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Original Article

Incidences of micro-deletion/duplication 22q11.2 detected by multiplex ligation-dependent probe amplification in patients with congenital cardiac disease who are scheduled for cardiac surgery

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Abstract *Background:* 22q11.2 microdeletion is the most common microdeletion in the global population. Congenital cardiac disease is the most frequently observed feature of this syndrome. The prognosis of patients with 22q11.2 copy number aberrations varies from those without 22q11.2 deletion or duplication. *Methods:* We enrolled 241 patients from Nanjing Drum Tower Hospital and Nanjing Sick Children's Hospital, 227 being scheduled for cardiac surgery, and 14 cases being fetuses aged from 24 to 36 gestational weeks. We performed karyotypic analysis and multiplex ligation-dependent probe amplification in all cases. *Results:* Karyotypic analysis demonstrated 3 cases with trisomy 21, and 1 case with mosaic trisomy 8 [47,XY,+8/46,XY(1:2)]. Multiplex ligation-dependent probe amplification analysis revealed 10 cases (4.15%) with changes in the number of copies within the region of 22q11.2, of which 7 cases were hemizygous interstitial microdeletion from CLTCL1 to LZTR1, 1 case with deletion of the region from CLTCL1 to PCQAP, and 2 cases with 22q11.2 duplication, one of which spanned from ZNF74 to LZTR1, and simultaneously showed trisomy 21 by karyotyping analysis, and the other spanned from HIC2 to TOP3B. The phenotypes of the cardiac lesions included 3 cases of ventricular septal defect, 3 of tetralogy of Fallot, 2 of combined ventricular and atrial septal defects, and 2 with pulmonary arterial stenosis. *Conclusions:* Patients with congenitally malformed hearts who are scheduled for cardiac surgery, as well as fetuses with congenital cardiac disease, should routinely undergo karyotypic analysis and examination for 22q11.2 aberrations. Multiplex ligation-dependent probe amplification has been proven to be a cost-effective diagnostic technique for 22q11 deletion syndrome.

Keywords: 22q11 deletion syndrome; DiGeorge syndrome; velocardiofacial syndrome; cat-eye syndrome

The syndrome of 22q11.2 microdeletion is the most common involving microdeletion in the global population, with an incidence of around 1 per 4000,[1] usually found as new deletions. Although the spectrum of the ensuring clinical phenotype is extensive, and includes cleft palate, hypocalcaemia, thymic or immune deficiencies, developmental delay, difficulties in feeding, ocular abnormalities, and psychiatric illness,[2] congenital cardiac disease is the most frequently observed clinical manifestation, occurring in three-quarters of patients.[3]

In a recent study, such children were shown to be at high risk for mortality and morbidity.[4]

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The prognoses are rather different for those with congenitally malformed hearts in the absence of this kind of genetic abnormalities.[5] Accurate preoperative or prenatal genetic diagnosis, therefore, should prove helpful in health care and for genetic counseling of the patients and their families.

The currently available method for detection of the aberrations is fluorescence in situ hybridization, but this method is labour intensive and quite expensive, and may be unreliable in detecting some atypical deletions.[6] Recent research has indicated that multiplex ligation-dependent probe amplification is a more cost-effective and accurate diagnostic tool,[7] and also identifies cases not detected by standard fluorescence in situ hybridization.[6] In our study, therefore, we used a new multiplex ligation-dependent probe amplification probe set to detect the deletions and duplications of 22q11.2 in patients scheduled for congenital cardiac surgery, as well as in fetuses with congenital cardiac malformations diagnosed prenatally by echocardiography.

Materials and methods

Patients

From November, 2006, through April, 2008, we enrolled patients for study from Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, and Nanjing Children's Hospital, a member hospital of Nanjing Drum Tower Hosptial Group. Appropriate approval was obtained for this study from the Committee for Ethical Clinical Research of both hospitals. Detailed data regarding the cardiac malformations was achieved primarily by echocardiography. Having obtained consent for genetic examination, we systematically collected 227 samples of peripheral blood from the patients scheduled for the cardiac surgery. Of these samples, 108 samples were from males and 119 from females, with 9 patients being neonates, 145 aged under 14 years, 5 aged 14 to 18 years, and 68 were adults, aged from 18 to 63 years. In the other 14 fetal cases with congenitally malformed hearts, we obtained samples of umbilical cord blood. Amongst all 241 cases, we found 100 cases of ventricular septal defect, 36 cases of atrial septal defect, 36 cases of tetralogy of Fallot; 10 cases of atrial septal defect combined with ventricular septal defect, 6 cases of pulmonary arterial stenosis, 3 cases of ventricular septal defect combined with pulmonary arterial stenosis, 5 cases of atrial septal defect combined with pulmonary arterial stenosis, 5 cases of patency of the arterial duct, 6 cases of ventricular septal defect combined with patency of the arterial duct, and 34 instances of other types of defect (see Fig. 1)

Cases with 22q11.2 deletion

In order to have a preview of the multiplex ligation-dependent probe amplification probe set, we used 4 cases previously diagnosed by short tandem repeat analysis and fluorescence in situ hybridization. They included one case of no deletion, two cases with a typical 3 Megabase deletion, and one case with 1.5 Megabase deletion.[8]

Cell culture and karyotyping

Karyotypic analysis was performed on all patients, including the fetuses. We cultured the lymphocytes from peripheral blood or fetal umbilical cord blood. The traditional G-banding analyses, at about 400 bands level were completed.

DNA extraction

DNA was extracted from nucleolated cells of the blood following the standard phenolchloroform DNA extraction method.[9] The DNA samples were diluted using Tris-EDTA (Ethylenediamine Tetraacetic Acid) buffer. All samples were quantified by ultraviolet Spectrophotometer (Eppendorf).

Multiplex ligation-dependent probe amplification analysis

The SALSA MLPA kit P250 DiGeorge (Lot 0907) (MRC-Holland, Amsterdam) was used in this study to detect the variation of copy number in 22q11.2 region. This kit contains thirty probes on 22q11.2, including fourteen probes for commonly deleted DiGeorge region, five probes in Cat Eye Syndrome Region, eleven probes from LCR22-D to LCR22-G. The remaining 18 probes are located on 22q13, two probes, chromosome 10, 8, 4, 9 and 17 as controls. The experiments were performed following the instructions of the manufacturer, instructions and all runs included a normal control DNA for calibrating. The polymerase chain reaction products were loaded on an ABI 3130 Genetic Analyzer (Applied Biosystems) for collection of data. For sizing and peak area obtaining, GeneMapper software v3.7 was applied. The texts of data were exported and transferred to Coffalyser v7 (MRC-Holland) for analysis.

Results

Karyotypc analysis

We identified 4 aneuploidies, 3 instances of trisomy 21, and a mosaic trisomy 8 [47,XY,+8/46,XY(1:2)]. No other obvious structural abnormalities were found on the metaphase spreads.

Multiplex ligation-dependent probe amplification

In the 4 samples already having definite genetic diagnoses, we confirmed the findings by multiplex

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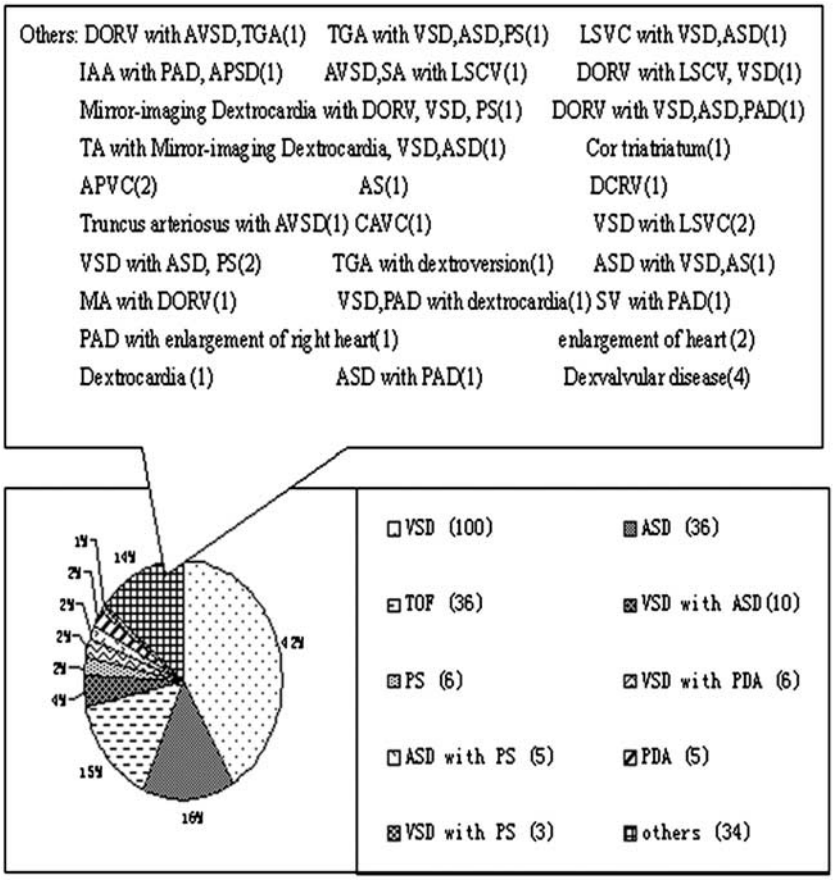

Figure 1.
The spectrum of phenotypes of cardiac anomalies in our patients with congenitally malformed hearts. Abbreviations: DORV: double outlet right ventricle; AVSD: atrioventricular septal defect; TGA: concordant atrioventricular and discordant ventriculo-arterial connections; LSCV: left superior caval vein; VSD: ventricular septal defect; ASD: atrial septal defect; IAA: interruption of aortic arch; SA: single atrium; TA: tricuspid atresia; APVC: anomalously pulmonary venous connection; AS: aortic stenosis; DCRV: double chambered right ventricle; SV: functionally single ventricle; MA: mitral atresia; PAD: patency of arterial duct; TOF: tetralogy of Fallot.

ligation-dependent probe amplification. In 2 cases with typical "3 Megabase" deletion previously detected by short tandem repeat and fluorescence in situ hybridization, we found a homozygous value less than 0.7 from CLTCL1 to LZTR1 (LCR22-A to LCR22-D), about 2.0–2.5 Megabase deletions. The case with a 1.5 Megabase deletion gave a hemizygous result for the region from CLTCL1 to DGCR8, about 0.9–1.5 Megabase in size. The normal case showed ratios ranging from 0.78 to 1.13 (see Fig. 2).

In the 241 cases, 7 cases were hemizygous from CLTCL1 to LZTR1. In 1 case, we found a different deletion region, from CLTCL1 to PCQAP, equal to LCR22-A to LCR22-C, about 1.6–1.9 Megabase in size. In these 8 cases, the cardiac phenotypes included 3 instances of ventricular septal defect, 2 of tetralogy of Fallot, 2 of combined atrial and ventricular septal defects, and 1 case of pulmonary arterial stenosis. We also found duplications in 2 cases. One spanned from ZNF74 to LZTR1 (LCT22-B to LCR22-D). This case also was a patient with trisomy 21, whose cardiac defect was pulmonary arterial stenosis. The other duplication spanned from HIC2 to TOP3B (LCR22-D to LCR22-E), and the patient exhibited tetralogy of Fallot (Table 1). Other probes on the referred chromosomes, such as chromosomes 4, 8, 9, 10, 17, did not reveal obvious changes in the number of copies.

Discussion

Though introduced only 5 years ago, multiplex ligation-dependent probe amplification has already proven an effective method for the detection of fragment deletions or duplications.[10] When studying 22q11.2 deletion, the SALSA MLPA kit P023B DiGeroge Syndrome/VCFS is more commonly used.[7,11] Recently, a high density multiplex ligation-dependent probe amplification probe set has specially been designed for detailed investigation on the deletion and duplication in 22q11.[6] The set used in our study also was of high density in the

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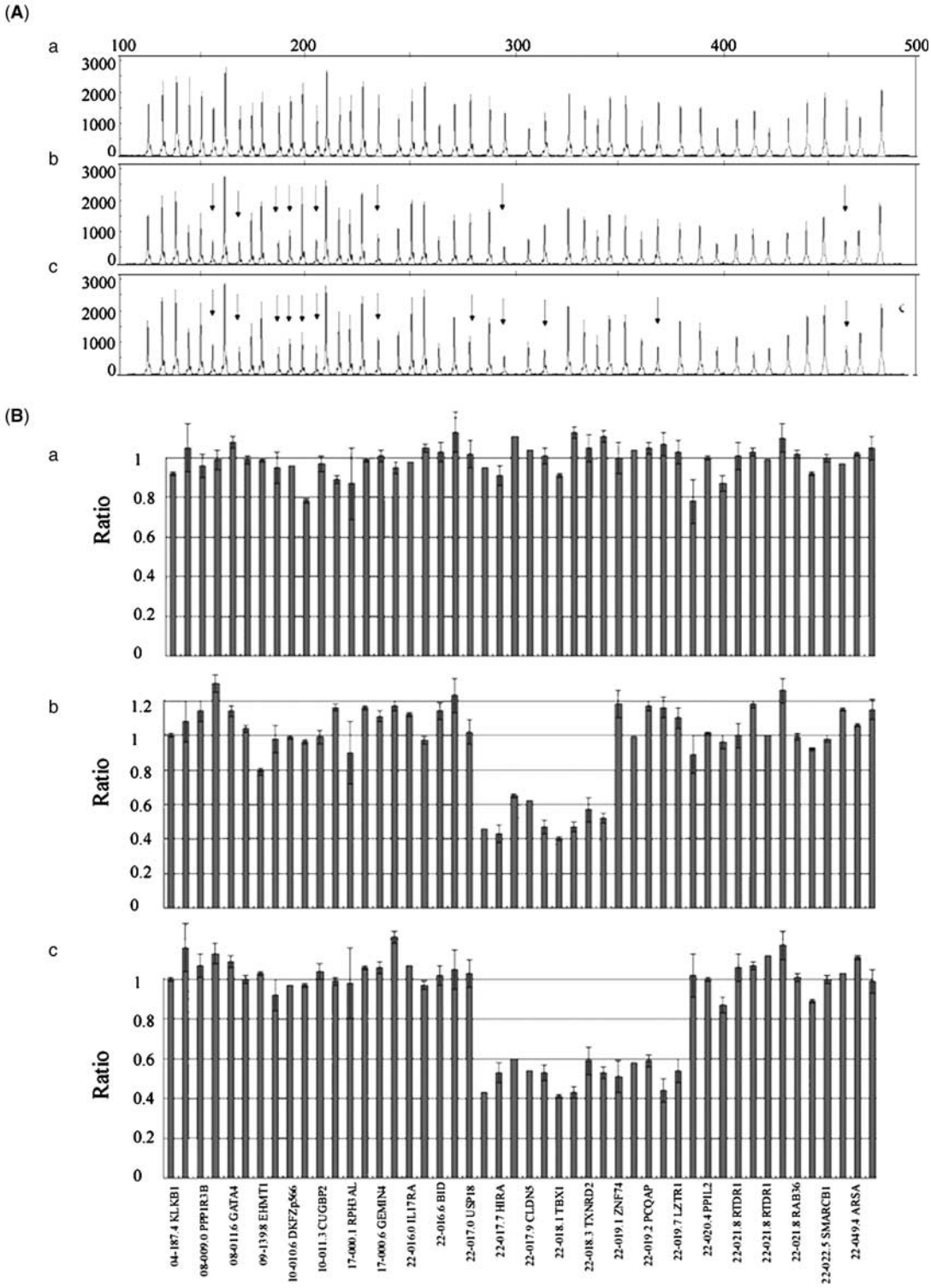

Figure 2.

*Panel **A** shows multiplex ligation-dependent probe amplification electropherograms of three samples: two patients and a control, previously diagnosed by fluorescence in situ hybridization and short tandem repeat polymerase chain reaction. Amplified probes were detected as fluorescent signals, the area of each peak were calculated by Coffalyser v7 software. **A**-a: control sample. b: sample with a 1.5 megabases deletion region of 22q11.2. c: sample with a 3 megabases deletion region of 22q11.2. The arrows point out the decreased signal intensity of the peaks, compared with the control sample. Panel **B** shows the ratio charts of the samples above. X-axis was marked by the names of ratio bars discontinuously. The Y-axis indicates the ratio. a, b, c are corresponding to the a, b, c of **A** respectively.*

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Table 1. The detailed information of the 10 patients with copy number aberrations within 22q11.2.

No.	Size of deletion or duplication	Age	Cardiac defects	Karyotype
107	Del: CLTCL1-LZTR1	22 years	Pulmonary arterial stenosis	46, XX
er13		3 month	Ventricular septal defect	46, XY
er65		6 years	Ventricular septal defect	46, XY
er96		6 years	Ventricular septal defect	46, XY
er103		1 month	Ventricular septal defect, atrial septal defect	46, XY
E2		1 month	Tetralogy of Fallot	46, XY
R0107		24 gestational weeks	Tetralogy of Fallot	46, XX
er51	Del: CLTCL1-PCQAP	5 years	Ventricular septal defect, atrial septal defect	46, XX
er60	Dup: ZNF74-LZTR1	2 years	Pulmonary arterial stenosis	47,XY,+21
56	Dup: HIC2-TOP3B	38 years	Tetralogy of Fallot	46, XY

Abbreviations: Del: deletion; Dup: duplication.

22q11.2 region. There were 21 probes located in the same gene, but with different sequences than the corresponding probes used previously.[6] Specifically, 2 probes within LCR22-A, and 1 within LCR22-B, are different, but in the same location. The probes we used to cover the LCR22-E zone were also greater than those used previously.[6]

Compared with our previous experience in detecting 22q11.2 deletion by short tandem repeat polymerase chain reaction,[8] fluorescence in situ hybridization and real-time polymerase chain reaction,[12] we found that use of multiplex ligation-dependent probe amplification offers numerous advantages. The procedure is as rapid and convenient as short tandem repeat polymerase chain reaction and real-time polymerase chain reaction, and the results can be achieved in 2 days. It is more practical than short tandem repeat polymerase chain reaction, since the absence of parental specimens is not a barrier for analysis. The probes on 22q11.2 are much denser than used in real-time polymerase chain reaction. As a result, multiplex ligation-dependent probe amplification can give more reproducible results, more detailed information about the size of the deletion or duplication, and can even detect atypical deletions. Fluorescence in situ hybridization is considered as a standard diagnostic method, but the commercial probes, being located at the proximal end of the typically deleted region, fail to detect some atypical deletions.

We detected 10 cases of micro-deletion and micro-duplication of 22q11.2 using the set. Of the 8 cases with 22q11 deletion, 7 are hemizygous from CLTCL1 to LZTR1. They had the same deletion region as the previously confirmed 3 megabase deletion sample. As we calculated the detailed base pairs throughout this region using our multiplex approach, we found the deletion to be about 2 megabases, and at most 2.5 megabases. This was unexpected. Previous investigators[7] identified the size of the region from HIRA to LZTR1 as 3 megabases using the SALSA MLPA kit P023B DiGeroge Syndrome/VCFS, which is a sparse probe set in 22q11.2. Others[11] identified a specified deletion region for all patients, albeit a little shorter. They described a "common 3 megabase hemizygous deletion". Since in these early periods, the only way to identify the break point of micro-deletion was fluorescence in situ hybridization, a more cursory probe, the initial definition of the commonly deleted region at 3 megabases should be considered approximate. Our study, and the other recent investigation,[6] have shown the deleted region to be less than 2.5 megabases, rather than 3 megabases.

We detected deletions or duplication in almost one-twentieth of our patients. This is higher than the rate previously detected in non-selected patients with congenital cardiac disease.[13] In our patients, we encountered ventricular septal defect, the lesion in combination with a defect in the oval fossa, tetralogy of Fallot, and pulmonary arterial stenosis. This spectrum is much different from that seen previously.[13] Our patients, however, reflect those already selected as requiring cardiac surgery, hence the propensity of septal defects. The more usually reported lesions in the setting of 22q.11 deletion, such as interruption of the aortic arch, or common arterial trunk, were rare in our series. The differences noted, therefore, probably reflect the bias introduced by the populations studied.

We also detected one uncommon region for deletion, and 2 cases of duplication. The deleted region was from CLTCL1 to PCQAP, while the duplicated regions mapped ZNF74 to LZTR1 and HIC2 to TOP3B respectively. Although not reported previously, the break points are close to the boundaries of known deletions or duplications. It is not unexpected, therefore, to find such variations. As HIC2 to TOP3B is downstream to the critical region of 1.5 deleted megabases, those seeking candidate genes for congenital cardiac disease should not focus exclusively on the 1.5 megabase region.

According to our study, the genetic anomalies are detected at a rate of about one in 20. These genetic

abnormalities can cause more malformations and functional disabilities than congenital cardiac disease presenting in isolation. Investigation of these genetic problems prior to surgery may, therefore, prove helpful in improving the diagnosis and prognostication. Genetic information will be necessary for planning care, as well as choosing suitable strategies for management.[5] Owing to the techniques for detection of micro-deletions being available for only a few years, the 22q11.2 deletion syndrome is not well known to many of those working in clinical practice. For example, in our study, the structural anomalies in one patient were recognized only after she was diagnosed by multiplex ligation-dependent probe amplification as having 22q11.2 deletion. This patient, aged 22 years, has an abnormally long face and unusually small ears. An absence of posterior nasal septum was detected by electronic nasoscope, and the left side of the face and the nasal pinna were larger than the right side. The patient also possesses a high arched palate, and she reports that one of her 2 brothers was born with cleft palate. These phenotypes were not noted in our first evaluation, which strongly suggests that patients with congenitally malformed hearts should undergo genetic screening during pre-operative investigations. In addition, prenatal genetic molecular diagnosis in those with recognized cardiac defects could also provide additional information, which might assist parents in choosing either to continue or terminate the pregnancy.

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References

1. Scambler PJ. The 22q11 deletion syndromes. Hum Mol Genet 2000; 9: 2421–2426.
2. Hay BN. Deletion 22q11: Spectrum of associated disorders. Semin Pediatr Neurol 2007; 14: 136–139.
3. McDonald-Mcginn DM, Kirschner R, Goldmuntz E, et al. The Philadelphia story: the 22q11.2 deletion: report on 250 patients. Genet Couns 1999; 10: 11–24.
4. Kyburz A, Bauersfild U, Schinzel A, et al. The fate of children with microdeletion 22q11.2 syndrome and congenital heart defect: clinical course and cardiac outcome. Pediatr Cardiol 2008; 29: 76–83.
5. Weismann CG, Gelb BD. The genetics of congenital heart disease: a review of recent developments. Curr Opin Cardiol 2007; 22: 200–206.
6. Jalali GR, Vorstman JAS, Errami A, et al. Detailed analysis of 22q11.2 with a high density MLPA probe set. Hum Mutat 2008; 29: 433–440.
7. Fernández L, Lapunzina P, Arjona D, et al. Comparative study of three diagnostic approaches (FISH, STRs and MLPA) in 30 patients with 22q11.2 deletion syndrome. Clin Genet 2005; 68: 373–378.
8. Yi L, Xu ZF, Mo XM, et al. New tetranucleotide STRP markers for detecting the 22q11.2 deletion. Mol Cell Prob 2006; 20: 359–365.
9. Hu YL, Zheng MM, Xu ZF, Wang XR, Cui HM. Quantitative real-time PCR technique for rapid prenatal diagnosis of Down syndrome. Prenat Diagn 2004; 24: 704–707.
10. Janssen B, Hartmann C, Scholz V, Jauch A, Zschocke J. MLPA analysis for the detection of deletions, duplications, and complex rearrangements in the dystrophin geen: potential and pitfalls. Neruogenenetics 2005; 6: 29–35.
11. Stachon AC, Baskin B, Smith AC, et al. Molecular diagnosis of 22q11.2 deletion and duplication by multiplex ligation dependent probe amplification. Am J Med Genet 2007; 143A: 2924–2930.
12. Zhang XQ, Xu ZF, Yi L, et al. Rapid detection of 22q11 microdeletion with quantitative real-time polymerase chain reaction. J Appl Clin Pediatr 2006; 21: 1409–1410.
13. Botto LD, May K, Fernhoff PM, et al. A population-based study of the 22q11.2 deletion: phenotype, incidence, and contribution to major birth defects in the population. Pediatrics 2003; 112: 101–107.