22q11.2 Microdeletion in a Fetus With Double-outlet Right Ventricle, Pulmonary Stenosis and a Ventricular Septal Defect: Prenatal Diagnosis by Array Comparative Genomic Hybridization

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A 30-year-old, primigravid woman was referred to the hospital at 24 weeks' gestation because of fetal cardiac abnormalities detected by sonography. The woman and her husband were nonconsanguineous and healthy, and there was no family history of congenital heart defects or DiGeorge syndrome (DGS). She did not have diabetes mellitus and denied any exposure to teratogenic agents or infectious diseases during this pregnancy. Level II ultrasound revealed a singleton fetus with a fetal biometry equivalent to 24 weeks' gestation, a normal thymus, a normal amount of amniotic fluid, double-outlet right ventricle, pulmonary stenosis, and a ventricular septal defect. Amniocentesis was performed. Bacterial artificial chromosome array comparative genomic hybridization (aCGH) and oligonucleotide aCGH were applied for genetic analysis using cultured amniocytes. Bacterial artificial chromosome aCGH demonstrated a 22q11.21 microdeletion (RP11-690P21 → RP11-1116M14; (Figure 1). Oligonucleotide aCGH demonstrated a 2.6-Mb deletion in 22q11.21 (chromosome 22, 17141308-19792353 bp; Figure 2). G-banded chromosome analysis at the 850-band level revealed a 46,XX karyotype. Fluorescence *in situ* hybridization (FISH) using dual color DNA probes showed a heterozygous deletion of the DGS/velocardiofacial syndrome (DGS/VCFS) region (Figure 3). FISH chromosome analysis revealed a 46,XX.ish del

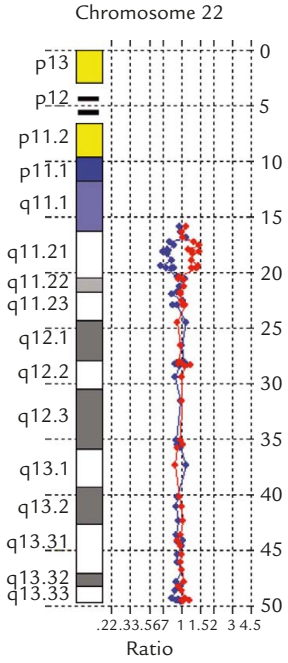

Figure 1. Bacterial artificial chromosome array comparative genomic hybridization using CMDX bacterial artificial chromosome array comparative genomic hybridization CA2500 chips (CMDX, Irvine, CA, USA) showing a 22q11.21 microdeletion [arr cgh 22q11.21q11.21 (RP11-690P21 → RP11-1116M14) × 1].

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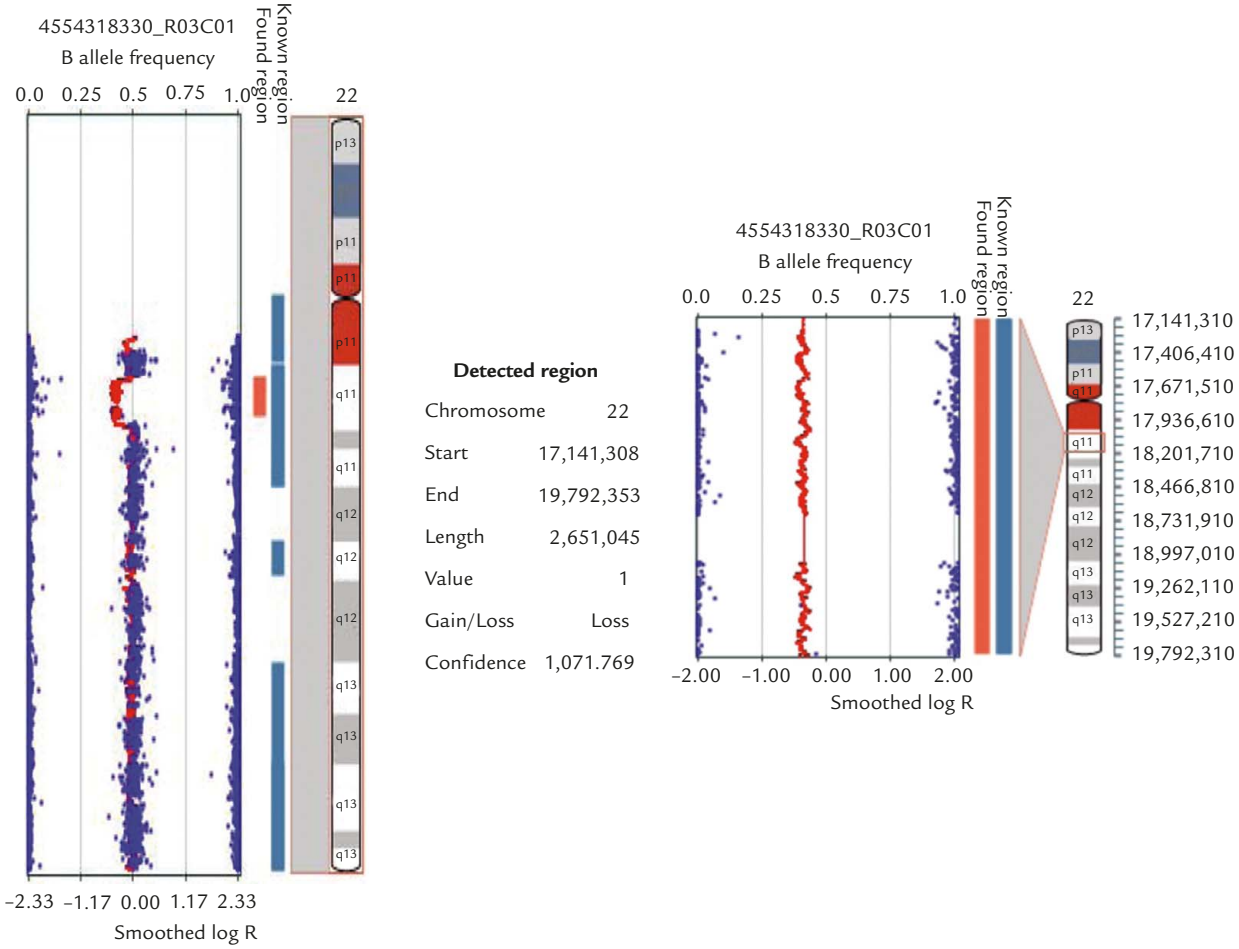

Figure 2. Oligonucleotide array comparative genomic hybridization using HumanCytoSNP-12 v1 BeadChips (Illumina, San Diego, CA, USA) showing a 2.6-Mb deletion in 22q11.21 [arr 22q11.21 (17141308-19792353) × 1].

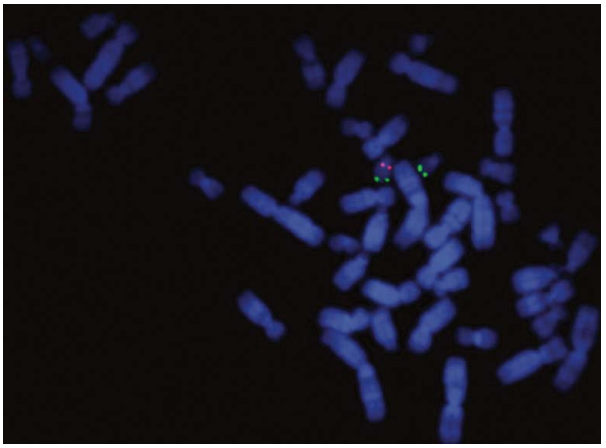

Figure 3. Fluorescence *in situ* hybridization using Vysis LSI DiGeorge/velocardiofacial syndrome region/ARSA dual color DNA probes with a 22q13.3 telomere probe (LSI ARSA; green signal) and a DiGeorge critical region probe at 22q11.2 (LSI DiGeorge/velocardiofacial syndrome; red signal) (all probes by Abbott Laboratories, Abbott Park, IL, USA) showing a normal chromosome 22 (one red signal and one green signal) and a del(22)(q11.2q11.2) chromosome (green signal only) in a metaphase amniocyte.

(22)(q11.2q11.2)(D22S553–) karyotype. Microarray-based chromosome analysis showed arr 22q11.21 (17141308-19792353) × 1.

The 22q11.2 deletion has an estimated incidence of 1 per 4,000 live births [1] and has been detected in the majority of patients with DGS, VCFS and conotruncal anomaly face syndrome, as well as in some patients with Opitz G/BBB syndrome and Cayler cardiofacial syndrome [2–5], all of which are referred to as the 22q11.2 deletion syndrome [5]. The 22q11.2 deletion syndrome is characterized by congenital heart defects, aplasia or hypoplasia of the thymus with immune deficiency, small or absent parathyroid glands with hypocalcemia, palatal abnormalities with speech disorders, and cognitive difficulties [5]. Indications for prenatal testing for the 22q11.2 deletion syndrome include a previous child with DGS/VCFS or a 22q11.2 deletion, an affected parent with DGS/VCFS or a 22q11.2 deletion, and a sonographic finding of conotruncal cardiac defects [5]. Reported prenatal sonographic features associated with a 22q11.2 microdeletion

include conotruncal cardiac malformations, increased nuchal translucency (NT), polyhydramnios, intrauterine growth restriction, extracardiac anomalies, severe pulmonary artery anomalies, and thymic hypoplasia [6–12]. The present case did not present with increased NT, polyhydramnios, thymic hypoplasia or intrauterine growth restriction, but did show cardiac and pulmonary artery anomalies on prenatal ultrasound.

Lautrup et al [13] found that none of 146 fetuses with NT above the 99th centile (> 3.5 mm) and a normal karyotype was diagnosed with a 22q11 microdeletion by either multiplex ligation-dependent probe amplification or FISH, and they estimated that the prevalence of a 22q11 microdeletion in these otherwise normal fetuses with increased NT was below 2.7%. In a study of 540 fetuses with prenatally detected cardiac defects and a normal karyotype, Moore et al [14] found that 17 (3.15%) had a 22q11.2 microdeletion diagnosed by FISH. In a study of 27 fetuses with intrauterine growth restriction, congenital heart defects and a normal G-banded karyotype, Chen et al [15] found that three (11.1%) had a 22q11.2 microdeletion and two (7.4%) had a subtelomeric deletion, including monosomy 21q22.3 and monosomy 1p36.3. In a review of 52 patients with DGS, Minier et al [16] found that only six (11.5%) were diagnosed during the prenatal period, 12 (23.1%) were diagnosed by pathologic examination and 34 (65.4%) were diagnosed during infancy. There is marked phenotypic variability among patients with a 22q11.2 deletion [17]. In a study of 30 individuals (19 adults and 11 children) with a 22q11.2 deletion following the diagnosis in their relatives, McDonald-McGinn et al [17] found that 60% (18/30) had no visceral anomalies, and only 32% (6/19) of the adults and 55% (6/11) of the children with a 22q11.2 deletion had major findings.

The 22q11 region is susceptible to chromosomal rearrangements leading to DGS/VCFS, cat-eye syndrome and t(11;22)der(22) syndrome, of which all three breakpoint regions harbor similar low-copy repeat sequences, also known as *LCR22*s [18,19]. Homologous recombination events between LCR22s during meiosis have been implicated in DGS/VCFS and cat-eye syndrome, and the sites of chromosome breakage on 11q23 and 22q11 in der(22) syndrome occur in the unstable AT-rich palindromic sequences leading to non-homologous recombination mechanisms [19–22]. About 90% of the patients with a 22q11.2 microdeletion have a common approximately 3-Mb deletion, and 7% of the patients have a smaller, nested, approximately 1.5-Mb recurrent deletion, all located in the 22q11.2 proximal deletion region [23,24]. Recently, Ben-Shachar et al [24] identified atypical deletions located in the 22q11.2 distal region. Patients with an atypical deletion have a 22q11.2 microdeletion distal to the common deletion region and manifest a phenotype distinct from DGS/VCFS [24].

DGS (OMIM 188400), a developmental field defect of the third and fourth pharyngeal pouches, is characterized by hypocalcemia arising from parathyroid hypoplasia, thymic hypoplasia, and outflow tract defects of the heart including tetralogy of Fallot, truncus arteriosus, and interrupted aortic arch. Most cases with DGS result from a deletion of chromosome 22q11.2. However, about 10–15% of patients do not have the standard 22q11.2 deletion but have other genetic causes, such as distal deletions of chromosome 10p and chromosome 4q, unbalanced chromosomal translocations, atypical deletions of 22q11.2, and *UFD1L* haploinsufficiency [25–32]. Although FISH, ultrarapid FISH and multiplex ligation-dependent probe amplification are highly accurate tests for detecting the standard 22q11.2 microdeletion [33–35], routine aCGH has the advantage of detecting uncharacterized chromosomal imbalances and atypical 22q11.2 microdeletions, as well as refining the 22q11.2 deletion breakpoints [36,37] in the event of *in utero* detection of a fetus with congenital heart defects.

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