were statistically significant compared with those of chromosome karyotype analysis alone ($p < 0.001$). The results of chromosome karyotype analysis combined with CMA detection had no statistical significance compared with those of CMA detection alone ($p > 0.05$). The results of CMA alone were statistically significant compared with those of chromosome karyotype alone ($p < 0.001$). Kappa consistency test was used to analyze the consistency of the two methods (Kappa = 0.447). CMA has more advantages in detecting chromosome microdeletion or microduplication. Karyotype analysis can detect some chromosome structural abnormalities such as chromosomal translocation, inversion and insertion that cannot be identified by CMA. Therefore, combining karyotype analysis and CMA for prenatal diagnosis of elderly pregnant women, both of which complement each other, is crucial.

The results of 22 cases with no abnormalities in chromosomal testing and pathogenic CNV results in CMA, case A202246, were analyzed, and duplication of ~2.02 Mb was detected in sample 22q11.21, a pathogenic CNV. This duplication overlaps with the 22q11.2 duplication region, and the ClinGen database was queried to

find a clear triple dose effect for the 22q11.2recurrent DiGeorge/palatine facial syndrome (DGS/VCFS) region (proximalA-B, chr22:18,912,231-20,287,208), and the individuals carrying this duplication have varying clinical symptoms. The 22q11 repeat is inherited from phenotypically normal or near-normal parents in ~70% of cases. Rosenfeld *et al*. [21] performed a Bayesian analysis of data from a large sample of people and showed that the 22q11.2 microrepeat has ectopic incompleteness with an ectopic rate of ~21.9%. This study's patient had a history of adverse pregnancy and delivery with two embryonic stoppages; we performed CMA on the peripheral blood of the parents, but the microarray analysis did not reveal any clinically significant chromosomal abnormalities, and the pregnant fetus was born with pathological jaundice without significant abnormalities in appearance and is currently growing well.

A202677 detected a deletion of ~1.77 Mb in the 17q12 region of the sample, a pathogenic copy number variant. This deletion region is involved in 17q12 deletion syndrome, also known as renal cysts and diabetes syndrome; the key gene is *HNF1B*, which is expressed in all renal tubular epithelial cells that constitute the renal units and collecting ducts, controls and participates in membrane transport, cellular differentiation, and expression of metabolic genes, and has a role in the regulation of important renal genes such as *PKHD1* and *PKD2* [22]. The clinical presentation of patients with this syndrome varies widely among individuals, with the main clinical features including abnormal kidney or urogenital development (polycystic kidney, renal insufficiency), diabetes in late adolescence (MODY5), neurodevelopmental/neuropsychiatric disorders (autism, schizophrenia, anxiety, and epilepsy), developmental delay, language backwardness, and mental retardation. 17q12 deletion syndrome is dominantly inherited, with 70% and 30% of deletions being de novo variants and inherited from the parents, respectively, and the epistatic rate of 34.4%. A study [23] showed a significant correlation between enhanced renal parenchymal echo and 17q12 microdeletion syndrome. Quintero-Rivera *et al*. [24] reported the first case of duodenal atresia associated with 17q12 microdeletion revealing for the first time that the phenotypic spectrum of 17q12 microdeletion syndrome should include duodenal atresia. Here, the case patient had fetal ultrasound findings of bilateral renal pelvis separation, and the fetal outcome at gestational follow-up was a healthy boy delivered alive without significant abnormalities.

A204280 detected an ~910.84 kb duplication in sample Xq28, a pathogenic CNV. This duplication is involved in Xq28 duplication syndrome with the key gene *MECP2*, which is an X-linked neurodevelopmental disorder where patients exhibit severe psychomotor retardation, hypotonia, language development disorders, progressive muscle spasms, various seizures, and in some cases, developmental regression, ataxia, choreiform movements, sleep disorders, and recurrent respiratory infections. Males are 100% ectopic, whereas female carriers are usually unaffected or show only psychoneurological abnormalities. Subsequent lineage analysis suggested maternal inheritance, and the fetal sample Xq28 with ~910.84 kb of repeat inheritance from the mother was confirmed through CMA analysis, followed by selection for labor induction.

A210791 detected a deletion of ~8.07 Mb in sample 5p15.33p15.31, a pathogenic CNV. The deletion overlaps most of the region with the cri-du-chat syndrome (also known as catcalling syndrome), which has multiple pathogenic and possibly pathogenic reports in DECIPHER and ClinVar databases. The clinical phenotypes of these patients include intrauterine growth retardation, prematurity, feeding difficulties, short stature, peculiar facial features, mental retardation, and voice abnormalities; the deletion region contains 41 proteins, including *TERT*. The gene encoding *TERT* was mentioned in the ClinGen database as possibly having a single-dose effect (Haploinsufficiency Score: 1), but the evidence was insufficient. A duplication of 4.68 Mb was also detected in sample 10p15.3p15.1, of unknown clinical significance. This duplication was queried in the patient databases, DECIPHER and ClinVar, for 3 cases of (probable) pathogenicity reported and unqueried in the general population database, DGV; this region contains 12 protein-coding genes, including IDI2, and the ClinGen database was unqueried for the above gene/region duplication dose effect. The pregnancy outcome in this pregnancy was direct induction of labor.

A203673 detected ~2.5 Mb deletion in the sample 22q11.21, which was a pathogenic CNV. This deletion involves 22q11.2 deletion syndrome, which is mainly classified into three subtypes as follows: DGS syndrome (#188400), VCFS(#192430), and vertebral shaft abnormal facial syndrome (CAFS). Among them, DGS is mainly characterized by congenital heart disease, immunodeficiency, and hypocalcemia and is common in newborns; VCFS mainly shows cleft palate, congenital heart disease, special face, slender fingers, and mental and behavioral abnormalities, among others; CAFS mainly shows special face and heart deformities. This female patient had a history of adverse pregnancy with billet abortion, and a genealogical analysis suggested that this mutation was a de novo mutation, and the fetus was induced.

Another 17 cases detected a heterozygous deletion of ~17.19 kb in sample 16p13.3, a pathogenic CNV. This deletion contained at least 4 OMIM genes, including *HBM*, *HBA2*, *HBA1*, and *HBQ1*, of which *HBA1* and *HBA2* are key genes for α-thalassemia (referred to as α-thalassemia). Individuals with heterozygous deletion of *HBA1* and *HBA2* genes ($-/\alpha\alpha$) are those with mild α-thalassemia, also known as α-thalassemia gene carriers, who are usually asymptomatic or may have mild anemia manifestations. This is because Guangxi, Guangdong, is a high-prevalence area for thalassemia, and more carriers of the gene for thalassemia exist among pregnant women. In

these 17 cases, one fetus was induced after diagnosing thalassemia major in the elderly pregnant women; the remaining 16 cases were delivered alive without any significant abnormalities at 6 months of follow-up.

Here, seven cases of CNV of unknown clinical significance were detected, and all seven fetuses with CNVs of unknown clinical significance were delivered alive. In our study, 7 cases with unknown clinical significance accounted for 3.33% of CNVs, higher than the result of a foreign study of 1.89% [13]. This is consistent with the report of another foreign study that VOUS accounts for no more than 5% [25]. In the case of CNVs of unknown significance, it has been suggested that this may cause significant stress and even panic among pregnant women and their families and may lead to unnecessary labor induction in some cases. Therefore, the clinical indications for prenatal diagnosis are strictly defined before performing CMA, the pregnant women and their families are fully informed of the possible outcome, and consent is obtained. Furthermore, genetic counseling is adequately conducted before the prenatal diagnosis is performed [26]. In addition, most of the older pregnant women are the second child or more, so the proportion of cesarean section is larger, further increasing the risk of pregnancy. Recently, Vimercati *et al*. [27] used an innovative ultrasound parameter to measure the level of scar-vesicovaginal fold distance to determine the risk of uterine rupture in pregnant women. Combined with prenatal diagnosis, this method will better predict the occurrence of adverse pregnancy outcomes.

Summarily, the detection rate of chromosome abnormalities in CMA was significantly higher than that in the routine karyotype analysis if the CMA technique was used alone in this study. However, CMA will miss some chromosomes with structural abnormalities, such as chromosome translocation and inversion, which may cause the risk of infertility and miscarriage [28]. Here, the effect of CMA in detecting chromosome aneuploidy variation is similar to that of chromosome karyotype analysis. CMA has more advantages in detecting chromosome microdeletions and microrepeats; therefore, CMA detection is recommended for elderly pregnant women, regardless of whether they are combined with other indications, but CMA can not identify some chromosome structural abnormalities such as chromosome translocation, inversion, and insertion, among other. Since CMA cannot replace karyotype analysis, it is suggested that CMA should be combined with various detection methods.

5. Conclusions

For the prenatal diagnosis of fetal amniotic fluid in elderly pregnant women, the combined application of chromosome karyotype analysis and CMA detection technology can further improve the detection rate of abnormal chromosomes and reduce the rate of missed diagnosis, thus, reducing the birth defects rate and achieving the goal of eugenics.

Availability of Data and Materials

The data used to support the findings of this study are included within the article.

Author Contributions

GTL, CO and WWL designed the research study. CO and WWL provided the idea for the research. GTL and WWL analyzed the data and performed the literature search. GTL, CO and WWL provided oversight and were responsible for the study organization and implementation, and writing of the manuscript. GTL, CO and WWL revised the manuscript's intellectual content. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

All those who underwent invasive prenatal diagnosis signed an informed consent form before the procedure and were approved by the ethics committee of Medical Ethics Committee of Yulin Maternal and Child Health Hospital (Approval number: YLSFYLL2021-04-29-04). The sample-related data analyzed for this manuscript was entirely retrospective with no patient or patient-related identifiers included in the analysis.

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Conflict of Interest

The authors declare no conflict of interest. We declare there is no conflict of interest between this research and Perkin Elmer Co., Ltd.

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