Clinical Application of Chromosomal Microarray Analysis for Fetuses with Craniofacial Malformations

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Research

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Abstract

Background This study aimed to evaluate the applicability of chromosomal microarray analysis (CMA) for prenatal diagnosis of craniofacial malformations (CFMs). We also investigated the potential correlations between chromosomal abnormalities and CFMs. To this end, 118 fetuses with CFMs were enrolled in the study and underwent both G-banded chromosome analysis and CMA.

Results Of the 118 cases in this study, 39.8% were isolated CFMs (47/118) whereas 60.2% were non-isolated CFMs (71/118). The detection rate of chromosomal abnormalities or submicroscopic chromosomal abnormalities in non-isolated CFM fetuses was significantly higher than that in isolated CFM fetuses (26/71 vs. 7/47, p = 0.01). Compared to the 16 fetuses (16/104; 15.4%) with pathogenic chromosomal abnormalities detected by karyotype analysis, CMA identified a total of 33 fetuses (33/118; 28.0%) with clinically significant findings. These 33 fetuses included cases with aneuploidy abnormalities (14/118; 11.9%), microdeletion/microduplication syndromes (9/118; 7.6%), and other pathogenic CNVs only (10/118; 8.5%). We further explored the CNV/phenotype correlation and found a series of clear or suspected dosage-sensitive CFM genes.

Conclusion CMA is a rapid and reliable molecular technique to identify fetal chromosomal aberrations associated with CFMs. Identification of the genetic basis of CFMs contributes to the understanding of their pathogenesis and etiology.

Background

Major structural malformations occur in 2–3% of newborns[1,2]. Among these, craniofacial malformations (CFMs) are among the most common congenital birth malformations in humans, with orofacial clefts accounting for approximately 13% of congenital malformations in all live births[3]. The majority of CFMs occur in fetuses without a family history. Thus, it is particularly important to evaluate the integrity of craniofacial structures of fetuses using ultrasonographic screening. Generally, fetal CFMs include defects such as cranial malformations, ocular malformations, nasal dysplasia, and orofacial defects, among others. These defects may be isolated variations or may occur in combination with other congenital structural abnormalities such as central nervous system abnormalities, cardiac defects, abdominal wall defects, skeletal defects, and so on. Due to the similar phenotypes of craniofacial syndromes with and without multiple organ involvement, prenatal genetic counseling can be challenging. Although the causes of CFMs are currently unclear, genetic analysis can help to provide a genetic basis for prenatal diagnosis and can also contribute to the understanding of the pathogenesis of CFMs.

Conventional karyotyping is the classic method for detecting aneuploidy or chromosomal rearrangements[4,5]. However, this approach has gradually been replaced by newer genetic testing technologies due to its low resolution and low detection efficiency. Recently, chromosomal microarray analysis (CMA) has been recommended for prenatal diagnosis due to its advantages when fetal abnormalities are detected by ultrasound[6]. Copy number variations (CNVs) in simple cranial or facial malformations have also been reported, confirming that CNVs are involved in the genetic pathogenesis of CFMs[7,8]. However, the comprehensive CMA assessment of fetuses with CFMs and its advantage over conventional chromosome analysis is limited. In the present study, we reviewed the clinical and molecular findings of 118 fetuses with CFMs to explore their molecular etiology and to retrospectively assess the clinical significance of CMA in each case. This study aimed to provide useful information for prenatal diagnosis of CFMs and related genetic counseling.

Results

Fetal ultrasound findings

As shown in Table 1, CFMs including cranial malformations (41, 34.7%), orofacial clefts (44, 37.3%), ocular and orbital malformations (6, 5.1%), nasal deformity (5, 4.2%), ear abnormality (3, 2.5%), macroglossia (1, 0.8%), micrognathia (1, 0.8%), and complex CFMs (17, 14.4%) were observed in 118 fetuses that underwent CMA in our unit. Of these 118 cases, 47 (39.8%) were isolated CFMs whereas 71 (60.2%) were non-isolated CFMs. Complex CFMs refer to abnormalities involving two or more different cranial or facial features.

Table 1
Phenotypic characteristics of 108 fetuses with CFMs

Abnormalities	Isolated CFM (N)	Non-isolated CFM (N)	Referred cases (N)
Cranial malformations			*41(34.7%)*
Microcephaly	5	4	9
Macrocephaly	1	5	6
Defect in the skull bone	0	13	13
Abnormal skull shape	3	10	13
Orofacial clefts			*44(37.3%)*
cleft lip	6	4	10
cleft palate	0	1	1
cleft lip and palate	19	14	33
Ocular and orbital malformations			*6(5.1%)*
Hypertelorism	0	3	3
Hypotelorism	0	2	2
Microphthalmia□Cataract	0	1	1
Nasal deformity	5	0	*5(4.2%)*
Ear abnormality	2	1	*3(2.5%)*
Macroglossia	0	1	*1(0.8%)*
Micrognathia	1	0	*1(0.8%)*
Complex CFMs	5	12	*17(14.4%)*
Total	47(39.8%)	71(60.2%)	118(100%)

CFM, craniofacial malformation.

Conventional G-banded cytogenetic analysis findings

Samples for karyotype analysis were obtained from 104 fetuses from singleton pregnancies (40 samples from amniocentesis and 64 samples from cordocentesis); another 14 samples from aborted fetuses were excluded. Successful karyotyping results indicated that 16 (16/104; 15.4%) fetuses had chromosomal abnormalities; 12 cases showed abnormal chromosome number including trisomy 13 (n = 5), trisomy 18 (n = 4), trisomy 21 (n = 1), and mosaicism 45,X[32]/46,XY[3] (n = 1) and 45,XN,der(14)t(14;20)(p13;p11.2),–20[17]/46,XN (n = 1). Among these, mosaicism 45,X[32]/46,XY[3] was found to be monosomy X by CMA. Another 4 cases showed chromosomal structural aberrations: 46,XN,der(13)t(4;13)(q35;q31); 46,XN,der(13)t(13;16)(q32;q23); 46,XN,del(7)(q34); and 46,XN,rec(6)dup(6q)inv(6) p25q22).

Table 2

CFMs fetuses with chromosomal aneuploidy abnormalities identified by CMA and karyotype analysis

Case	Karyotype	CMA results	Craniofacial malformations	Other malformations
1	47,XN,+13	arr(13)×3	CLP	Gallbladder enlargement; ES; Hyperechogenic kidneys; Strephenopodia; Small stomach bubble
2	47,XN,+13	arr(13)×3	Microphthalmia、CLP	Gallbladder enlargement; DW; Hyperechogenic kidneys
3	47,XN,+13	arr(13)×3	Abnormal skull shape, CLP	HPE, DK, TOF
4	47,XN,+13	arr(13)×3	Lemon-shaped skull	Bilateral cerebral ventriculomegaly; SB
5	47,XN,+13	arr(13)×3	CLP	—
6	NA	arr(13)×3	Skull defect	Encephalocele
7	47,XN,+18	arr(18)×3	Abnormal skull shape	HPE; absent radius; VSD; SUA
8	47,XN,+18	arr(18)×3	Microtia, Abnormal pinna	CHD, CH, abnormal hand posture, SUA, polyhydramnios
9	NA	arr(18)×3	Midface depression	Limb body wall complex
10	47,XN,+18	arr(18)×3	CLP	CPC; VSD
11	47,XN,+18	arr(18)×3	Strawberry-shaped skull	Overlapping hands、CPC、LPCM、SUA
12	47,XN,+21	arr(21)×3	Abnormal skull shape	—
13	45,X[32]/46,XY[3]	arr(X)×1	CL	—
14	NA	arr(X) ×1~2, (Y) ×1	Skull defect	Anencephaly; Enlarged bladder

Table 3

CFMs fetuses with microdeletion/microduplication syndromes and other pCNVs

Case	Craniofacial malformations	Other findings	Karyotype	Candidate Gene	Clinical significance	CMA results
15	CLP	—	46,XN	*TBX1*	P (22q11 proximal deletion syndrome)	22q11.21(18895227_21460220)×1
16	CLP	CHD	46,XN	*TBX1*	P (22q11 proximal deletion syndrome)	22q11.21(18895227_21445064)×1
17	CL	RVE, RAE, APVD	46,XN	*MAKP1*	P (22q11.2 distal deletion syndrome)	22q11.2(21798907_22762651)×1
18	CLP	—	NA	*TBX1*	P (22q11 duplication syndrome)	22q11.2(18648855_21800471)×3
19	Skull defect	Anencephaly	NA	_	P (7q11.23 duplication syndrome)	7q11.23(72722981_74494207)×3
					VOUS	14q12(25333115_26945366)×3
20	CLP	—	46,XN	*PCYT1A*、*DLG1*	P (3q29 microdeletion syndrome)	3q29(195678474_197340833)×1
21	Macrocephaly	Hydronephrosis	46,XN	*LHX1*	P (Rrenal cysts and diabetes syndrome)	17q12(34462281_36249565)×1
22	CLP	IUGR	46,XN	_	VOUS	14q32.33(104871320_106251148)×1
					LP (16p11.2 microduplication syndrome)	16p11.2(29681582_30190029)×3
23	Abnormal skull shape	Absent gallbladder, RD, PE, IUGR, HPE	NA	*ZIC2*	P	4q32.2q35.2(163980423_190880409)×3
					P	8p23.3p23.1(176818_6974050)×3
					P(8p23.1 duplication syndrome)	8p23.1(8101641_11394233)×3
					P	13q31.3q34(92884370_115106996)×1
					P	Xq26.2q28(130488944_154929412)×1
24	Abnormal skull shape	HPE	46,XN,der(13)t(4;13)(q35;q31)	*ZIC2*	P	4q35.1q35.2(183907715_190880409)×3
					P	13q31.3q34(94514343_115106996)×1
25	CLP	VWT	46,XN,der(13)t(13;16)(q32;q23)	*ZIC2*	P	13q32.1q34(96311577_115106996)×1
					P	16q23.2q24.3(81148438_90148796)×3
26	CL	DWM	46,XN	*SHH*	P	7p22.3p21.2(43376_15044564)×3
					P	7q34q36.3(142326472_159119707)×1
27	Abnormal skull shape, Hypotelorism, beaked nose	HPE, absent radius	46,XN,del(7)(q34)	*SHH*	P	7q34q36.3(138831707_159119486)×1
28	Midfacial hypoplasia	BPC、Hyperechogenic kidneys, LM, LPCM	46,XN,rec(6)dup(6q)inv(6)(p25q22)	*FOXC1*	P	6p25.3p25.1(156974_5395099)×1
					P	6q22.32q25.3(126253838_170914297)×3
29	Microcephaly	RAA、Persistent LSVC	45,XN,der(14)t(14;20)(p13;p11.2),-20[17]/46,XN[17]	*SNRPB, CSNK2A1*	P	20p13p11.21(61661_21268329)×1[0.4]
30	CP	PFL, NT thickening, Omphalocele	NA	*HPGD* (AR)	P	4q32.3q35.2(169998230_190880409)×1
					P	6q25.3q27(158387117_170898549)×3
31	Midfacial hypoplasia,	MCDK、VSD、Persistent	46,XN	*CREBBP*	P	11q24.1q25(122446233_134944006)×1

	flat nose, prominent maxilla	LSVC、SUA			P	16p13.3p13.12(105320_12986742)×3
32	Hypertelorism	ICL、Arachnoid cyst	46,XN	*FOXC1*	P	6p25.3p25.2(1482077_2681511)×1
33	Micrognathia	—	46,XN	*SF3B4*	P	1q21.2(149815079_150260948)×1

APVD, anomalous pulmonary venous drainage; BPC, blake's pouch cyst; CFM, craniofacial malformation; CHD, complex heart disease; CL, cleft lip; CLP, cleft lip and palate; CMA, chromosomal microarray analysis; CNV, copy number variant; CP, cleft palate; DWM, Dandy-Walker malformation; HPE, Holoprosencephaly; ICL, intracranial cystic lesions; IUGR, intrauterine growth retardation; LM, Limb Malformations; LP, likely pathogenic; LPCM, low placed conus medullaris; LSVC, left superior vena cava; MCDK, multicystic dysplastic kidney; NA, not available;NT, nuchal translucency; P, pathogenic; PE, pericardial effusion; PFL, posterior Fossa Lesions; RAE, right atrial enlargement; RAA, right aortic arch; RD, Renal dysplasia; RVE, right ventricular enlargement; SUA, single umbilical artery; VOUS, variant of unknown significance; VWT, ventricular wall thickening; VSD, ventricular septal defect; XN, XX or XY.

Table 4

Detection rate of clinically significant chromosomal aberrations in fetuses with various CFMs

Malformation	Simple CFMs							Complex CFMs (n/N)	Referred cases (n/N,%)
	Cranial malformation (n/N)	Orofacial cleft (n/N)	Nasal deformity (n/N)	Ocular malformation (n/N)	Ear abnormality (n/N)	Macroglossia (n/N)	Micrognathia (n/N)		
Isolated CFMs	1/9	5/25	0/5	0/0	0/2	0/0	1/1	0/5	7/47, 14.9%
Non-isolated CFMs	10/32	8/19	0/0	1/6	1/1	0/1	0/0	6/12	26/71, 36.6%
Total	11/41	13/44	0/5	1/6	1/3	0/1	1/1	6/17	33/118, 28.0%

CFM, craniofacial malformation.

CFM, craniofacial malformation; CH, cerebral hernia; CHD, complex congenital heart disease; CLP, cleft lip and palate; CL, cleft lip; CMA, chromosomal microarray analysis; CPC, choroid plexus cysts; DK, duplex kidney; DWM, Dandy-Walker malformation; ES, esophageal stenosis; HPE, Holoprosencephaly; LPCM, low placed conus medullaris; SB, Spina bifida; SUA, single umbilical artery; TOF, tetralogy of Fallot; VSD, ventricular septal defect; XN, XX or XY.

CMA findings

An interpretable CMA profile was obtained for all 118 tested genomic DNA samples. Clinically significant results (P or LP CNVs) were found in 33 cases (33/118; 28.0%) including 14 cases (14/118; 11.9%) with chromosomal aneuploidies and 19 cases (19/118; 16.1%) with P/LP CNVs.

In the 14 cases with CMA results indicating chromosomal aneuploidies, 6 had trisomy 13, 5 had trisomy 18, 1 had trisomy 21, 1 had monosomy X and 1 had copy gain of X chromosome in approximately 20% of cells (Table 2). Of the 19 cases with P/LP CNVs, we identified 9 fetuses (9/118; 7.6%) with CNVs related to known microdeletion or microduplication syndromes (MMSs); these included 22q11 deletion syndrome (n = 3), 22q11 duplication syndrome (n = 1), 7q11.23 duplication syndrome (n = 1), 3q29 microdeletion syndrome (n = 1), 16p11.2 microduplication syndrome (n = 1), renal cysts and diabetes syndrome (n = 1), and 8p23.1 duplication syndrome (n = 1). In addition to MMSs, we identified a further 20 pathogenic CNVs from 11 fetuses; these CNVs involved deletions of 1q21, 4q32q35, 6p25p25, 7q34q36, 11q24q25, 13q31q34, 20p13p11, and Xq26q28, and duplications of 4q32q35, 4q35, 6q22q25, 6q25q27, 7p22p21, 8p23p23, 8p23, 16q23q24, and 16p13p13 (Table 3 and Figure 1). Among these cases, fetus 23 were found to have 8p23.1 duplication syndrome as well as another 4 pathogenic CNVs.

All 33 fetuses identified with P/LP CNVs were terminated pregnancies or intrauterine deaths. Additionally, we found that 9 (9/118; 7.6%) fetuses only had VOUS CNVs. The remaining 76 cases (76/118; 64.4%) had no CMA abnormalities or only LB/B CNVs.

Identification of CFM-associated CNVs and genes

We further analyzed the associations between CFMs and these CNVs and identified the potential candidate genes within these regions. We screened several dosage-sensitive or suspected dosage-sensitive genes, including gene *TBX1* (locus 22q11.21), *MAPK1* (locus 22q11.22), *PCYT1A (*locus 3q29) and *DLG1* (locus 3q29) related to cleft lip/palate, *LHX1* (locus 17q12) related to macrocephaly,*SF3B4*locus 1q21.2) related to micrognathia, *FOXC1 (*locus 6p25.3) related to ocular hypertelorism and midfacial hypoplasia, *ZIC2 (*locus 13q32.3) related to cleft lip/palate and abnormal skull shape, *SHH* (locus 7q36.3) related to multiple CFMs, *CREBBP* (locus 16p13.3) related to complex CFMs, and genes *SNRPB* or *CSNK2A1*locus 20p13) related to microcephaly.

Comparison of chromosomal abnormality detection rates

Overall, as shown in Table 4, the detection rate of chromosomal abnormalities in non-isolated CFM fetuses was significantly higher than in isolated CFM fetuses (26/71 vs. 7/47; p = 0.01). There was no significant difference in the detection rate of chromosomal abnormalities in complex CFMs compared to in simple CFMs (6/17 vs. 27/101; p > 0.05). There was also a statistically significant difference in the detection rate between karyotype analysis and CMA (16/104 vs. 33/118; p = 0.024).

Discussion

Single nucleotide polymorphism (SNP) array testing has been widely used in prenatal molecular diagnosis due to its high resolution and advantage in detecting loss of heterozygosity and uniparental disomy. Recently, there have also been several successive research publications on prenatal diagnosis of fetal cleft lip/palate and cranial anomalies using CMA. However, the progress of research into the clinical relevance of CNVs lags far behind development of the CMA technology itself. Despite the existence of various databases, the clinical pathogenicity of many CNVs is still unclear. Therefore, in this study we attempt to identify chromosomal abnormalities and CNVs in fetuses with CFMs using CMA and karyotype analysis, in order to determine the potential correlation of CNVs with CFMs.

Craniofacial anomalies are common in postnatal cases, especially in patients with nervous system disorders. However, their prenatal detection rate is low due to limitations of prenatal imaging technology and fetal development status. Nicolaides et al(1993) reported a 7% incidence of facial defects in fetal malformations[9], of which orofacial clefts were most common with prevalence of approximately 1 case per 700 deliveries[10]. The detection rate of prenatal cranial abnormalities is unclear, but indicates a high incidence in neonates[11]. Consistent with the above reports, in the 118 CFMs cases in this study it was observed that cranial abnormalities and orofacial clefts were most common, accounting for 40.6% and 43.6%, respectively, in fetuses with single CFMs. In our study, cytogenetic karyotyping revealed abnormal karyotypes in 15.4% of fetuses and the detection rate increased by 12.6% with CMA. In the current study, the incidence of chromosomal aberrations and CNVs was significantly higher than the 4.6% and 6.3%, respectively, reported in a recent study of fetal structural abnormalities[12]. This is because, in contrast to previous reports[13], our study did not merely focus on isolated CFMs or on cases with simple CFMs. When we focused only on isolated CFMs, more similar results of 6.4% and 8.5% were obtained, respectively.

At present, chromosomal karyotype analysis is unable to achieve the same results as CMA for regions less than 10 Mb. However, it failed to detect any abnormalities in case 31 by karyotype, while a 12.49 Mb deletion in 11q24.1q25 and a 12.88 Mb duplication in 16p13.3p13.12 were found by CMA. A similar situation occurred in Case 26 as well; the failure of chromosomal karyotype analysis could have been due to the deletion and duplication regions being similar in size and in band. In addition, chromosomal karyotype analyses were not performed in 14 aborted fetal samples here, including 7 cases of chromosome abnormalities subsequently revealed by CMA. It is well known that cytogenetic karyotype analysis is often made unavailable due to the frequent culture failures of non-sterile tissues or the selective overgrowth of maternal cells during tissue culturing[14]. Although CMA offers obvious advantages in improving the detection rate and identifying the pathogenicity of CNVs, it is limited in detecting balanced translocations and low-level mosaicism. Specifically, we found the enrolled cases of fetal abnormality caused by parental translocation accounted for a certain proportion in this study. Morever, mosaic 45,X[32]/46,XY[3] identified in case 13 by traditional karyotyping was determined to be monosomy X by CMA; this may have a slight impact on genetic counseling. Taken together, these results suggest that the combination of CMA with cytogenetic analysis can achieve more accurate and comprehensive results.

This study showed 9 cases (cases 1523) with CNVs relating to known chromosomal MMSs, of which 22q11 deletion/duplication syndrome was the most common with an overall prevalence of 3.4% (4/118; 2 proximal deletion, 1 proximal duplication and 1 distal deletion). 22q11 proximal deletion, also known as DiGeorge syndrome or velocardiofacial syndrome, involves more than 30 Mendelian genes; potential genes such as *TBX1, COMT, UFD1L, GNB1L, TRXR2, MED15,* and *RANBP1* were researched to explore the phenotype/CNV correlation. Cleft palate is among the most common problems in patients with this microdeletion, while cleft lip is only occasionally found[15]. According to previous reports[16], *TBX1* is considered to be responsible for cleft lip/palate phenotypes in either 22q11 deletion or 22q11 duplication. Fetus 17 with heart abnormalities and cleft lip was found to carry 22q11 distal deletion syndrome. Heart problems are a usual finding but cleft lip only, without cleft palate, has never been reported within the clinical spectrum of this syndrome. This suggested that simple cleft lip may need to be included in the phenotype spectrum of 22q11 distal deletion syndrome. Although researchers such as Spineli-Silva supported that the cause of CHDs and craniofacial anomalies in patients with distal 22q11 deletion may be associated with haploinsufficient *MAPK1* expression[17], the underlying mechanisms are still largely unknown. We identified five distinct CNVs associated with rare MMSs including two microdeletion syndromes (3q29 and 17q12) and three microduplication syndromes (7q11.23, 8p23.1 and 16p11.2). These syndromes are associates with a range of mental and physical disabilities as well as craniofacial abnormalities. We screened several candidate genes located in these regions involved in craniofacial development, such as *PCYT1A* (locus 3q29)[18], *DLG1* (locus 3q29)[19], and *LHX1* (locus 17q12)[20,21]. Although *TBX6* is considered to be a key gene that results in several major phenotypes in 16p11.2 duplication, potential genes associated with orofacial cleft of this region still need further exploration. Additionally, there is no reported correlation between 7q11.23 duplication and skull defects resulting from anencephaly, but this fragment has been confirmed as a pathogenic CNV of central nervous system development.

Other rare CNVs detected in the present study are also believed to contribute to the pathogenesis of CFMs. *ZIC2* in 13q23.3 (cases 2325) has been identified as a key gene associated with several major CFMs resulting from holoprosencephaly[22]. Deletion in chromosome 7q34q36.3 encompassing gene *SHH* was identified in cases 26 and 27; *SHH* is involved in the organization and morphology of the developing embryo and is known to be a key gene in craniofacial abnormalities such as microcephaly, hypotelorism, midface hypoplasia, and cleft lip/palate[23]. In case 33 with isolated micrognathia, a 0.44 Mb deletion in region 1q21.1 was identified; haploinsufficiency of gene *SF3B4*in 1q21.1 has been confirmed to be associated with micrognathia[24]. Additionally, there has been evidence of the pathogenicity of haploinsufficient *FOXC1* expression as well. Heterozygous deletion of *FOXC1* in 6p25.3 (cases 28 and 32) can lead to Axenfeld-Rieger syndrome (6p25 deletion syndrome); ocular hypertelorism and flat midface were prevalent in the affecting postnatal cases[25]. Case 31, which was characterized by a maxillary protrusion, midface hypoplasia, and flat nose, had a 12.49 Mb deletion in chromosome 11q24.1q25 and a 12.88 Mb duplication in chromosome 16p13.3p13.12 involving the gene *CREBBP.* There are several literature reports suggesting that duplication of the 16p13.3 region containing the *CREBBP* gene results in a distinct similar facial dysmorphism[26], but to date still no case with duplication only encompassing the *CREBBP* gene has been reported. Case 29 had microcephaly < 2 SD and had a 21.21 Mb mosaic deletion in chromosome 20p13p11.21. Among 141 protein coding

genes within this deletion region, mutations only in *SNRPB* and *CSNK2A1* had been reported as associated with autosomal dominant microcephaly[27,28]. However, to date there is no evidence supporting their pathogenicity in haploinsufficiency. In case 30, we could not identify a gene specifically associated with the observed cleft palate; we only identified an autosomal recessive gene, *HPGD,* associated with a high-arched palate and without dose-sensitive reports[29]. We suspected a single mutation on the other chromosome may explain the observed phenotype.

There are several limitations of our study that should be discussed. First, we detected chromosomal abnormalities in fetuses with ultrasonographic features of cranial or facial abnormality; however, the majority of enrolled cases were cranial malformations and orofacial clefts due to the rarity of other CFMs features. Second, the function of candidate genes within the identified CNVs was not further investigated. Finally, not all parental samples were available, resulting in insufficient information for the interpretation of CNVs identified in these fetuses.

Conclusion

To the best of our knowledge, this is the first comprehensive study performed among a Chinese population to examine fetuses with CFMs that underwent genomic analysis with both karyotyping and CMA. We analyzed chromosomal abnormality results from 118 CFM cases and pointed out the detection rates of each CFM type. We detected several CNVs including nine MMS regions associated with CFMs, further explored the CNV/phenotype correlation, and found a series of clear or suspected dosage-sensitive CFM genes including *TBX1*, *MAPK1*, *PCYT1A*, *DLG1*, *LHX1*, *SHH*, *SF3B4, FOXC1*, *ZIC2*, *CREBBP*, *SNRPB*, and *CSNK2A1*. Our study enriches understanding of the potential causative CNVs and genes in CFMs and demonstrates that CMA is a rapid and reliable molecular technique to identify fetal pathogenic CNVs associated with CFMs.

Methods

Study subjects

The present study was approved by the institutional research ethics committee of our unit. All parents agreed to participate in the study and were provided written informed consent. We retrospectively analyzed a cohort of 118 fetuses with CFMs that were diagnosed at the Wenzhou Prenatal Diagnosis Center between 2012 and 2019. All the pregnant women underwent prenatal ultrasound examination or magnetic resonance imaging performed by experienced maternal fetal medicine specialists and ultrasound technicians. The pregnant women ranged in age from 21 to 43 years, with their gestational week ranging between 13 to 32 weeks. The conditions eligible for inclusion in this study were isolated CFMs and non-isolated CFMs (with other structural abnormalities or sonographic soft markers). The CFMs included cranial malformations (such as abnormal size, shape, and integrity of the skull) and various facial structure abnormalities (such as orbits, nose/nostrils, mouth, the facial profile, lips, ears) according to the ultrasonographic report of prenatal CFMs[30]. Of note, nasal bone absence or dysplasia as sonographic soft markers were excluded from facial malformations. Fetal samples were obtained from aborted fetuses (14 cases) or were collected by amniocentesis (40 cases) or cordocentesis (64 cases) based on the gestational week at the time of invasive prenatal testing.

Karyotype analysis

A total of 104 fetal samples (14 fetal tissues from abortions were excluded) were analyzed using standard G-banded karyotyping at 320–450 bands resolution to diagnose overall chromosomal abnormalities. At least 20 metaphase cells from each sample were carefully examined by an experienced technician to detect chromosomal structural abnormalities and numerical abnormalities.

Chromosomal microarray analysis

CMA was performed on all samples from the 118 cases using the Illumina Human CytoSNP-12 Array or the Affymetrix CytoScan 750k Array according to the respective manufacturers' instructions. The results were analyzed with Chromosome Analysis Suite software. All detected CNVs were compared with known CNVs in the scientific literature and in the following publicly available databases: DECIPHER (http://decipher.sanger.ac.uk/), Database of Genomic Variants (DGV, http://dgv.tcag.ca/dgv/app/home), Online Mendelian Inheritance in Man (OMIM, http://www.omim.org), ClinGen Dosage Sensitivity Map (https://www.ncbi.nlm.nih.gov/projects/dbvar/clingen), and International Standards for Cytogenomic Arrays (ISCA, https://www.iscaconsortium.org/).

According to the American College of Medical Genetics Standards and Guidelines, the CNVs were classified as pathogenic (P), likely pathogenic (LP), benign (B), likely benign (LB), or variant of unknown significance (VOUS)[31,32]. Given the reliability of the minimum detection range, the reporting threshold for P or LP CNVs was 100 Kb; LB/B CNVs and VOUS CNVs smaller than 500 Kb deletion or 1000 Kb duplication were not reported, consistent with the Canadian College of Medical Geneticists (CCMG)-Society of Obstetricians and Gynaecologists of Canada (SOGC) recommendations[33]. All reported CNVs were according to the National Center for Biotechnology Information human genome build 37 (hg 19). CMA or quantitative real-time polymerase chain reaction was also performed on parental DNA samples if their DNA were available to determine whether the CNVs detected were inherited or de novo.

To identify CFM-associated CNVs and candidate genes, we further examined and analyzed the genes within identified CNVs by retrieving related literature and examining related databases.

Statistical analysis

Statistical analysis was performed with SPSS 23.0 (IBM Corporation, USA). The CMA detection rates of P/LP variants were compared between various isolated and non-isolated CFM fetuses, and simple and complex CFM fetuses; $p < 0.05$ was considered statistically significant.

List Of Abbreviations

CMA: chromosomal microarray analysis

CFM: craniofacial malformation

CNV: copy number variation

P: pathogenic

LP: likely pathogenic

LB: likely benign

B: benign

VOUS: variant of unknown significance

MMS: microdeletion or microduplication syndrome

Declarations

Ethics approval and consent to participate

This research was approved by the Ethics Committee of Wenzhou Central Hospital. It is a retrospective study without any identifiers related with patients. All patients participating in the study wrote informed consent.

Consent for publication

All patients in this study provided their consent for publication.

Availability of data and materials

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

Competing interest

The authors declare no conflict of interests regarding the publication of this paper.

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Authors' Contributions

C. Y. Xu and S. H. Tang conceived the idea, Y. B. Xiang and X. Q. Dong collected the samples, H. Z.Li and X. Q.Xu performed the experiments, L. L. Zhou and C. Y. Xu performed data analyses, C. Y. Xu and Y. B. Xiang wrote the manuscript. All authors have read and approved the final manuscript.

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Figures

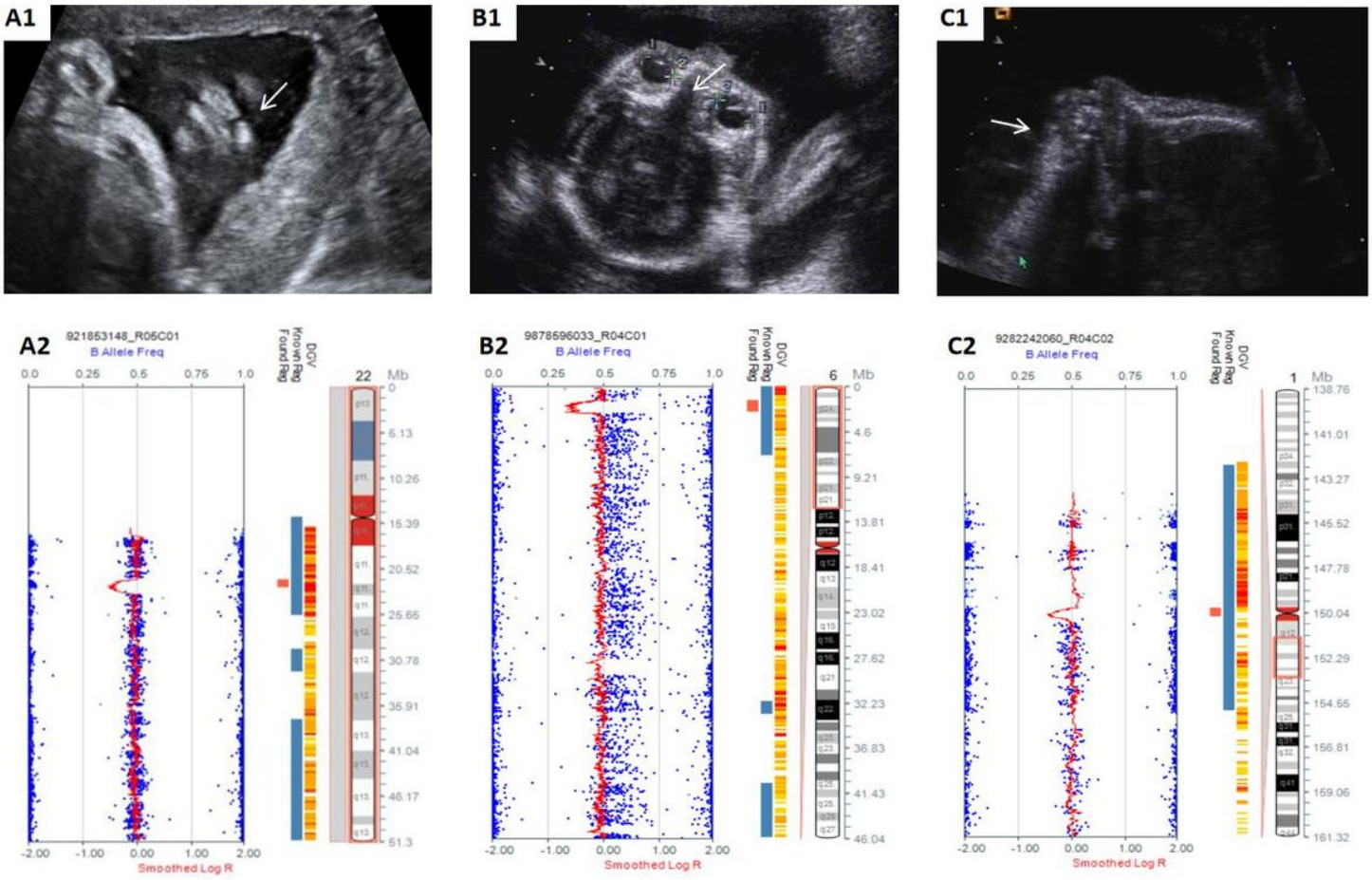

Figure 1

Abnormal ultrasonographic findings and the identified pCNVs of three selected fetuses. (A) A 0.96 Mb deletion related to 22q11.2 distal deletion syndrome (A2) was identified in fetus 17 with cleft lip (A1). (B) A 1.20 Mb deletion of 6p25.3p25.2 (B2)was identified in fetus 32 with hypertelorism (B1). (C) A 0.44 Mb

deletion of 1q21.11q21.2 (C2) was identified in fetus 33 with micrognathia (C1).

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