单绒毛膜双羊膜囊双胎心脏畸形拷贝数变异分布的差异

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摘　要：【目的】分析拷贝数变异在单绒毛膜双羊膜囊双胎(MCDA)合并先天性心脏病病例中分布的差异。【方法】MCDA 双胎合并先心病病例接受胎儿染色体核型检查和染色体微阵列检测，采用 Affymetrix CytoScan 高分辨微阵列平台。【结果】从 2010 年至 2016 年，114 例 MCDA 双胎合并心脏畸形且类型不一致，4 例合并心脏畸形且类型一致。114 例 MCDA 双胎合并心脏畸形且类型不一致病例中，72 例接受胎儿染色体核型检查。胎儿染色体核型检测结果如下：5 例双胎核型不一致，1 例双胎均是 47, XYY，1 例双胎均是 21 三体，65 例双胎染色体核型均正常。36 例 MCDA 双胎合并心脏畸形类型不一致且染色体核型、22q11.2 微缺失检测均正常病例中，拷贝数变异不一致比率是 5.6%。所有先心病中致病性拷贝数变异和临床意义未明拷贝数变异的检出率分别是 2.2%和 2.2%。4 例 MCDA 双胎合并心脏畸形类型一致病例中，1 例未行胎儿染色体核型检查，其余 3 例染色体核型和染色体微阵列检测结果均正常。【结论】合子后的拷贝数变异可解释 5%MCDA 双胎合并心脏畸形类型不一致的发生原因。

关键词：拷贝数变异；单绒毛膜双羊膜囊双胎；不一致；先天性心脏病
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Copy Number Variations in Monochorionic Diamniotic Twins with Discordant and Concordant Congenital Heart Diseases in Prenatal Cohort

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Abstract: 【Objective】To investigate the distribution of copy number variations (CNV) in monochorionic diamniotic (MCDA) twins with discordant and concordant congenital heart diseases (CHD). 【Methods】MCDA twins with CHD were analyzed with conventional fetal karyotyping and chromosomal microarray analysis (CMA), which was performed with Affymetrix CytoScan HD array. 【Results】Between 2010 and 2016, 114 MCDA twins with discordant CHD and four with concordant CHD were identified. Among the 114 MCDA twins with discordant CHD, fetal karyotyping was performed in 72 cases. The findings of fetal karyotyping were as follows: five with discordant chromosomal anomalies, one with concordant 47, XYY, one with trisomy 21 and 65 with accordant normal karyotype. In the 36 cases with discordant CHD, normal karyotype and normal FISH results for DiGeorge region, the prevalence of discrepant CNV was 5.6%. Uncertain of clinical significance CNV and pathogenic CNV occurred in 2.2% (1/45) and in 2.2% (1/45) fetuses with CHD. With regard to the four cases with concordant CHD, fetal karyotype was not performed in one. Normal karyotype and normal CMA results were confirmed in the remaining three cases. 【Conclusions】Post-zygotic CNV events in MCDA twins may be associated with about 5% twin discordant for CHD in the investigated cases.

Key words: copy number variations; monochorionic diamniotic twins; discordant; congenital heart disease
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Monozygotic (MZ) twin pairs are generally presumed as genetically identical and discordant phenotype is caused by environmental factors. Since the first published report on discordance for monosomy X[1] and for Trisomy 21[2] in MZ twins, more and more literatures on discordance for aneuploidies have been published. Chromosomal microarray analysis (CMA) could identify microdeletions and microduplications, which are called as copy number variations (CNV). Microarray analysis increases the detection rate of pathogenic submicroscopic rearrangement of chromosome and has already been the first-line diagnostic test for clinical evaluation of patients with unexplained intellectual disability, autism spectrum disorder and multiple congenital malformations[3-4]. Prognosis of CHD might be poor if coexisting with chromosomal aberrations or genetic syndromes. CMA has been used to evaluate the association between CNV and CHD in prenatal settings recently[5-7]. Prevalence of CHD at birth was much higher when compared singletons with MC twin pairs[8]. Breckpot et al had explored the occurrence of copy number differences in MZ twins discordant for the presence of CHD in postnatal series[9]. To the best of our knowledge, the published literatures about whether post-zygotic CNV play an important role in the incidence of discordant CHD or not are limited. In this study, our aim was to investigate the distribution of CNV in MCDA twins with discordant and concordant CHD and to explore whether difference of CNV was associated with discordant of presence or severity of cardiac malformations.

1 Material and Methods

1.1 Objectives

This retrospective study included all the MCDA twins that were detected with congenital heart diseases in our institution between January 2010 and January 2016. Fetuses with isolated cardiomegaly (n=8), tachycardia (n=1), cardiac tumor (n=1) and myocardial hypertrophy (n=4) were excluded from our cohort. MCDA twin pairs with identical cardiac defects were considered as concordant, whereas MCDA twin pairs with discordance of presence of CHD or different type of cardiac defects were considered as discordant. The Ethics Committee of the institution has approved this study.

1.2 Methods

Intracardiac anatomy and extracardiac structures were evaluated in detail in all cases. After two-dimensional echocardiography examination, which was performed with an ultrasound system equipped with pulsed, continuous, and color Doppler capability, fetal cardiac volume datasets were obtained by using spatiotemporal image correlation (STIC). Off-line analysis of the recorded datasets was carried out on a personal computer with dedicated software (4D-viewer 7.0; GE Medical Systems). The operators were experienced in prenatal ultrasonographic screening. Presence of CHD or not, category and severity of CHD was recorded. After ultrasonographic evaluation, fetal karyotyping and chromosomal microarray analysis was recommended. All prenatal samples were submitted to a single laboratory for G-band karyotyping and microarray analysis, which was performed by using Affymetrix CytoScan HD array. The resolution of the whole genome analysis was 100 kb. Interpretations of CNV were compared with the public databases. In addition, the published articles that were associated with the relevant segment were reviewed. The CMA results were classified as benign CNV, CNV of uncertain significance (VOUS) and pathologic CNV. Pathologic CNV included pathological CNV that were independent of the fetal phenotype and pathological CNV explaining the fetus's phenotype. Quantitative fluorescent PCR (QF-PCR) was used to confirm the zygosity of the MCDA twins. QF-PCR or fluorescence in-situ hybridization (FISH) was carried out for verification of copy number gains/losses when necessary. CNV between the MCDA twin pairs were considered as discordant if the discrepancy was larger than 100 kb. Specially, the parents were also furnished with a detailed counseling on the prognosis

of CHD from cardiac surgery.

Selective fetal reduction was carried out in the fetuses with CHD of unfavorable prognosis and expectant management was suggested for the MCDA twins with CHD of good prognosis. Till delivery, outpatient prenatal surveillance with ultrasound was suggested with an interval of two weeks. Clinical outcomes data were collected and recorded. Prenatal diagnosis of CHD and extracardiac anomalies were confirmed by postnatal echocardiography examinations, surgery or autopsy. All live-born infants had a detailed neonatal examination by trained pediatricians.

2 Results

A total of 1 317 MCDA twins were identified during the study period in our institution, among which 246 MCDA twins were evaluated as with discordant malformations. One hundred and forty-five MCDA twins were suspected with CHD prenatally. In ten cases, perinatal outcomes were not obtained. In addition, three cases were still undergoing pregnancy. Excluding fetuses with isolated cardiomegaly, tachycardia, cardiac tumor or myocardial hypertrophy (secondary to twin to twin transfusion syndrome or selective fetal growth restriction), the remaining 118 MCDA twins with CHD, including 114 cases with discordant CHD and four with concordant CHD, formed the study population in this study. Gestational weeks at the time of ultrasound screening were ranging from 15^{+5} to 31^{+3}. Termination of pregnancy occurred in 33 cases and three cases were ended as spontaneous abortion. In 13 cases, selective reduction was performed in the anomalous fetuses. Overall survival rate was 59.2% (135/228) and 25.0% (2/8) for MCDA twins with discordant CHD and concordant CHD. The survival rate of at least one twin was 65.8% (75/114) and 25.0% (1/4), respectively.

Among 114 MCDA twins with discordant CHD, 105 cases were discordant for presence of CHD and nine were discordant for severity of CHD. Ultimately, one hundred and twenty-three fetuses with CHD were enrolled into this study. Genetic data could not be obtained in forty-two cases. Fetal karyotyping was performed in both amniotic sacs in 72 MCDA twins. The findings of fetal karyotyping were as follows: five cases with discordant chromosomal anomalies (four with discordant 45, X and one twin pair with 47, XXY karyotype in one fetus and 45, X[7]/46, XY[43] karyotype in the other fetus), one twin pair with concordant 47, XYY, one twin pair with trisomy 21 and 65 with accordant normal karyotype. More detail about the five cases with discordant karyotype was shown in Table 1. CMA was performed in 43 cases, including 36 MCDA twins with concordant normal karyotype and the seven cases with discordant or concordant abnormal karyotype. All these 43 MCDA twin pairs were monozygotic, which was confirmed by QF-PCR.

Of these 36 MCDA twins with discordant CHD, normal karyotype and normal FISH results for the DiGeorge region, 31 cases (86.1%, 31/36) were evaluated as with no microdeletions or microduplications by using CMA. On microarray, CNV were interpreted as common benign in 6.7% (3/45) fetuses with CHD. Microarray analysis detected CNV of uncertain clinical significance in 2.2% (1/45) of the cases with isolated CHD and non-isolated CHD combined. CNV were determined to be pathogenic in 2.2% (1/45) fetuses with CHD. In addition, discrepant CNV occurred in 5.6% (2/36) of the 36 MCDA twins with discordant CHD and normal karyotype. The CMA findings, ultrasonographic findings and clinical outcomes of these six cases with discordant cardiac defects and abnormal results on microarray are displayed in Table 2. With regard to the four MCDA twins with concordant CHD, fetal karyotyping and CMA was rejected in one case. Normal karyotype and no microdeletions or microduplications on microarray analysis were confirmed in the remaining three cases.

Concordant pathological CNV was detected in one case (Case 35), exhibiting a 3.706 Mb deletion of chromosomal band 17p in the fetus with

Table 1 Ultrasonographic characteristics and cytogenetic results in the five cases with discordant congenital heart disease and discordant cytogenetic results

Case	Ultrasonographic findings of pair 1	Ultrasonographic findings of pair 2	Cytogenetic findings of pair 1	Cytogenetic findings of pair 2	Outcomes
70	Hypoplastic left heart syndrome, cutaneous edema, pleural effusion	Normal	45, X	46, XX	Underwent selective reduction with RFA at 19^{+4} weeks; vaginal delivery at 39 weeks, female, 2 980g, phenotypically normal
119	Hypoplastic left heart syndrome, cutaneous edema, lymphohygroma, pleural effusion	Normal	45, X	46, XX	Both fetuses suffered with IUD, TOP
131	Lymphohygroma, pleural effusion, cardiomegaly	Normal	45, X	46, XX	TOP
71	Ventricular septal defect, hypoplastic left heart syndrome, hydrops fetalis, lymphohygroma,	Normal	45, X	46, XY	Underwent selective reduction with RFA at 20^{+1} weeks; C.S at 36^{+4} weeks, male, 2980g, phenotypically normal
77	Persistent left superior vena cava, omphalocele	Normal	47, XXY	45,X[7]/46, XY[43]	Underwent selective reduction with BCC at 25^{+6} weeks, C.S at 33^{+4} weeks, male, 2 450 g, admitted to NICU, phenotypically normal

ventricular septal defect and pulmonary stenosis and a 3.672 Mb deletion in the co-twin with fetal growth restriction. Chromosome 17p11.2 comprises the SREBF1 and RALI1 genes and mutations of these genes have been shown to be associated with Smith-Magenis syndrome. Both of these two fetuses suffered with intrauterine death at 30 weeks. Chromosomal microarray analysis did not identify additional discrepancy of genomic anomalies that could potentially be responsible for the phenotypic differences and we concluded that these could possibly be related to the variable expressivity of the microdeletion of 17p11.2.

Among the 36 MCDA twins with discordant CHD, normal karyotype and normal FISH results for the DiGeorge region, only one case (Case 56) was identified with accordant CNV of VOUS in both fetuses, involving a small de no deletion (244 kb) in chromosome 16p11.2 in the fetus with aortic coarctation and hypoplasia of the nasal bone and a de no microdeletion (241 kb) in chromosome 16p11.2 in the co-twin with single umbilical artery. Public databases and published reports were reviewed and we found that the clinical presentations of the reported cases were not similar to this case. After post-test counseling, the parents decided to continue with the pregnancy. The neonate with aortic coarctation was performed an operation at about 20 days after delivery. Now the infant was twenty months, however, delayed cognitive performance and motor development was present, whereas, motor development, verbal development and cognitive performance was normal in the co-twin.

Discordant CNV between MCDA twins with discordant CHD were indentified in two cases (Case 31 and Case 45). Case 31 involved a 593 kb deletion at the chromosome 14q11.2 in the fetus with lymphohygroma and hypoplasia of the left heart but no microdeletions or microduplications were identified in the phenotypic normal fetus. The female neonate with hypoplasia of the left heart suffered with death at one month after delivery. However, we

Table 2　Characteristics of fetuses with copy number variations among 36 monochorionic diamniotic with discordant congenital heart disease, normal karyotype and negative for 22q11.2 microdeletion syndrome

Case	Cardiac defect and extracardiac anomalies in pair 1	Cardiac defect and extracardiac anomalies in pair 2	CMA findings of pair 1 and relevant genes	CMA findings of pair 2 and relevant genes	Pathological significance	DECIPHER: Syndromes overlapping	Outcomes
35	Ventricular septal defect, pulmonary stenosis	Selective intrauterine growth restriction	arr17p11.2 (16, 727, 264-20, 433, 502)×1, 3.706 Mb; TNFRSF13B, COPS3, MED9, RALI1, TOM1L2……	arr17p11.2 (16, 761, 814-20, 433, 502)×1, 3.672 Mb; TNFRSF13B, COPS3, MED9, RALI1, TOM1L2……	Yes	Smith-Magenis Syndrome, Potocki-Lupski syndrome (17p11.2)	Both fetuses suffered with IUD at 30 weeks
31*	Hypoplasia of the left heart, nuchal fold thickening, lymphohygroma, oligohydramnios	Normal	arr14q11.2 (22, 385, 287-22, 978, 349)×1, 593 kb; No relevant genes	No CNV or LOH	No	No	The anomaly twin suffered with NND, the co-twin phenotypically normal
56	Aortic coarctation, hypoplasia of the nasal bone, fetal growth restriction	Single umbilical artery	arr16p11.2 (28, 807, 417-29, 051, 191)×1, 244 kb; ATXN2L, ATP2A1, CD19, NFATC2IP, SNPS1, SH2B1, RABEP2, LAT	arr16p11.2 (28, 810, 324-29, 051, 191)×1, 241 kb ATXN2L, ATP2A1, CD19, NFATC2IP, SNPS1, SH2B1, RABEP2, LAT	VOUS	16p11.2-p12.2 microdeletion syndrome	Anomalous twin with aortic coarctation was performed operation, no symptom
45*	Ventricular septal defect, hypoplasia of the nasal bone	Normal	arr2q13 (110, 504, 318-110, 980, 108)×3, 476 kb; RGPD5, LIMS3, MALL, NPHP1	arr2q13 (110, 496, 601-111, 400, 649)×3, 905 kb; RGPD5, LIMS3, MALL, LIMS3L, NPHP1, RGPD6	No	No	Vaginal delivery at 34 weeks, size of the VSD decrease gradually
49	Atrial septal defect	Meconium peritonitis, polyhydramnios	arr2q13 (110, 504, 318-111, 369, 264)×3, 865 kb; RGPD5, LIMS3, MALL, LIMS3L, NPHP1, RGPD6	arr2q13 (110, 504, 318-111, 369, 264)×3, 865 kb; RGPD5, LIMS3, MALL, LIMS3L, NPHP1, RGPD6	No	No	The fetus with atrial septal defect was undertaken expectant management at present and the co-twin was NND at about 10 days

VOUS: Variations of uncertain significance

considered that the neonatal death may be associated with CHD but not the microdeletion on chromosome 14q. In Case 45, one fetus with ventricular septal defect and hypoplasia of the nasal bone exhibited a 476 kb duplication on chromosome 2q13 and a 905 kb microduplication in the co-twin with heterogeneous echo pattern of the liver. Published articles reported that duplication involving 2q13 are associated with dysmorphic features, development delay and abnormal head size[10]. Duplications of this region on chromosome 2q13 are not reported in normal CNV databases[11]. At present, intelligence, motor development and cognitive performance are normal in these two infants. So we concluded that

microduplications on chromosome 2q13 are likely benign. The size of microduplications was different and so the findings of CNV in this case were identified as discrepant.

Furthermore, Case 49 was classified as with concordant benign CNV, which involved a 865 kb microduplication in chromosome 2q13 in the fetus with an atrial septal defect and a same microduplication in the co-twin with meconium peritonitis.

3 Discussions

3.1 The detection rate of pathological CNV in the fetuses with CHD

Monochorionic twins are at increased for CHD than dichorionic twins, with an incidence rate of 5%-7% in MCDA twins [12-13]. Almost 98% CHD are caused by the combination effect of environmental and genetic factors. Microduplications or microdeletions may be missed by standard G-band karyotype analysis on account of the limitation of resolution. Microarray analysis could perform whole-genome screening for chromosomal imbalances with a resolution of 50 kb. The reported incremental yield of submicroscopic rearrangements with pathological significance was varying from 4.4% to 12.8% for non-isolated CHD and isolated CHD combined [6-7, 14]. In our study, excluding cases with chromosomal aberrations, clinical significant CNV were detected in one fetus with CHD. In this study, minor cardiac malformations, such as persistent left superior vena cava, were included as CHD. In most published studies, minor cardiac malformations were not considered as CHD. The detection rate of clinical significant CNV was lower in the current study may be caused by the different selection criteria. The twin pairs with even minor different malformations were considered as discordant phenotype so we included minor cardiac defects as CHD.

3.2 The causes of discordant CHD in MCDA twins

Discordant CHD in MCDA twins may be caused by placental vascular anastomoses, post-zygotic genetic or post-zygotic epi-genetic factors. In our data, five cases were considered as heterokaryotypia. Heterokaryotypic twinning appears to be a rare event in MCDA twins. A likely hypothesis to explain the pathogenesis may be that of a post-zygotic event in the very early embryonic period [15] or as a post-zygotic non-disjunction event leading to chromosomal mosaicism. Post-zygotic non disjunction is a reasonable explanation for the twin pair with a 47, XXY karyotype in one fetus and a 45, X[7]/46, XY[43] karyotype in the other fetus, but the three cases with discordant 45, X/46, XX and one case with 45, X/46, XY twin may be interpreted as the combined effect of idiochromosome loss due to anaphase lag and the 'recognition and repulsion' of discordant blastomeres [16].

Breckpot et al [9] have supposed that differences in copy number variations between discordant monozygotic twins as a model for exploring chromosomal mosaicism in CHD. In this study, discordant or concordant CHD in MCDA twins were analyzed to evaluate the relationship between differences in CNV and discordant presence of CHD or severity of CHD and to identify post-zygotic CNV events in the occurrence of discordant MCDA twins. To best of our knowledge, this is the first study to report on the assessment of microarray analysis in the examination of discordant and concordant CHD in MCDA twins in prenatal cohort. In postnatal settings, CNV differences in discordant MZ twins have been analyzed in twins with discordant CHD [9], omphalocele [7], urorectal malformations [18], and congenital diaphragmatic hernia, esophageal atresia [19]. Except for congenital malformations, copy number variations distribution in twins discordant for schizophrenia [20], Parkinson disease [21] and attention problems [22] were also reported. The result of Breckpot et al [9] demonstrated that only one in six MZ twin pairs discordant for the presence of CHD showed copy number differences, demonstrating the incidence of somatic CNV events in MZ twins. However, they still recommended that copy number,

genome and epigenome analyses in a larger MZ twin cohort were required to explore the role of somatic CNV and mutations in the genesis of CHD. In contrary, Zhang et al[17] analyzed nine MZ twin pairs discordant for CHD, endocrine disorders, omphalocele, or congenital diaphragmatic hernia and suggested that somatic mutational events in coding regions do not seem to play a major role in the phenotypic expression of MZ discordant twin pair. The other two studies made a similar conclusion[19,23]. In our study, only two cases with discordant CHD and normal karyotype were evaluated as showing discrepancy on CNV but without clinical significance. For example, a 476 kb duplication on chromosome 2q13 in the fetus with ventricular septal defect and hypoplasia of the nasal bone and a 905 kb microduplication in the fetus with heterogeneous echo pattern of the liver were detected in case 45. At present, motor development and cognitive performance are normal in these two infants. So we concluded that microduplications on chromosome 2q13 are likely benign. Although the size of CNV was different, clinical significance was almost similar. In most cases, the results showed no prove for post-zygotic CNV events to MCDA twins discordance for CHD. In the other side, the twin pairs with identical CMA results were identified as with discordant CHD by using prenatal ultrasound screening.

Our study has, several limitations, one being that several defects, for example, skin leukoplakia, hypothyroidism or mental retardation could not be evaluated by using ultrasound examinations prenatally. In addition, karyotype or CNV information of the cases that rejected fetal karyotyping or CMA was not known. We are not sure that whether these cases would change our results or not.

Our study demonstrated that about 10% MCDA twins with discordant CHD might be associated with heterokaryotype and 5% might be associated with discrepant CNV. Post-zygotic CNV events in MCDA twins may be attributed to about 5% twin discordant for CHD in the investigated cases.

References

[1] Turpin R, Lejeune J, Lafoucade J, et al. Presumption of monozygotism in spite of a sexual dimorphism: XY male subject and haploid X neuter subject [J]. C R Hebd Seances Acad Sci, 1961, 252(8): 2945-2946.

[2] Wolff K, Scharer K, Lejeune J. Contribution to the study of monogoloid twins. A case of heterokaryotic monozygotism [J]. Helv Paediatr Acta, 1962, 17(8): 301-328.

[3] Sagoo GS, Butterworth AS, Sanderson S, et al. Array CGH in patients with learning disability (mental retardation) and congenital anomalies: Updated systematic review and meta-analysis of 19 studies and 13, 926 subjects [J]. Genet Med, 2009, 11(3): 139-146.

[4] Miller DT, Adam MP, Aradhya S, et al. Consensus statement: Chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies [J]. Am J Hum Genet, 2010, 86(14): 749-764.

[5] Schmid M, Stary S, Blaicher W, et al. Prenatal genetic diagnosis using microarray analysis in fetuses with congenital heart defects [J]. Prenat Diagn, 2012, 32(4): 376-382.

[6] Yan Y, Wu Q, Zhang L, et al. Detection of submicroscopic chromosomal aberrations by array-based comparative genomic hybridization in fetuses with congenital heart disease [J]. Ultrasound Obstet Gynecol, 2014, 43(4): 404-412.

[7] Liao C, Li R, Fu F, et al. Prenatal diagnosis of congenital heart defect by genome-wide high-resolution SNP array [J]. Prenat Diagn, 2014, 34(9): 858-863.

[8] Karatza AA, Wolfenden JL, Taylo RMJ, et al. Influence of twin-twin transfusion syndrome on fetal cardiovascular structure and function: Prospective case-control study of 136 monochorionic twin pregnancies [J]. Heart, 2002, 88(3): 271-277.

[9] Breckpot J, Thienpont B, Gewillig M, et al. Differences in copy number variation between discordant monozygotic twins as a model for exploring chromosomal mosaicism in congenital heart defects [J]. Mol Syndromol, 2012, 2(2): 81-87.

[10] Riley KN, Catalano LM, Bernao JA, et al. Recurrent deletions and duplications of chromosome 2q11.2 and 2q13 are associated with variable outcomes [J]. Am J Med Genet Part A, 2015, 167A(11): 2664-2673.

[11] Itsara A, Cooper GM, Baker C, et al. Population analysis of large copy number variants and hotspots of human genetic disease [J]. Am J Hum Genet, 2009, 84(2): 148-161.

[12] Bahhiyar MO, Dulay AT, Weeks BP, et al. Prevalence of congenital heart defects in monochorionic/diamniotic twin gestations: A systematic literature review [J]. J Ultrasound Med, 2007, 26(11): 1491-1498.

[13] Manning N, Archer N. A study to determine the incidence of structural congenital heart disease in monochorionic twins [J]. Prenat Diagn, 2006, 26(11): 1062-1068.

[14] Mademont-Soler I, Morales C, Soler A, et al. Prenatal diagnosis of chromosomal abnormalities in fetuses with abnormal cardiac ultrasound findings: Evaluation of chromosomal microarray-based analysis [J]. Ultrasound Obstet Gynecol, 2013, 41(4): 375-382.

[15] Cheng PJ, Shaw SW, Shih JC, et al. Monozygotic twins discordant for monosomy 21 detected by first trimester nuchal translucency screening [J]. Obstet Gynecol, 2006, 107(2): 538-541.

[16] Machin GA. Some causes of genotypic and phenotypic discordance in monozygotic twin pairs [J]. Am J Med Genet, 1996, 61(3): 216-228.

[17] Zhang R, Thiele H, Bartmann P, et al. Whole-exome sequencing in nine monozygotic discordant twins [J]. Twin Res Hum Genet, 2016, 19(1): 60-65.

[18] Shetty A, Smith APM. The sonographic diagnosis of chorionicity [J]. Prenat Diagn, 2005, 25(9): 735-739.

[19] Veenma D, Brosense DE, Jong E, et al. Copy number detection in discordant monozygotic twins of congenital diaphragmatic hernia (CDH) and esophagel atresia (EA) cohorts [J]. Eur J Hum Genet, 2012, 20(3): 298-304.

[20] Castellani CA, Awamleh Z, Melka MG, et al. Copy number variation distribution in six monozygotic twin pairs discordant for Schizophrenia [J]. Twin Res Hum Genet, 2014, 17(2): 108-120.

[21] Bruder CE, Piotrowski A, Gijsbers AA, et al. Phenotypically concordant and discordant monozygotic twins display different DNA copy-number variation profiles [J]. Am J Hum Genet, 2008, 82(3): 763-771.

[22] Ehli EA, Abdellaoui A, Hu Y, et al. De novo and inherited CNV in MZ twin pairs selected for discordance and concordance on attention problems [J]. Eur J Hum Genet, 2012, 20(10): 1037-1043.

[23] Baranzini SE, Mudge J, Van Velkinburgh JC, et al. Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis [J]. Nature, 2010, 464(7293): 1351-1356.

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