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Chromosomal microarray analysis in the prenatal diagnosis of orofacial clefts

Experience from a single medical center in mainland China

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Abstract

The aim of this study was to investigate the value of chromosomal microarray analysis (CMA) for the prenatal diagnosis of orofacial clefts.

A total of 143 fetuses with oral clefts were detected by ultrasound during prenatal exam between 2012 and 2017 in our center. We categorized the cases into 4 groups: isolated cleft lip (CL) (CL only), isolated cleft palate (CP only), isolated cleft lip and palate (CLP) (CLP only), and syndromic CLP (combined with other malformations). The CMA was performed in all cases, while 139 fetuses were referred for G-banded chromosome analysis.

There were 42 male and 10 female fetuses were born, with a sex ratio of 4.2:1. The isolated CLP group accounted for 74.1% (106/143) of cases, while the isolated CL, isolated CP, and syndromic CP groups accounted for 13.9% (20/143), 2% (3/143), and 10% (14/143), respectively. A total of 11 fetuses had pathogenic copy number variants (CNVs, 7.7%), including isolated CP (1/143, 0.7%), isolated CLP (5/143, 3.5%), and syndromic CLP (5/143, 3.5%). Compared with the CMA results, 5 fetuses were found to have an abnormal karyotype (5/139, 3.6%). However, no abnormalities were found in either karyotype analysis or CMA in the isolated CL group.

CMA is a valuable tool for identifying submicroscopic chromosomal abnormalities in the prenatal diagnosis of oral clefts. An excellent outcome can be expected for fetuses with isolated CL that are negative for chromosomal abnormalities.

Abbreviations: CL = cleft lip, CLP = cleft lip and palate, CMA = chromosomal microarray analysis, CNVs = copy number variants, CP = cleft palate, OFCs = Orofacial clefts, VOUS = variants of unknown significance.

Keywords: chromosomal microarray analysis, isolated orofacial clefts, prenatal diagnosis

1. Introduction

Orofacial clefts (OFCs) are one of the most common congenital birth malformations in humans, accounting for approximately 1/700 live births.[1] Although the causes of oral clefts are currently unclear, environmental exposure, gene mutation, and chromosomal defects are currently related.[2,3] Based on the point in time at which the oral cavity fails to close during embryogenesis, OFCs are classified into 3 categories: cleft lip only (CL), cleft palate only (CP), or cleft lip with cleft palate (CLP).[4] These defects may be

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HJ, YC, and ZL contributed equally to this work.

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syndromic, combined with congenital abnormalities, or may be isolated variations. Genetic testing can help to enhance the specificity and sensitivity of the prenatal diagnosis of OFCs between syndromic and isolated malformations.[5] Recently, conventional chromosome analysis and chromosomal microarray analysis (CMA) have been applied to prenatal diagnosis. Compared with traditional chromosome analysis, CMA can prenatally discern between submicroscopic genomic imbalances.

Based on this technology, Shaffer et al found that 10.3% of the prenatal CL/palate diagnoses had clinically significant findings, and suggested that CMA could increase the detection rate of genomic imbalances for non-syndromic cases.[6] However, the specific information about detections rates and its advantage over conventional chromosome analysis is limited.

Hence, the purpose of our study was to investigate the value of CMA in the prenatal diagnosis of isolated OFCs through a retrospective analysis. Such information will be useful for the future genetic counseling for parents of fetuses with isolated OFCs.

2. Methods

Our retrospective analysis of fetuses with OFCs was carried out in Guangzhou Women and Children's Medical Center for prenatal diagnosis between 2012 and 2017. The study included all the offspring of women who underwent fetal ultrasound at our center and were diagnosed with OFCs. All examinations were performed by experienced maternal fetal medicine specialists and ultrasound technicians. All examinations were performed using a Voluson Expert E8 (GE Healthcare), using curvilinear 2.0 to 5.0 MHz transducters. The ultrasound standard we choose is based on the prenatal ultrasound classification system proposed by Nybg et al.[7] All parents received a written explanation of the study and signed a consent form prior to their participation. Ethical approval was not required for this retrospective study.

A total of 143 fetuses with oral clefts were initially referred for CMA. G-banded chromosome analysis following standard protocols was performed for 139 cases. The ages of the pregnant women ranged from 18 to 39 years, with a gestational age between 21 and 32 weeks. Based on the location of the CLP and whether or not it was associated with other malformations, we categorized cases into 4 groups: isolated CL (CL only), isolated CP (CP only), isolated CLP (CLP only), and syndromic CLP (combined with other malformations).

The CMA was performed using CytoScan HD Array (Affymetrix, Santa Clara, CA) according to the manufacturer's instructions, and the reporting threshold of the copy number variations (CNVs) was set at 100kb with a marker count of ≥50. The results were analyzed with Chromosome Analysis Suite software. The detected copy number gains or losses were aligned with known CNVs listed in the publicly available databases, including the Database of Genomic Variants (DGV, http://projects.tcag.ca/variation/), UCSC (http://genome.ucsc.edu/), OMIM (http://www.omim.org), the DECIPHER database (https://devipher.sanger.ac.uk/), and ISCA (https://www.iscaconsortium.org/). According to the guidelines,[8,9] the CNVs were classified as benign, pathogenic, or variants of unknown significance (VOUS). The disease-associated analysis and biological analysis were also performed.

3. Results

A total of 143 fetuses diagnosed with oral cleft by fetal ultrasound were included in this retrospective study. As shown in Table 1, the oral clefts were categorized into 4 groups: isolated CL, isolated CP, isolated CLP, and syndromic CLP and compromised 20 (13.9%), 3 (2%), 106 (74.1%), and 14 (10%) cases, respectively. Among these, the percentage of isolated CLP was high. In addition, among the syndromic cases, central nervous system abnormalities (CNS, 50%) and cardiac defects (21.4%) were the most frequently associated structural anomalies, compared with genitourinary malformation (14.4%), abdominal wall defects (7.1%), and skeletal defects (7.1%).

Table 1

Summary of 143 oral cleft cases.

	No. of cases	%
Isolated cleft lip	20	13.9
Isolated cleft palate	3	2
Isolated cleft lip and palate	106	74.1
Syndromic cleft lip and palate		10
CNS abnormalities	7	
Cardiac defects	3	
Abdominal wall defects	1	
Genitourinary malformation	2	
Skeletal defects	1	
Total	143	100

CNS = central nervous system.

In order to further analyze whether or not these cases were associated with chromosomal abnormalities, CMA and chromosome karyotype analysis were performed. A total of 143 prenatal oral cleft fetuses were analyzed by CMA and 139 fetuses were detected by karyotype analysis. Table 2 shows that 11 of the fetuses had pathogenic copy number variants (CNVs, 7.7%), including isolated CP (1/143, 0.7%), isolated CLP (5/143, 3.5%), and syndromic CLP (5/143, 3.5%). A further 15 were VOUS (VOUS, 10.5%), and the remaining 117 were benign (81.8%). The number of isolated CLP cases was higher than those of isolated CP, but was the same as the number of syndromic CLP cases. Furthermore, no pathogenic CNVs were found in the isolated CL group (Table 2). Compared with CMA, we also found 5 fetuses with abnormal karyotype (5/139, 3.6%), including isolated CP (1/139, 0.7%), isolated CLP (1/139, 0.7%), and syndromic CLP (3/139, 2.2%). Similarly, we failed to detect any karyotype abnormalities in the isolated CL group. The sensitivity of CMA (7.7%) was higher than that of traditional chromosome analysis (3.6%).

As shown in Table 3, we further analyzed the 11 fetuses with pathogenic CNVs, with a gestational age range from 24 to 30 weeks. Two of the fetuses had trisomy 13 (46, XX, +13, der [13;14] [q10; q10] and 46, XX, der [13]), and another 2 had trisomy 18 (46, XX, der [18] and 46, XX, der [18]). Seven cases with microdeletion and microduplication syndromes were also

Table 2

Summary of fetuses detected by chromosomal microarray analysis and karyotype analysis.

	Benign (n)	Pathogenic (n, %)	VOUS (n)	Abnormal karyotype (n, %)
Isolated cleft lip	20	0 (0)	0	0 (0)
Isolated cleft palate	2	1 (0.7)	0	1 (0.7)
Isolated cleft lip and palate	87	5 (3.5)	14	1 (0.7)
Syndromic cleft lip and palate	8	5 (3.5)	1	3 (2.2)
Total	117	11 (7.7)	15	5 (3.6)

VOUS = variants of unknown significance.

Table 3

Characteristics of cases with pathogenic copy number variants.

Case	Type	Gestational weeks	Cytoband	Chromosome region	Size (Kb/Mb)	Copy number	Karyotype	Outcome
1	Isolated CP	25	18q21.31~q23	55040745~78014123	22.97Mb	Loss	46, XX, der (18)	TOP
2	Isolated CLP	28	7q36.3	157615631~159119707	1.5Mb	Loss	46, XX, der (7) t	TOP
			11q21~q25	96130099~134937416	38.81Mb	Gain	(7;11)(q36;q21) mat	
			Xp22.31	6440776~8135568	1.69Mb	Gain		
3	Isolated CLP	27	22q11.21~q11.22	21464763~22706413	1.24Mb	Loss	46, XN	TOP
4	Isolated CLP	30	10q26.3	131063320~135426386	4.36Mb	Gain	46, XN	TOP
5	Isolated CLP	26	10q22.2~q22.3	76652946~78419911	1.77Mb	Loss	–	TOP
6	Isolated CLP	27	15q14	33875755~37706384	3.83Mb	Loss	–	TOP
7	Syndromic CLP	25	13q11~q13.3	19436286~38379248	19.94Mb	Gain	46,XX, +13, der	TOP
			13q13.3~q31.3	39391721~92255853	52.86Mb	Gain	(13;14)(q10;q10)	
			13q31.3~q33.3	92260684~108679534	16.42Mb	Gain		
			13q33.3~q34	108695728~115107733	6.4Mb	Gain		
8	Syndromic CLP	26	7q14.1	116532360~116679009	147Kb	Gain	–	TOP
9	Syndromic CLP	28	7q35~q36.3	14510614~159119707	13.41Mb	Gain	–	TOP
10	Syndromic CLP	24	13q11~q34	19436286~115107733	95.67Mb	Gain	46, XX, der (13)	TOP
11	Syndromic CLP	23	18p11.32~q23	136227~78013728	77.88Mb	Gain	46, XX, der (18)	TOP

CLP = cleft lip and palate, CP = cleft palate, TOP = termination of pregnancy.

found that were not found with traditional chromosome analysis, including 22q11.2 microdeletion syndrome, 15q14 microdeletion, 10q26.3 microduplication, 10q22.2 microdeletion, 7q35 (Pierre Robin sequence, PRS), and 7q14.1 microdeletion (Details are shown in Table 3). The outcome for these fetuses was termination of the pregnancy.

Due to the facial abnormalities, most patients chose to terminate the pregnancy; in our study, 83 cases resulted in the termination of pregnancy (TOP), of which 70 cases choose TOP despite no genetic aberrations, 41 in vaginal delivery, 11 in cesarean section, and the other 8 are still under observation during pregnancy (Table 4). Among the newborns, there were 42 males and 10 females.

4. Discussion

Oral clefts, which can include CL, CP, or CL with CP, are being prenatally diagnosed with increasing frequency. These phenotypes are believed to be etiologically distinct, based on differences in epidemiologic characteristics and embryological development.[10,11] An oral cleft has a significant impact on the individual, as well as the parents and community, in terms of physical and affective well-being and medical costs.[12] The etiology of oral clefts is not fully clear, but most of the current studies suggest that both genetic and environmental factors may be involved. Several environmental factors during pregnancy have been associated with an increased risk of oral clefts, including early drug use, maternal smoking, and alcohol use. In addition, racial and regional differences also play a certain role.[13–16] Furthermore, about 500 diseases are associated with CLP and can be found through the OMIM database, including more than 100 kinds of Mendelian syndrome, chromosome deletion or duplication, and familial inheritance, which may occur as isolated findings or in association with more than 180 genetic syndromes.[17]

Currently, fetal ultrasonography plays a significant role in determining the structural malformation of CLP. In this study, 143 cases of CLP were found by fetal ultrasound, of which CL with or without palate accounted for approximately 88% (126/143), which is like the results from studies by Souza et al.[18] At the same time, ultrasonography can also be used to detect whether other structural malformations are merged. In our study, we found 14 fetuses with CNS abnormalities (50%) and cardiac defects (21.4%), again similar to the results from other studies.[19] Ye et al also found CNS abnormalities, cardiac defects, and muscle and skeletal defects were the most highly associated structural anomalies among syndromic cleft cases.[20] However, ultrasonography has not been able to detect chromosomal abnormalities.

Conventional karyotyping has been the gold standard in prenatal diagnosis. In this study, 139 fetuses underwent chromosomal karyotype analysis, of which 3.6% (5/139) had chromosomal abnormalities. Most of the abnormalities were in the syndromic CLP group (2.2%), but isolated CP (0.7%) and CLP (0.7%) were also present. In these chromosomal abnormalities, we found that there were 4 cases of trisomy 18 and 13, accounting for approximately 80% of the chromosomal abnormalities (4/5). Trisomy 13 is associated with a variety of

Table 4

Follow up of fetuses with prenatal oral cleft (n).

	Isolated cleft lip	Isolated cleft palate	Isolated cleft lip and palate	Syndromic cleft lip and palate	Total
TOP	14	1	61	7	83
Vaginal delivery	5	2	30	4	41
Cesarean delivery	1	0	9	1	11
In observation	0	0	6	2	8
Sex (M/F)	4 M/2 F	1 M	36 M/7 F	1 M/1 F	52

F = female, M = male, TOP = termination of pregnancy.

characteristic malformations, of which CLP account for 45% to 71%.[21,22] Tonni et al suggested that approximately 10% of fetuses with CP also had chromosome abnormalities, usually in association with trisomy 18 or 13, conditions accounting for 40% and 60% of cases, respectively.[23] All the fetuses with an isolated CL were chromosomally normal.

At present, chromosomal karyotype analysis is unable to identify abnormal chromosomes less than 10Mb. CMA can detect the CNV of the genome of the whole genome. Compared with the traditional karyotype analysis, the resolution of CMA is higher. At the same time, it can detect mosaic, loss of heterozygosity and uniparental disomic in the genome. In recent years, several centers have recommended CMA as a diagnostic tool in prenatal diagnosis.[24–27] The occurrence of CL with/or without CP corresponds with chromosomal microdeletion. Peredo et al[28] reported a CLP in a female with a 1.63Mb microdeletion in 5q35.2-q35.3. Sing et al also detected a case of *de novo* microdeletion of 15q24.3-q25.2 in an infant with an oral cleft and suggested that this may be a critical region for orofacial development. Furthermore, they also emphasized that microarray was useful for the evaluation of children with congenital anomalies.[29] However, current research is mainly focused on children and infants, with few reports of prenatal diagnosis. In our study, 58% (83/143) of the pregnant women whose babies had fetal CL with or without palate, as detected by ultrasound, chose to terminate the pregnancy. Methods with which to provide better and more accurate prenatal counseling for these women are very important. To further analyze the CNV of the fetuses with oral clefts, 143 were analyzed with CMA. Eleven fetuses with pathogenic CNVs were discovered, accounting for approximately 7.7% of the cases, with these pathogenicity fragments distributed on chromosome 18q21.31~q23, 7q36.3, 11q21~q25, Xp22.31, 22q11.21~q11.22, 10q26.3, 10q22.2~q22.3, 15q14, 13q11~q13.3, 13q13.3~q31.3, 13q31.3~q33.3, 13q33.3~q34, 7q14.1, 7q35~q36.3, 13q11~q34, and 18p11.32~q23. These fragments did not show any regularity. Among them, 5 cases were smaller than 10Mb, 2 of which were not detected in the karyotype analysis, and 3 of which were not analyzed. We found a case of 22q11.2 microdeletion syndrome (DiGeoege syndrome) in the pathogenicity fragments; CP is one of the main manifestations of this syndrome and is also associated with congenital heart disease, hypocalcemia, abnormal face, and cognitive, behavioral, and mental disorders. In addition, a fragment of 7q35-q36 we also found in the PRS, accounting for 58% to 90% infants with a wide U-shaped CP,[30] which is also associated with micrognathia, glossoptosis, and airway obstruction. Interestingly, no abnormalities were found in either the karyotype analysis or CMA in the isolated CL group. In infants, the proportion of males (42) was higher than that of females (10).

5. Conclusions

Syndromic CLP is more frequently associated with chromosomal abnormalities, which are found in approximately 50.7% of the cases compared to 0.9% of the cases of isolated cleft.[31] Comparable results were found in our current research. Compared with traditional karyotyping, CMA has superior sensitivity (7.7% vs 3.6%) and a faster turn-around time, but there are also some disadvantages. CMA is unable to detect balanced chromosomal aberrations and cannot reveal the chromosomal location of extra chromosomal material. A higher resolution scan of the genome would result in a greater chance of revealing VOUS, which may cause problems in counseling patients. Despite this, CMA is highly recommended in prenatal testing for both syndromic oral cleft and isolated cases.

Author contributions

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