Analysis of chromosome 22q11 copy number variations by multiplex ligation-dependent probe amplification for prenatal diagnosis of congenital heart defect

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Abstract

Background: Congenital heart defects (CHD) represent one of the most common birth defects. This study aimed to evaluate the value of multiplex ligation-dependent probe amplification (MLPA) as a tool to detect the copy number variations (CNVs) of 22q11 in fetuses with CHD.

Results: A large cohort of 225 fetuses with CHD was screened by fetal echocardiography. Once common chromosome abnormalities in 30 fetuses were screened out by conventional G-banding analysis, the CNVs of chromosome 22q11 in the remaining 195 fetuses were determined by MLPA for prenatal genetic counseling. In 195 CHD fetuses with normal karyotype, 11 cases had pathological CNVs, including 22q11.2 deletion (seven cases), the deletion of 22q11 cat eye syndrome (CES) region (one case), 22q11.2 duplication (one case), 22q13.3 deletion (one case) and 17p13.3 deletion (one case). In total, our findings from MLPA screening represented 4.9% in our cohort. Among these, three cases were inherited CNVs, and eight cases were de novo. These CNVs were further verified by single nucleotide polymorphism (SNP)-array analysis, and their chromosomal location was refined.

Conclusion: This study indicated that MLPA could serve as an effective test for routine prenatal diagnosis of 22q11 in fetuses with CHD.

Keywords: Congenital heart defects, 22q11 deletion syndrome, Prenatal diagnosis, MLPA

Background

Congenital heart defects (CHD) usually refer to the abnormalities in the heart’s structure or function that arise before birth [1]. It represents the most frequent birth defects and the leading cause of death from a congenital structural abnormality worldwide, causing more than 220,000 deaths globally every year [2]. In China, epidemiological studies have suggested a noticeable increase in trend of CHD mortality with the overall mortality rate increasing from 141 in 2003 to 229 in 2010 per 10,000,000 person-years [3].

The pathogenesis of CHD is largely unknown; however, current studies have indicated a multiple interaction between genetic and environmental factors. Specifically, associations between CHD and chromosomal abnormalities have been well recognized, which accounts for about 16 ~ 56% of CHD [4]. Moreover, copy number variants (CNVs) have also been identified as a significant factor in CHD development and the most common example is the 22q11 deletion syndrome, which is estimated to affect approximately 1/4000 to 1/6000 in live births [5, 6]. In China, fetal echocardiography is performed after the second trimester ultrasound screening. It is well known that abnormal ultrasound finding is one of most common indications for amniocentesis or other invasive examination [7, 8]. Therefore, the amniocentesis or cordocentesis...
is suggested to diagnose chromosomal abnormalities and CNVs for fetuses with CHD.

Conventional fluorescence in situ hybridization (FISH) with commercial probes (TUPLE1 or N25) has been developed for the prenatal diagnosis of 22q11 chromosome deletion by many prenatal services. Mademont-soler et al. [9] compared FISH and MLPA techniques for detection of 22q11 chromosome deletion. The results showed that the use of MLPA had not increased the number of diagnosis of 22q11 deletion, and the author suggested that MLPA should not replace FISH as a conventional technology for prenatal diagnosis of 22q11 chromosome deletion. However, many reports have pointed out the advantage of MLPA in the postnatal study [10–12]. To date, there is little data about using MLPA in a large cohort prenatal study. In our report, we present a large cohort of 225 CHD fetuses to evaluate the application value of MLPA in prenatal detection of CNVs in 22q11.

Results

Conventional G-banding analysis detected all 225 fetuses, and then MLPA screening was performed in the remaining fetuses with normal karyotype. In total, chromosomal abnormalities represented 30 cases (13.3 %) in our cohort (Table 1), including 11 fetuses with trisomy 18, 14 fetuses with trisomy 21, one fetus with 45, XO, two fetuses with chromosomal polyploidy, one fetus with 46, XY, add(1) (p36) and one fetus with balanced translocation (46, XY, t(1:2) (p32;q35)) respectively. Of 30 cases with chromosomal abnormalities, 19 cases had isolated CHD and 11 cases had multiple congenital anomalies.

For remaining 195 CHD fetuses with normal karyotype, MLPA analysis revealed that 11 cases had CNVs (Table 1). All the 11 cases had isolated CHD, including ten conotruncal defects and one septal defect (Table 1). Fetuses 1–6 showed typical 22q11.2 deletions located from LCR-A to LCR-D regions, fetus seven showed 22q11.2 deletions located from LCR-A to LCR-B regions, and fetus eight showed the deletion based on the 22q11 cat eye syndrome region while Fetus 9–11 showed 22q11.2 duplication, 22q13.3 deletion, and 17p13.3 deletion, respectively (Fig. 1) (Table 2). Succeedent SNP-array analysis verified all of the positive results from MLPA and the concordance rate is 100 % (Table 2). For fetus 8, SNP-array analysis revealed a 980 kb heterozygous deletion mapping to position 17,067,005-18,047,231 on chromosome 22q11 (Fig. 2). Further study indicated that each CNV of fetuses 7–9 were inherited from one’s asymptomatic mother or father, while the others were de novo.

Discussion

In this study, a large cohort of 225 fetuses with CHD was detected by traditional karyotyping and MLPA to identify the chromosome abnormality and CNVs on chromosome 22q11. The results demonstrated 30 fetuses (13.3 %) had a chromosomal abnormality, and 11 fetuses (4.9 %) had CNVs. All positive findings from MLPA were in agreement with those from SNP-array.

Our study used the MLPA P250 DiGeorge kit to identify the CNVs on different chromosomes and found that 3.1 % of CHD cases (7/225) had deletions on 22q11 (Table 1). Several studies have also reported the frequency of 22q11 CNVs in CHD fetuses and the data ranged from 1.6 ~ 11.5 % [4, 13]. Our data was similar to that reported by Moore et al. [14] (17/540, 3.1 %). Of the seven cases with 22q11 deletions, six cases had conotruncal defects. Our results showed high detection rate of 22q11 CNVs in cases with a conotruncal defect (7.1 %, 6/85), which was close to the findings by Galindo et al. [15] (8.7 %) and Breitelle et al. [16] (4.7 %). All these results indicated that the conotruncal defect was mostly associated with 22q11 CNVs [15].

In addition to 22q11, MLPA P250 DiGeorge kit also contains the probes targeting other regions including 22q13, 10p14, 8p23, 9q34, 17p13.3 and 4q34. Thus, we also found the deletions in both 22q13.3 and 17p13.3 (Table 2). The 22q13.3 deletion syndrome was also defined as a Phelan-McDermid syndrome and mainly manifested as global developmental delay, hypotonia, delayed or absent speech, and autistic behavior [17, 18]. Only one study [19] reported one CHD fetus with 22q13.3

| Types of CHD          | Number of fetuses | Number of fetuses with aneuploidy | Number of fetuses with CNVs | Total |
|-----------------------|-------------------|-----------------------------------|-----------------------------|-------|
| Conotruncal defect    | 85                | 8                                 | 6                           | 1     | 1   | 1   | 18   |
| Septal defect         | 104               | 17                                | 1                           | 0     | 0   | 0   | 18   |
| Left-heart defect     | 7                 | 0                                 | 0                           | 0     | 0   | 0   | 0    |
| Right-heart defect    | 1                 | 0                                 | 0                           | 0     | 0   | 0   | 0    |
| Other heart defect    | 28                | 5                                 | 0                           | 0     | 0   | 0   | 5    |
| Total                 | 225               | 30 (13.3 %)                       | 7 (3.1 %)                   | 1 (0.4 %) | 1 (0.4 %) | 1 (0.4 %) | 41 (17.8 %) |

CHD congenital heart defects, CNVs copy number variants, CES cat eye syndrome
deletion by prenatal diagnosis, supporting the findings of our study. The 17p13.3 deletion syndrome is also known as Miller-Dieker Lissencephaly Syndrome. The syndrome is characterized by nervous system anomalies, facial abnormalities, IUGR, mental retardation and other malformations including cardiac defects. To date, at least 29 prenatal cases with the 17p13.3 deletion syndrome have been reported. Among them, only four cases presented with CHD [20]. In our study, the case 11 with 17p13.3 deletion showed cardiac defects including DORV, VSD and PA. These results suggested that MLPA technology could comprehensively and rapidly detect pathogenic CNVs in several different chromosomes regions.

Previous studies found that about 6 ~ 28 % of prenatal 22q11 deletions were inherited from one parent [21]. Two cases with pathological CNVs were inherited from one parent in our study (Table 1). The SNP-array analysis indicated that the position of the fetus was identical with that of the parent. One case in this study had 22q11 duplication inherited from his father (Table 2). Though 22q11 CNVs were present in the three parents, they did not display any mental disorder based on physical examination. Furthermore, their internal organs especially heart revealed no abnormalities by sonographic examination. Several reasons may explain the difference in phenotypes with the same genetic changes, such as allelic variation at the haploid locus, self-repair and environmental effects [22, 23]. Since healthy carriers of chromosomal deletions or duplications have 50 % chance to pass on to the next generation in each pregnancy, two couples who had the fetus with pathogenic CNVs in our study may have a high risk in their next pregnancy. Among the three families, the mother of case seven had a normal child with no CNVs, the mother of case nine had a miscarriage and the mother of case eight had not been pregnant again.

The proximal portion of chromosome 22q was a hot region for chromosomal rearrangement. Cat eye syndrome (CES) is a rare chromosome disorder in human caused by the deletion of chromosome 22q11. The CES critical region covered approximately 2 Mb from the centromere to the locus D22S57 [24], but the deletion of this region is rarely reported. According to DECIPHER database (https://decipher.sanger.ac.uk), only eight cases were detected to carry genomic deletion encompassing the region, and none of them displayed CHD. Kriek et al. [25] firstly reported that a fetus, carrying different rearrangements on chromosome 22q, including the deletion of CES critical region, had the manifestation of developmental delay but no history of cardiac problems. Kriek et al. also suggested that the deletion of CES critical region had little clinical relevance because the normal familial members were carrying this deletion. In our study, we firstly report a fetus with CHD showing a deletion of 980 K spanning the CES region from genomic position 17,067,005 to 18,047,231 (Fig. 2). This deletion region contained 14 genes, including six OMIM genes (CECR7, CECR2, CECR1, IL17RA, XKR3, and SLC25A18). Xie et al. [26] identified that

### Table 2: MLPA and SNP-array results of 11 fetuses with CHD

| Case | Age | Weeks of gestation | Cardiac ultrasound findings | MLPA results | SNP-array results | Type of mutation |
|------|-----|-------------------|---------------------------|--------------|------------------|-----------------|
| 1    | 29  | 25                | IAA, VSD                  | 22q11.2      | Del. CLTCL1 ~ LZTR1 | 18877787 ~ 21798907 | 2.92 M de novo |
| 2    | 24  | 24                | TGA, VSD                  | 22q11.2      | Del. CLTCL1 ~ LZTR1 | 18877787 ~ 21462353 | 2.58 M de novo |
| 3    | 37  | 25                | TA, VSD                   | 22q11.2      | Del. CLTCL1 ~ LZTR1 | 18877787 ~ 21462353 | 2.58 M de novo |
| 4    | 23  | 27                | TA, VSD                   | 22q11.2      | Del. CLTCL1 ~ LZTR1 | 18895227 ~ 21462353 | 2.56 M de novo |
| 5    | 29  | 24                | TOF                       | 22q11.2      | Del. CLTCL1 ~ LZTR1 | 18877787 ~ 21462353 | 2.58 M de novo |
| 6    | 29  | 23                | VSD                       | 22q11.2      | Del. CLTCL1 ~ LZTR1 | 18895227 ~ 21462353 | 2.56 M de novo |
| 7    | 28  | 23                | TOF, PA                   | 22q11.2      | Del. CLTCL1 ~ LZTR1 | 18895227 ~ 20306993 | 1.4 M inherited |
| 8    | 26  | 23                | DORV, VSD, PA             | 22q11.12q11.2 | Del IL17RA, SLC25A18 | 17067005 ~ 18047231 | 980 k inherited |
| 9    | 31  | 24                | AH, VSD                   | 22q11.2      | Dup. CLTCL1 ~ LZTR1 | 18623108 ~ 21462353 | 2.8 M inherited |
| 10   | 29  | 24                | IAA, VSD, ASD             | 22q13.3      | Del. ARSA, SHANK3 | 49045728 ~ 51169045 | 2.1 M de novo |
| 11   | 26  | 22                | DORV, VSD, PA             | 17p13.3      | Del. RHR3, GEMIN4, YWHAE | 18901 ~ 2633324 | 2.61 M de novo |

VSD ventricular septal defect, IAA interrupted aortic arch, TGA transposition of conducting arteries, TA truncus arteriosus, TOF trilogy of fallot, PA pulmonary atresia, AH aortic hypoplasia, ASD atrial septum defect, DORV double outlet right ventricle
IL17RA was related to myocardial disease while there were no other genes having been reported to be related to cardiac disease. Besides the duplication of CES region can cause the manifestation of CHD, our finding firstly indicated a possible relationship between CHD and the deletion of CES region. Further collecting of more cases is still needed to confirm if the deletion of CES region may cause CHD.

**Conclusion**

In summary, this study confirmed that MLPA could rapidly and efficiently detect pathogenic CNVs associated with CHD in our cohort. Thus, it is an economical, fast and accurate method for the prenatal genetic diagnosis of CHD for clinical application.

**Methods**

**Case recruitment**

The study was performed at the Department of Prenatal Diagnosis in Nanjing Maternity and Child Health Care Hospital (Jiangsu, China) between 2011 and 2014. In our hospital, the second trimester ultrasound screening was carried out in all pregnancies. After structural heart defects were found, prenatal echocardiography was offered...
for detail diagnosis. In total, 225 fetuses presenting with CHD were enrolled in the study. The distribution of different clinical manifestations was shown in Table 1. The CHD cases included in the study were: conotruncal defects (85/225), septal defects (104/225), left-heart defects (7/225), right-heart defect (1/225), and other heart defects (28/225). In all 225 fetuses, 211 cases were with isolated CHD, and the other 14 cases were with extra cardiac anomalies. G-banding analysis was first carried out in all 225 fetuses. Once chromosome abnormalities had been excluded in a fetus with a CHD, the CNV of chromosome 22q11 were investigated by MLPA. The mean age of these pregnancies was 29 ± 4.33 years old and the average gestation age at invasive prenatal diagnosis was 24 ± 2.56 weeks. All the pregnant women signed the informed consent form. This study was approved by the medicine ethics committee of Nanjing Maternity and Child Health Care Hospital.

Cytogenetic analysis
All samples of amniotic fluid and fetal cord blood were detected using G-banding according to the standard procedure described previously [27].

Multiplex ligation-dependent probe amplification
Fetal DNA was extracted from uncultured amniotic fluid or fetal blood cells according to the illustrations of the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). The SALSA MLPA P250 kit was performed to detect deletion or duplication of the 22q11 chromosomal region. This kit includes 24 probes targeting DiGeorge syndrome region, five probes targeting cat eye syndrome region, and 19 probes for DiGeorge anomaly related chromosomal regions such as 22q13, 10p14, 8p23, 9q34, 17p13.3, and 4q34.

About 100 ~ 150 ng DNA of each sample was involved in the experiment. MLPA analysis was performed following the manufacturer’s instructions. The MLPA products were examined by ABI 3130 genetic analyzer (Thermo Fisher, USA) and quantitative data were analyzed using the software of Coffalyser V8.0 (http://www.mlpa.com/coffalyser). The 30 % increase or decrease of the relative peak area of the probe showed the duplication or deletion of the targeted region, respectively.

SNP-array analysis
Fetus DNA was examined by Human cyto12 SNP-array scanning (Illumina, USA), which comprised about 300,000 SNPs across the whole genome. SNP-array experiments were carried out as previously described [28] and molecular karyotype analysis was performed by KaryoStudio Software V 1.3.11 (Illumina, USA).

Consent
Written informed consent was obtained from the pregnant women for publication of this paper. This research was approved by the ethics committee of Nanjing Maternity and Child Health Care Hospital.

Abbreviations
CHD: congenital heart defects; CNV: copy number variation; MLPA: multiplex ligation-dependent probe amplification; CES: cat eye syndrome; SNP: single nucleotide polymorphism; FISH: fluorescence in situ hybridization; VSD: ventricular septal defect; IAA: interrupted aortic arch; TGA: transposition of conducting arteries; TA: truncus arteriosus; TOF: trilogy of fallot; PA: pulmonary atresia; AH: aortic hypoplasia; ASD: atrial septum defect; DORV: double outlet right ventricle.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
JJZ, DYM, PH, ZFX designed the study and gave the final approval of the manuscript. JJZ and DYM interpreted the data of MLPA and drafted the paper. YW, FCQ and CYL performed SNP-array. AL, LL, YL and GL were responsible for the conventional cytogenetic analysis. LC and YW performed fetal echocardiography. All of the authors read and approved the final manuscript.

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