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ORIGINAL RESEARCH

Chromosomal Microarray Analysis for the Prenatal Diagnosis in Fetuses with Nasal Bone Hypoplasia: A Retrospective Cohort Study

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Background: Previous studies have shown a strong correlation between fetal nasal bone hypoplasia and chromosomal anomaly; however, there is little knowledge on the associations of fetal nasal bone hypoplasia with chromosomal microdeletions and microduplications until now. Chromosomal microarray analysis (CMA) is a high-resolution molecular genetic tool that is effective to detect submicroscopic anomalies including chromosomal microdeletions and microduplications that cannot be detected by karyotyping. This study aimed to examine the performance of CMA for the prenatal diagnosis of nasal bone hypoplasia in the second and third trimesters.

Subjects and Methods: A total of 84 pregnant women in the second and third trimesters with fetal nasal bone hypoplasia, as revealed by ultrasound examinations, were enrolled, and all women underwent karyotyping and CMA with the Affymetrix CytoScan 750K GeneChip Platform. The subjects included 32 cases with fetal nasal bone hypoplasia alone and 52 cases with fetal nasal bone hypoplasia combined with other ultrasound abnormalities, and the prevalence of genomic abnormality was compared between these two groups.

Results: Karyotyping detected 21 cases of chromosomal anomaly in the 84 study subjects (21/84, 25%), including trisomy 21 (14 cases), trisomy 18 (3 cases), 46, del (4)(p16) karyotype (2 cases), 47, XYY syndrome (1 case) and 46, XY, del (5) (p15) karyotype (1 case). CMA detected additional four fetuses with pathogenic copy number variations (CNVs) and six fetuses with uncertain clinical significance (VOUS). No significant difference was detected in the prevalence of genomic abnormality in fetuses with nasal bone hypoplasia alone and in combination with other ultrasound abnormalities (13/32 vs 18/52; $\chi^2 = 0.31$, $P > 0.05$). The pregnancy was terminated in 21 fetuses detected with chromosomal abnormality and 4 fetuses detected with pathogenic CNVs. Among the other six fetuses detected with VOUS, the parents chose to continue the pregnancy, and the newborns all had normal clinical phenotypes.

Conclusion: In addition to chromosomal abnormalities identified in 21 fetuses by karyotyping, CMA detected additional 10 fetuses with abnormal CNVs (10/84, 11.9%) in the study population. CMA is a promising powerful tool for prenatal diagnosis that may provide valuable data for the accurate assessment of fetal prognosis and the decision of pregnancy continuation during the prenatal clinical counseling.

Keywords: nasal bone hypoplasia, chromosomal microarray analysis, prenatal diagnosis, copy number variation

Introduction

Ultrasonography is an important part of prenatal screening.[1] Currently, the common genetic ultrasound soft markers include nuchal translucency thickening, nasal bone

hypoplasia and shortening of fetal long bones,[2] and these soft markers show diverse values in prediction of fetal abnormalities.[3]

Fetal nasal bone hypoplasia is defined as absence or dysplasia of the nasal bone on the mid–sagittal plane in the second trimester as detected by ultrasonography.[4] Previous studies have shown a strong correlation between fetal nasal bone hypoplasia and chromosomal anomaly,[5–7] and the highest incidence of nasal bone hypoplasia is detected in fetuses with aneuploidy,[8–10] notably in Down syndrome (trisomy 21).[5,11,12] However, there is little knowledge on the associations of fetal nasal bone hypoplasia with chromosomal microdeletions and microduplications until now.[13,14]

Chromosomal microarray analysis (CMA) is a high-resolution molecular genetic tool that is effective to detect chromosomal microdeletions and microduplications that cannot be detected by karyotyping. This retrospective cohort study aimed to examine the performance of CMA for the prenatal diagnosis of nasal bone hypoplasia in the second and third trimesters.

Subjects and Methods

Subjects

We collected 84 fetuses diagnosed with fetal nasal bone hypoplasia, as revealed by ultrasound examinations at the Center of Prenatal Diagnosis, Fujian Maternity and Child Health Hospital (Fuzhou, China) during the period from December 2016 through December 2018. The pregnant women had ages of 20 to 41 years, and had gestational ages of 18 to 36 weeks. All pregnant women underwent karyotyping and CMA with amniotic fluid and umbilical blood samples. Among the 84 prenatal samples collected, there were 53 amniotic fluid samples and 31 umbilical blood samples. The subjects included 32 cases with fetal nasal bone hypoplasia alone and 52 cases with fetal nasal bone hypoplasia combined with other ultrasound abnormalities. The subjects' medical records were retrospectively reviewed.

Karyotyping

Chromosome karyotyping analysis was routinely performed as described previously.[15] Briefly, amniotic fluid and umbilical cord blood samples were collected, cultured, harvested and subjected to G-band karyotype analysis, with additional C- and N-band karyotyping performed if required. Amniocentesis was performed at 18 to 24 weeks of gestation, while cordocentesis was performed after 24 weeks of gestation. Karyotyping was done on a Leica GSL-120 automatic slide scanning system (Leica Biosystems Richmond, Inc.; Richmond, IL, USA). Each sample was detected for 40 karyotypes, with 5 used for banding, and additional detections were performed if required.

CMA

Approximately 10 mL of amniotic fluid and umbilical cord blood specimens were collected, centrifuged and the sediment was collected. Genomic DNA was isolated from amniotic fluid cells using the QIAamp DNA Blood Mini Kit (Qiagen; Hilden, Germany). All CMA analyses were performed using the Affymetrix CytoScan 750K GeneChip Platform (Affymetrix, Santa Clara, CA, USA) with copy number variation (CNV) and single nucleotide polymorphism (SNP) probes following the manufacturer's instructions. All CMA data were processed with the software Chromosome Analysis Suite version 3.2 (Affymetrix; Santa Clara, CA, USA), and the analysis was capable of detecting CNVs with clinically relevant genes and genome-wide backbone resolution of greater than 100 kb. The CNVs were interpreted using public databases, including the database of genomic variants (http://dgv.tcag.ca/dgv/app/home), the DECIPHER database (https://decipher.sanger.ac.uk/), Online Mendelian Inheritance in Man (https://www.omim.org/), International Standards for Cytogenomic Arrays (ISCA) Consortium (http://www.iscaconsortium.org/) and the Cytogenomics Array Group CNV Database (CAGdb database; http://www.cagdb.org/), as well as National Center for Biotechnology Information (NCBI). All CNVs detected were classified as pathogenic, benign or variants of uncertain clinical significance (VOUS) according to the American College of Medical Genetics standards and guidelines.[16] In addition, the peripheral blood was sampled from the parents of fetuses with VOUS for CMA analysis, and the type of CNVs was validated by means of CMA and pedigree analysis.

Ethical Consideration

This study was approved by the Ethics Review Committee of Fujian Maternity and Child Health Hospital. All participants were informed of the purpose, experimental procedures and potential risks of the study, and signed an informed consent. All experiments were performed in accordance with the Declaration of Helsinki and National

Regulations for Ethics of Biological Medical Sciences on Human Studies released by Ministry of Health, China.

Statistical Analysis

All statistical analyses were performed using the statistical software SPSS version 22.0 (SPSS, Inc.; Chicago, IL, USA). Differences of proportions were tested for statistical significance with chi-square test or Fisher's exact test, and a P value of <0.05 was considered statistically significant.

Results

Chromosome Karyotyping

There were 84 prenatal samples with karyotyping analysis, including 53 amniotic fluid specimens and 31 cord blood specimens. G-band karyotype analysis detected 21 cases of chromosomal anomaly in the 84 study subjects (21/84, 25%), and fetal chromosomal anomaly included trisomy 21 (14 cases), trisomy 18 (3 cases), 46, del (4)(p16) karyotype (2 cases), 47, XYY syndrome (1 case) and 46, XY, del (5) (p15) karyotype (1 case). Table 1 shows the karyotype, ultrasound findings and pregnant outcomes in fetuses with chromosomal anomaly.

CNVs Detected by CMA

All fetuses in our study were further tested for chromosomal anomaly using CMA. In addition to the 21 fetuses identified with chromosomal anomaly described above, CMA detected additional CNVs in 10 fetuses, including 4 fetuses with pathogenic CNVs and 6 fetuses with VOUS. The pathogenic CNVs included 15q13.2q13.3 microdeletion, 16p12.2 microdeletion, 17p12 microdeletion and 15q24.1q24.2 microdeletion, and VOUS included 15q13.3 microduplication, 16p13.13p13.12 microduplication, 2p22.3 microduplication, 15q11.2 microdeletion, Xq21.33 microduplication and 15q26.1 microdeletion (Table 2).

There was no significant difference was detected in the prevalence of genomic abnormality in fetuses with nasal bone hypoplasia alone and in combination with other ultrasound abnormalities (13/32 vs18/52; $\chi^2 = 0.31$, $P > 0.05$) (Table 3).

Pregnant Outcomes

All 84 fetuses were successfully followed up. The pregnancy was terminated in 21 fetuses detected with chromosomal abnormality and 4 fetuses detected with pathogenic CNVs. Among the other six fetuses detected with VOUS, five cases were had pedigree analysis, the parents chose to continue the pregnancy, and the newborns all had normal clinical phenotypes (Tables 1 and 2).

Discussion

The genetic etiology of fetal nasal bone hypoplasia has been extensively investigated,[17] and a close association has been identified between fetal nasal bone hypoplasia and chromosomal abnormality,[5–7] notably with aneuploidy.[8–10] In this study, we detected trisomy 21 in 14 cases, trisomy 18 in 3 cases and 47, XYY syndrome in one case among the 84 fetuses with nasal bone hypoplasia, and the prevalence of aneuploidy was 21.43% in the study subjects. In a previous study recruiting 239 fetuses at gestational ages of 15 to 20 weeks, absence of a nasal bone was detected in 37% of fetuses with trisomy 21 and 0.5% of normal fetuses, yielding a likelihood ratio of 83, and the findings suggested that absence of a nasal bone is the most sensitive ultrasound soft marker for trisomy 21.[18] Sonek and colleagues reported a 1% prevalence of absent nasal bones in normal fetuses and 37% prevalence in fetuses with trisomy 21 in the second trimester, yielding a positive likelihood ratio of 41 and negative likelihood ratio of 0.64, and they concluded that absence of a nasal bone shows a high predictive value for trisomy 21.[19] In the current study, we detected a lower prevalence of aneuploidy in the subjects as compared to previous reports. In our center, if the pregnant women harboring aneuploidy fetuses present a high risk of trisomy 21 as detected by the blood testing or non-invasive prenatal test (NIPT) in the first trimester, and they may lose the timing to directly receive prenatal diagnosis by amniocentesis without ultrasound screening. If the fetus is definitively diagnosed with aneuploidy, induction of labor is given, and the diagnosis of aneuploidy fetuses with absence of a nasal bone may be missed. Therefore, the prevalence of aneuploidy detected in the fetuses with nasal bone hypoplasia was lower in this study than previous reports.

CMA has a high resolution to detect microdeletions and microduplications,[20–22] which supplements the shortcomings of conventional G-band karyotyping in misdiagnosis of small chromosomal segments.[23] In this study, CMA detected microdeletions and microduplications in 10 fetuses with nasal bone hypoplasia, which increased the detection of genomic abnormalities by 11.9% as compared to G-banding karyotype analysis. We detected no significant difference in the prevalence of genomic abnormality in fetuses with nasal bone hypoplasia alone and in combination with other ultrasound abnormalities

Table 1 Chromosome Karyotyping Detects Abnormal Karyotypes in 21 Fetuses with Nasal Bone Hypoplasia

Fetus No.	Invasive Procedure	Chromosome Karyotype	Ultrasound Findings	Pregnant Outcome
1	AS	Trisomy 21	Fetal nasal bone hypoplasia	Induction of labor
2	AS	Trisomy 21	Fetal nasal bone hypoplasia	Induction of labor
3	AS	Trisomy 21	Fetal nasal bone hypoplasia	Induction of labor
4	CS	Trisomy 21	Fetal nasal bone hypoplasia	Induction of labor
5	AS	Trisomy 21	Fetal nasal bone hypoplasia and nuchal translucency thickening	Induction of labor
6	AS	Trisomy 21	Fetal nasal bone hypoplasia and nuchal translucency thickening	Induction of labor
7	AS	Trisomy 21	Fetal nasal bone hypoplasia and nuchal translucency thickening	Induction of labor
8	AS	Trisomy 21	Fetal nasal bone hypoplasia and nuchal translucency thickening	Induction of labor
9	AS	Trisomy 21	Fetal nasal bone hypoplasia and cysts of the choroid plexus of bilateral ventricles	Induction of labor
10	AS	Trisomy 21	Fetal nasal bone hypoplasia, echogenic bowel and hyperechogenic foci in the left ventricle	Induction of labor
11	AS	Trisomy 21	Fetal nasal bone hypoplasia, echogenic bilateral renal parenchyma, and echogenic hepatic parenchyma	Induction of labor
12	AS	Trisomy 21	Fetal nasal bone hypoplasia, nuchal fold thickening, and aberrant right subclavicular artery	Induction of labor
13	AS	Trisomy 21	Fetal nasal bone hypoplasia, abnormal blood flow signals in the right atrium, suspected right coronary artery-right atrial fistula and hyperechogenic foci in the left ventricle	Induction of labor
14	AS	Trisomy 21	Fetal nasal bone hypoplasia, nuchal translucency thickening, anasarca, endocardial cushion defect and deepening of notched A-wave on venous catheter	Induction of labor
15	CS	47, XYY	Fetal nasal bone hypoplasia	Induction of labor
16	AS	46, XY, del(5)(p15)	Absence of nasal bone	Induction of labor
17	AS	Trisomy 18	Fetal ventricular septal defect, aorta overriding, mild mitral and tricuspid regurgitation, and nasal bone hypoplasia	Induction of labor
18	AS	Trisomy 18	Artialseptal defect, fetal growth restriction, nasal bone hypoplasia and single umbilical artery	Induction of labor
19	AS	Trisomy 18	Fetal growth restriction, ventricular septal defect, high pulmonary artery to aorta ratio, nasal bone hypoplasia and overriding fingers	Induction of labor
20	CS	46, XX, del (4)(p16)	Fetal growth restriction, communicating branch of the portal vein, bilateral small kidney and nasal bone hypoplasia	Induction of labor
21	AS	46, XY, del (4)(p16)	Fetal nasal bone hypoplasia, small left ventricle, aortic stenosis, micrognathia, nuchal fold thickening and single umbilical artery	Induction of labor

Abbreviations: AS, amniocentesis; CS, cordocentesis.

Table 2 Chromosomal Microarray Analysis Detects Copy Number Variations in 10 Fetuses with Nasal Bone Hypoplasia

Fetus No.	Invasive Procedure	Reason for Referral	CMA Detection Results	Clinical Significance	Fragment Size (Mb)	OMIM Gene Number	Known Syndrome	Pregnant Outcome
1	AS	Nasal bone hypoplasia	arr[GRCh37]15q13.2q13.3 (30,386,398_32,444,261)×1	Pathogenic	2.0	7	15q13.3 microdeletion syndrome	Induction of labor
2	AS	Nasal bone hypoplasia	arr[GRCh37]16p12.2 (21,816,542_22,710,614) ×1 pat	Pathogenic	0.97	4	Recurrent 16p12.1 microdeletion (neurodevelopmental susceptibility locus)	Induction of labor
3	AS	Nasal bone hypoplasia	arr[GRCh37] 17p12 (14,070,219_15,484,335) ×1	Pathogenic	1.4	4	Hereditary Liability to Pressure Palsies (HNPP)	Induction of labor
4	AS	Nasal bone hypoplasia	arr[GRCh37] 15q13.3 (32,021,609_32,444,043) ×3 mat	VOUS	0.41	1	15q13.3 microduplication syndrome (neurodevelopmental susceptibility locus)	Normal phenotype after birth
5	AS	Nasal bone hypoplasia	arr[GRCh37] 16p13.13p13.12 (11,528,493_12,934,811) ×3	VOUS	1.4	9	—	Normal phenotype after birth
6	AS	Nasal bone hypoplasia	arr[GRCh37] 15q11.2 (22,770,421_23,276,833) ×1 pat	VOUS	0.50	4	15q11.2 recurrent region (BP1–BP2) (neurodevelopmental susceptibility locus)	Normal phenotype after birth
7	AS	Nasal bone hypoplasia	arr[GRCh37] 2p22.3 (34,002,379_35,076,738) ×3	VOUS	1.0	0	—	Normal phenotype after birth
8	AS	Fetal growth restriction, ventricular septal defect, pulmonary valve stenosis complicated by incompetence and nasal bone hypoplasia	arr[GRCh37]15q24.1q24.2 (72,965,465_75,567,135)×1	Pathogenic	2.6	38	15q24 recurrent microdeletion syndrome	Induction of labor
9	AS	Fetal nasal bone hypoplasia, biparietal diameter and humerus length of <2SD, and hyperechogenic foci in the left ventricle	arr[GRCh37] Xq21.33 (95,227,256_95,972,695)×3 pat	VOUS	0.7	0	—	Normal phenotype after birth
10	AS	Fetal nasal bone hypoplasia and ventricular septal defect	arr[GRCh37] 15q26.1 (90,211,822_91,080,606)×1 pat	VOUS	0.85	13	—	Normal phenotype after birth

Abbreviations: AS, amniocentesis; VOUS, variants of uncertain clinical significance.

Table 3 Comparison of Genomic Abnormality Prevalence Between Fetuses with Nasal Bone Hypoplasia Alone and in Combination with Other Ultrasound Abnormalities

Group	Total No. of Fetuses	Total No. of Fetuses with Abnormalities	No. of Chromosomal Abnormality	No. of Fetuses with Abnormal CNVs
Fetuses with nasal bone hypoplasia	32	13	6	7
Fetuses with nasal bone hypoplasia and other ultrasound abnormalities	52	18	15	3
Total	84	31	21	10

($P > 0.05$), which is inconsistent with previous reports.[24–26] It was reported that absence of a nasal nose, complicated with other fetal organ and structural abnormalities increased the risk of chromosomal abnormalities.[6,7] However, we detected a higher prevalence of genomic abnormality in fetuses with nasal bone hypoplasia alone than those with nasal bone hypoplasia and other ultrasound abnormalities (13/32 vs18/52). This may be because karyotyping analysis alone was employed in previous studies, while both karyotyping and CMA were performed in this study, thereby resulting in a rise in the detection of genomic abnormality; in addition, this variation may be attributed to the study subjects. Nevertheless, the prevalence of chromosomal abnormality was higher in fetuses with nasal bone hypoplasia and other ultrasound abnormalities (28.85%) than in those with nasal bone hypoplasia alone (18.75%), which is in agreement with previous studies.[6,7]

In this study, we detected CNVs in 10 fetuses with nasal bone hypoplasia, and pathogenic CNVs were identified in four fetuses, including 15q13.2q13.3 microdeletion (1 case), 16p12.2 microdeletion (1 case), 17p12 microdeletion (1 case) and 15q24.1q24.2 microdeletion (1 case). Previous studies have demonstrated that 15q13.2q13.3 microdeletion may cause 15q13.3 microdeletion syndrome, which is mainly manifested by developmental retardation, epilepsy, and finger and toe anomalies and minor facial abnormalities.[27–29] In this study, however, only nasal bone hypoplasia was found in the fetus with 15q13.2q13.3 microdeletions on sonography. A susceptibility locus of neurocognitive impairment has been identified in the region of 16p12.2 microdeletions, and the frequency of this susceptibility locus is estimated to be less than 1% in normal populations.[30] In the ClinGen database, the haploinsufficiency score of the recurrent 16p12.2 microdeletion is 2, while the overall penetrance is approximately 12%.[31] Patients with 16p12.2 microdeletions have diverse clinical manifestations, which mainly include developmental retardation, mild to moderate intellectual disturbance, congenital heart defects and epilepsy.[32] However, ultrasound examinations displayed nasal bone hypoplasia alone in the fetus with 16p12.2 microdeletions. 17p12 microdeletion is reported to link with hereditary neuropathy with liability to pressure palsies (HNPP).[33] To date, the penetrance of 17p12 microdeletions remains unknown, and many patients carrying 17p12 microdeletions present few and even no clinical symptoms; in addition, approximately 80% of deletions of the *PMP22* gene on chromosome 17p12 regions are estimated to be inherited from parents, where haploinsufficiency effect is observed with a score of 3;[34] however, only nasal bone hypoplasia was seen in the fetus with 17p12 microdeletions. 15q24.1q24.2 Microdeletion has been identified as a pathogenic factor of 15q24 microdeletion syndrome, which manifests as feeding intolerance, eye abnormality, widening of neck, nasal bone hypoplasia, muscle hypotonia, attention-deficit/hyperactivity disorder and autism.[35] In this study, ultrasound displayed fetal growth restriction, ventricular septal defect, pulmonary valve stenosis complicated by incompetence and nasal bone hypoplasia in the fetus with 15q24.1q24.2 microdeletions. These data indicate that chromosome karyotyping is likely to lead to missing diagnosis and misdiagnosis of genomic microstructural abnormality in fetuses with nasal bone hypoplasia detected by ultrasound. Our data suggest that CMA has an extensive range of indications to detect chromosomal microstructural abnormalities and shows a powerful value in prenatal diagnosis.

Nevertheless, there is a difficulty in the interpretation of the clinical significance of CMA detection results, notably in the interpretation of VOUS, and the huge rise in CMA detections interpretation will inevitably increase the burden of validations. Previous studies have shown a 1.1% to 6% detection of VOUS by CMA.[36–38] In this study, CMA detected in VOUS in 6 out of 84 fetuses with nasal bone hypoplasia, and the

VOUS prevalence (7.14%) was higher than previous reports.[36–38] Pedigree analysis confirmed that four cases were h inherited from healthy parents. In cases with VOUS, two cases were detected with neural susceptibility sites, which contained 15q13.3 microduplication and 15q11.2 recurrent region. If the CNVs were verified to be inherited, it would reduce the psychological burden of pregnant women, and pregnant women will be more willing to choose to keep the fetus. Recently, next-generation sequencing has been employed as a novel tool for genetic testing of single-gene mutations and CNVs, which may provide a more comprehensive prenatal genetic diagnosis for fetuses with nasal bone hypoplasia, and provide insights into a better assessment of fetal prognosis.[39–41]

Prenatal genetic testing determines the decision to terminate pregnancy.[42] In the present study, 84 fetuses with nasal bone hypoplasia were all successfully followed up, and pregnancy termination was performed in 21 fetuses detected with chromosomal abnormality and 4 fetuses detected with pathogenic CNVs, while the pregnancy continued in other 6 fetuses detected with VOUS, and these babies all had normal clinical phenotypes after birth. It is therefore considered that CMA may provide valuable data for the accurate assessment of fetal prognosis and risk of disease recurrence and the decision of pregnancy continuation during the prenatal clinical counseling.[22,43–45]

The present study has some limitations. First, this retrospective analysis was performed in a cohort including 84 fetuses with nasal bone hypoplasia recruited from a single center, and further multicenter studies recruiting more fetuses are needed. Second, not all cases with VOUS were given additional pedigree analyses, which is ineffective to provide better guidance for genetic counseling.

In summary, the results of the present study demonstrate that CMA increases the detection of CNVs in fetuses with nasal bone hypoplasia relative to conventional chromosome karyotyping. It is considered that CMA is a powerful tool used for prenatal diagnosis in fetuses with nasal bone hypoplasia.

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Disclosure

The authors declare no conflict of interests.

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