Short report

22q11.2 microduplication in a family with recurrent fetal congenital heart disease

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1. Introduction

It is well reported that region-specific low-copy repeats (LCRs) within the 22q11.2 region predispose to genomic disorders including DiGeorge/velocardiofacial syndrome (DGS/VCFS), cat-eye syndrome, Emanuel syndrome, and the 22q11.2 microduplication syndrome. 22q11.2 microduplication syndrome, which is characterized by an extra copy of 22q11.2 region with a length ranging from 1 to 6 Mb, was first reported by Edelmann et al.\textsuperscript{[1]}. 22q11.2 microduplication has been reported in many individuals.\textsuperscript{[2,3]} Reported clinical features of 22q11 microduplication syndrome include cardiovascular anomalies, velopharyngeal insufficiency with or without cleft palate, hearing loss, growth and development delay, learning disabilities, speech delay, behavioral problems and various dysmorphic features, some of which are overlapping with features of DGS/VCFS. Highly diverse inter- and intrafamilial outcomes were observed among patients harboring 22q11.2 microduplication, from almost normal to severely affected. This variance may be one of the reasons that 22q11.2 microduplication syndrome is a less frequently diagnosed than its corresponding microdeletion syndrome\textsuperscript{[2,4].}

Keywords: 22q11.2 microduplication
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People carrying a 22q11.2 microduplication display a phenotype varying from normal to severely affected. We report a phenotypically normal female presented with a fetus having a severe congenital heart defect with ventricular septal defect, tricuspid atresia, patent ductus arterial and interrupted aortic arch. The pregnant woman had a history of overall three consecutive aberrant pregnancies with tetralogy of Fallot. Standard G-banding karyotype analysis of the parents and the actual pregnancy were normal, while array comparative genomic hybridization (arrayCGH) analysis revealed a 22q11.2 microduplication within the fetus’ genome. Fluorescence in situ hybridization (FISH) and short tandem repeat polymorphism (STRP) tests indicated the affected fetus inherited the interstitial 22q11.2 microduplication from the mother. High-resolution oligonucleotide microarray analysis showed this microduplication is located in the common 3 Mb 22q11.2 deletion region between positions 17.298 Mb and 20.246 Mb with a length of 2.948 Mb. This report demonstrates the remarkable intrafamilial variability of a 22q11.2 microduplication phenotype. The 22q11.2 microduplication carried by one of the healthy parents has most likely contributed to the recurrent fetal heart defects.

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2. Case report

A 30-year-old pregnant mother (G3P0) had had in her previous pregnancies two female fetuses with tetralogy of Fallot (TOF). Ultrasound examination at 25th gestational week revealed that in the actual pregnancy this fetus also had severe heart defect including ventricular septal defect (VSD), tricuspid atresia and patent ductus arteriosus. Both parents were healthy and non-consanguineous. The pregnant woman was of normal height, did not display any behavioral or mental disorders and had a normal IQ score. She got her bachelor degree at the age of 23. On sonographic examination her internal organs did not reveal any abnormalities. She also did not show any dysmorphic facial features.

According to the poor prognosis suspected from the sonography, the parents decided for the termination of pregnancy. Autopsy after the interruption of pregnancy at gestational age of 32 weeks revealed a female fetus presenting VSD, tricuspid atresia, patent ductus arteriosus, interrupted aortic arch, imperforate anus and development delay. Both previous pregnancies were terminated at around 25
gestational weeks, and more detailed clinical informations are unavailable. Ultrasonic examinations of both previous fetuses at 22 gestational weeks indicated a normal growth size, and did not reveal any other abnormalities except for the tetralogy of Fallot.

3. Materials and methods

3.1. Cytogenetics

GTG-banding analysis at 320–400 band resolution on cultivated cells from Peripheral blood of both parents and umbilical cord blood from the fetus were performed.

3.2. BAC-based arrayCGH

ArrayCGH was performed using constitutional chip 4.0 (PerkinElmer, wallac, Finland) according to the protocol supplied. The slides were scanned on a ScanArray® Gx Plus, the outcome TIFF images were quantified using ScanArray Express microarray analysis software (PerkinElmer, wallac, Finland), and both GPR files were analyzed on SpectralWare v2.3 software online (http://service.spectralgenomics.com).

3.3. Method of confirmation

FISH analysis was carried out on interphase and metaphase cells from all three blood samples according the manufactures instructions (Vysis, Downers Grove, IL) to confirm the 22q11.2 duplication. As probes TUPLE1, specific for the DGS/VCFS critical region in 22q11.2 and a control probe ARSA at 22q13.3 region were applied. Competitive Fluorescent Multiplex STRP Assay (CFMSA) was conducted following a previously described protocol [6]. A set of primers aiming at 5 STRP markers with high heterozygosity (D22S873, 22D_5_1, 22D_4_5, 22D_4_4, 22D_4_3) located in the 22q11.2 region were applied. To investigate the accurate breakpoints of the microduplication, high density oligo arrayCGH was carried out using Nimblegen HG18 Chr22FT microarray (Roche Nimblegen, Madison, USA) containing unique 385 k probes across the human chromosome 22, with average probe spacing of 65 bp. The microarray chip was scanned by the GenePix 4000B Scanner (Molecular Devices, Foster, USA). Data analysis was conducted by the GenePix Pro6.0 software.

4. Results

Standard chromosome banding analysis at a resolution of 320–400 bands did not reveal any numerical or structural rearrangements, neither in both parents nor in the actual pregnancy. Genome-wide BAC-based array comparative genomic hybridization (arrayCGH) analysis was performed to investigate the possible presence of pathological submicroscopic genomic aberrations in the fetus’ genome. The result revealed a microduplication located in the 22q11.2 region with an estimated length of more than 1.5 Mb in the fetus (Fig. 1A). To confirm the result fluorescence in situ hybridization (FISH) using the commercially available probe TUPLE1 specific for the 22q11.2 with its control (ARSA in 22q13.3) was applied in interphase and metaphase analysis on cultivated blood cells of the fetus (Fig. 1B) and the parents. The results confirmed an interstitial 22q11.2 microduplication in the fetus and identified the same in the mother. A short tandem repeat polymorphism (STRP) analysis also indicated the mother carried a 22q11.2 microduplication which was transmitted to the fetus (Fig. 1C). Additional STRP analysis within the family of the adult female carrier indicated the 22q11.2 microduplication being de novo in her (date not shown).

To investigate the accurate breakpoints of the microduplication, high density oligo microarray was carried out. As shown in Fig. 1D, a single copy number gain spanning 17.298–20.246 Mb with a length of 2.948 Mb was detected in the fetus’ genome. The latter fully covers the typical DGS/VCFS region. The mother demonstrated the identical 22q11.2 microduplication.

5. Discussion

We report a familial case of 22q11.2 microduplication syndrome. ArrayCGH analysis revealed the phenotypically normal mother to be carrier of 22q11.2 microduplication. Her abnormal pregnancy history with three consecutive female fetuses having a severe heart defect is unique in literature. High-resolution microarray analysis indicated that the microduplication is located between 17.298 Mb (proximal) and 20.246 Mb (distal) with a length of 2.948 Mb. The microduplication was proven to be de novo in the healthy mother and transmitted to her third affected fetus.

Cardiac defects are observed in 22q11.2 microduplication syndrome at a frequency of around 15% [3]. The first 22q11.2 microduplication patient with CHD was reported by Ensenauer et al. [7]. Using FISH analysis, they carried out a systematic screening for microduplication 22q11.2 in a population of 650 patients referred for deletion analysis for DG/VCFS, and 10 of 650 patients were found to carry the microduplication. Two of the ten patients carrying 22q11.2 microduplication displayed heart defects, one had tetralogy of Fallot, and the other had hypoplastic left heart syndrome and interrupted aortic arch. Up to now, the reported spectrum of CHD reported in 22q11.2 microduplication syndrome includes VSD, aortic insufficiency/mitral valve prolapse, aortic coarctation, tetralogy of Fallot, hypoplastic left heart syndrome and interrupted aortic arch, truncus arteriosus, transposition of the great arteries associated with Ebstein’s anomaly. However, tricuspid atresia observed in our case has not been reported in 22q11.2 microduplication syndrome previously.

Anal anomalies including imperforate anus is frequently found in Cat-eye syndromes (CES). Imperforate anus is also reported in 22q11.2 deletion syndrome, although it is not a classical 22q11.2 deletion clinical presentation. Anal anomalies are rarely found in patients with 22q11.2 microduplication. To our knowledge, only one patient with distal 22q11.2 microduplication was found to have an imperforate anus. This 4-day-old male presented with dysmorphic features and multiple congenital anomalies including imperforate anus, hypoplastic left kidney, patent ductus arteriosus, patent foramen ovale and anomalous right subclavian artery and was identified to carry a LCR22-4 to LCR22-6 duplication [8].
The fetus we described here is the first proximal 22q11.2 microduplication case with imperforate anus.

The present microduplication is located in the most common 3 Mb region spanning from LCR22-1 to LCR22-4. 18 individuals with microduplications of 22q11.21-q11.23 distal to the classical 22q11.2 microduplication syndrome region have been reported, of which 13 duplications do not involve LCR22s 1–4 [8]. These individuals displayed variable phenotypic features including idiopathic mental retardation, dysmorphic features, cardiovascular and musculoskeletal defects. Two patients displayed congenital heart disease including tricuspid regurgitation and VSD.

This is the second prenatally diagnosed case of 22q11.2 microduplication syndrome. The previously reported fetus [5] had a complex heart defect including complex heart defects including single atrium, small left ventricle, large right ventricle, double outlet right ventricle with transposed great arteries, subpulmonary ventricular septal defect, persistent left superior vena cava, and total anomalous pulmonary venous return (TAPVR) in the right superior vena cava. In addition, abdominal situs inversus totalis with normal cardiac situs, thoracic heterotaxia with right predominance, bilateral tri-lobe lungs and mild facial dysmorphism, which did not present in our reported case, were found in that case.

Theoretically, a microduplication is expected to occur at a similar frequency as its corresponding microdeletion according to comparison with a large number of patients identified with 22q11.2 microdeletion. That may be partially explained by the remarkable phenotypic variability of 22q11.2 microduplication. Unlike DGS/VCFs, characterized by a typical facial appearance, conotruncal cardiac defects, velocardiofacial insufficiency, and learning disabilities, 22q11.2 microduplication demonstrated highly variable phenotypic features, as in the presented 30-year-old female without any clinical symptoms. Therefore, many individuals with 22q11.2 microduplications displaying unspecific or very mild clinical features might be under-diagnosed.

Intrafamilial phenotypic variability was observed in 22q11.2 microduplication previously. Ensenaer et al. [7] reported family members with identical 22q11.2 microduplication demonstrating variable phenotypes and mental impairment at different levels. In another report, a fetus with severe cardiovascular anomalies was found to carry a paternally derived 22q11.2 microduplication having mild cognitive impairment [5]. It is well known that parents with a mild phenotype may transfer a duplication to more severely affected offspring [10–12], the parents only being recognized after the diagnosis in the child is made. In this report, the adult female with 22q11.2 microduplication did not display any clinical features. The underlying mechanism is not clear by now for the variable penetrance and/or expressivity. Somatic mosaicism or modifier genes could be explanations.

In summary, 22q11.2 microduplications can be found in clinically healthy people without any clinical features as well as in prenatal cases with a heart defect. Thus, the 22q11.2 microduplication syndrome demonstrates highly variable phenotypic features, even in family members with identical chromosomal imbalance. It is recommended to test the 22q11.2 microduplication when dealing with recurrent congenital heart defects.

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