Research Letter

Tetralogy of Fallot With Absent Pulmonary Valve in a De Novo Derivative Chromosome 9 With Duplication of 9p13 → 9pter and Deletion of 9q34.3

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Received 17 February 2006; Accepted 29 June 2006

How to cite this article: Tansatit M, Kongruttanachok N, Kongnak W, Arunpan S, Maneeshote P, Buasorn V, Praphanphoj V, Shotelersuk V. 2006. Tetralogy of Fallot with absent pulmonary valve in a de novo derivative chromosome 9 with duplication of 9p13 → 9pter and deletion of 9q34.3. Am J Med Genet Part A 140A:1981–1987.

To the Editor:

The most common clinical manifestations of trisomy 9p syndrome include mental retardation, a wide fontanelle, microcephaly, downslanting and deep set eyes, prominent nasal root with a bulbous nasal tip, low-set abnormal ears, minor skeletal anomalies (hypoplastic phalanges, clinodactyly of the fifth finger and hypoplastic nails) and single palmar crease [Sutherland et al., 1976; Young et al., 1982; Haddad et al., 1996; Fujimoto et al., 1998; Sanlaville et al., 1999]. Intrauterine growth retardation, cleft lip/palate and congenital heart defect (CHD) are seen infrequently, unless the trisomic segments extend through 9q22–9q32 [Wilson et al., 1985].

In some cases of partial trisomy, analysis of the genotype–phenotype relationship is complicated by the presence of a complex chromosome rearrangement, which cannot be defined solely by conventional G-banding technique. Molecular cytogenetic methods, such as multicolor fluorescence in situ hybridization (mFISH) and multicolor banding (mBAND) analysis, may be required to define the interpretation [Chudoba et al., 2004]. Since small rearrangements involving chromosome end are not well represented in mFISH and mBAND, subtelomeric FISH probe sets can be applied to identify cryptic aberrations that might be the cause of dysmorphic features and idiopathic mental retardation in some patients [Knight et al., 2000].

We present a patient with clinical features resembling trisomy 9p in whom mFISH, mBAND and subtelomeric FISH demonstrated de novo trisomy 9p with an additional copy of 9p13 → 9pter attached to 9qter and a 9q34.3 subtelomeric deletion at the insertion breakpoint. While the 9p duplication explained several of the dysmorphic features, the 9q34.3 deletion might account for the conotruncal heart defects in this patient.

The proband was a female infant born at term, by vaginal delivery, to a 24-year-old, Gravida 0 Para 0 mother. Ultrasonography at 18 weeks gestational age showed polyhydramnios; and at 32 weeks gestational age a ventricular septal defect (VSD), pulmonary valve stenosis and tricuspid regurgitation were noted. Birth weight was 2,560 g (25th centile), length was 46 cm (25–50th centile), and head circumference was 30.5 cm (3rd centile).

Multiple dysmorphic features were noted at birth, including overlapping cranial sutures, bitemporal narrowing, sunken eyes with short and downslanting palpebral fissures, a bulbous nose, low-set ears, a right preauricular skin tag and over-folding of the

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DOI 10.1002/ajmg.a.31424

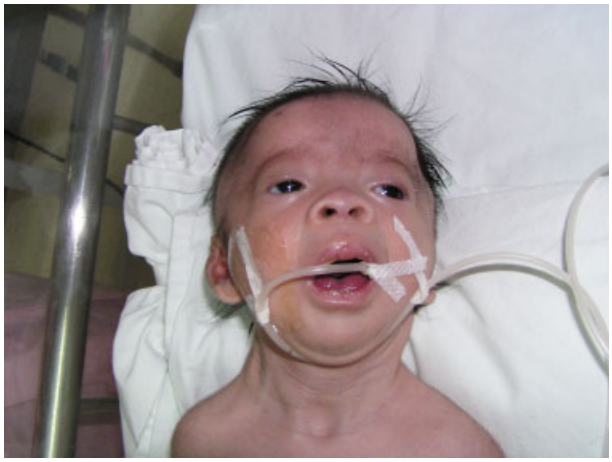

FIG. 1. Photograph of the patient at age 1 month. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

helices, redundant nuchal skin, webbed neck and a soft tissue mass at the midline of the anterior chest wall (Fig. 1). Also present were bilateral clinodactyly and hypoplasia of the middle phalanges of the fifth fingers and ulnar deviation of both thumbs. The toes were overlapping. The external genitalia appeared normal and there was a pilonidal sinus at the coccyx. Neurologically, the infant was hypoactive with hypotonia and normal neonatal reflexes.

Postnatal echocardiogram confirmed the prenatal findings of large VSD, atrial septal defect, patent ductus arteriosus and right ventricular hypertrophy consistent with Tetralogy of Fallot (TOF). Because the pulmonary valve leaflets were absent, annulus was hypoplastic and pulmonary trunk and proximal right and left pulmonary arteries were dilated, the heart was described as TOF with absent pulmonary valve syndrome. Family history was negative for CHDs and consanguinity. The patient died at age 4 months from cardio-respiratory failure related to the CHDs. Autopsy was declined.

Metaphase chromosomes were obtained from phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes, and G-banding was performed using standard methods [Watt and Steven, 1986].

mFISH was performed on metaphase chromosomes using a 24XCyte probe kit (MetaSystems, Altlussheim, Germany) according to the manufacturer's instructions.

mBAND was performed on metaphase chromosomes using an XCyte9 chromosome 9-specific mBAND probe (MetaSystems). Both mFISH and mBAND images were captured and analyzed using a Zeiss Axioplan 2 imaging microscope (Carl Zeiss, Jena, Germany) and the Isis software (MetaSystems).

The subtelomeric deletion of 9q was investigated by FISH using subtelomeric probes of 9p and 9q (BAC/PAC clones RP11–174M15 and RP11–885N19, respectively). Briefly, FISH was performed on metaphase chromosomes of the patient. Clones were directly labeled with SpectrumGreen or SpectrumRed (Vysis, Des Plaines, IL) by nick translation according to the manufacturer's specifications. Chromosomes were counterstained with DAPI.

TABLE I. Clones Used for 9qter Deletion and 9p13 Duplication Breakpoints Mapping

Clone 9q34.3	Location (Mb) from 9q telomere	Clone 9p21.1–13	Location (Mb) from 9p telomere
RP11–885N19	0.1	RP11–205M20	32.5
RP13–467E5	0.2	RP11–395N21	35.4
RP11–48C7	0.4	RP11–397D12	37.4
RP11–229P13	1.0		
RP11–413M3	1.4		
RP11–399H11	2.9		
RP11–374P20	4.1		

The locations of all clones correspond to the May 2004 draft sequence of the human genome on the UCSC Genome bioinformatics browser.

The 9q34.3 deletion and 9p duplication breakpoints were mapped by FISH in essentially the same manner using the clones listed in Table I.

Using the standard G-banding technique, the patient's karyotype was interpreted as 46,XX, der(9)t(9;?)(q34.3;?) at the 550-band level of resolution (Fig. 2). The derivative chromosome 9 had extra material attached to the 9qter. Both the paternal and maternal karyotypes were normal, suggesting a de novo event.

mFISH confirmed that the der (9) extra material was of chromosome 9 origin (Fig. 3A–C). mBAND analysis revealed an additional copy of 9p13 → 9pter attached to the 9qter in a direct fashion, resulting in partial trisomy of 9p (Fig. 3D and 4). To determine the presence of 9q subtelomeric region of the derivative chromosome, FISH using 9p and 9q subtelomeric probes was carried out. The results demonstrated a subtelomeric deletion at the insertion breakpoint on the 9qter (Fig. 5A). Mapping of the 9q34.3 deletion breakpoint was accomplished by systematically narrowing by FISH with genomic clones located within the most distal 4 Mb of 9qter. The 6 BAC clones used in mapping the deletion breakpoint and their locations from 9q telomere are listed in Table I. FISH demonstrated the deletion breakpoint lies between clone RP11–413M3 and RP11–229P13 (Fig. 5B,C). The deletion size was approximately 1.4 Mb. The 9p duplication breakpoint was mapped by FISH using clones spanning region 9p21.1–9p13 listed in Table I. The result indicated that the duplication size was about 35.4 Mb (Fig. 5D).

This patient with multiple dysmorphic features, TOF with absent pulmonary valve, and an abnormal karyotype showing a derivative chromosome 9 with an abnormal long arm was more precisely defined using mFISH and mBAND. The results revealed an unusual case of de novo trisomy 9p in which an additional copy of the 9p13 → 9pter was inserted at

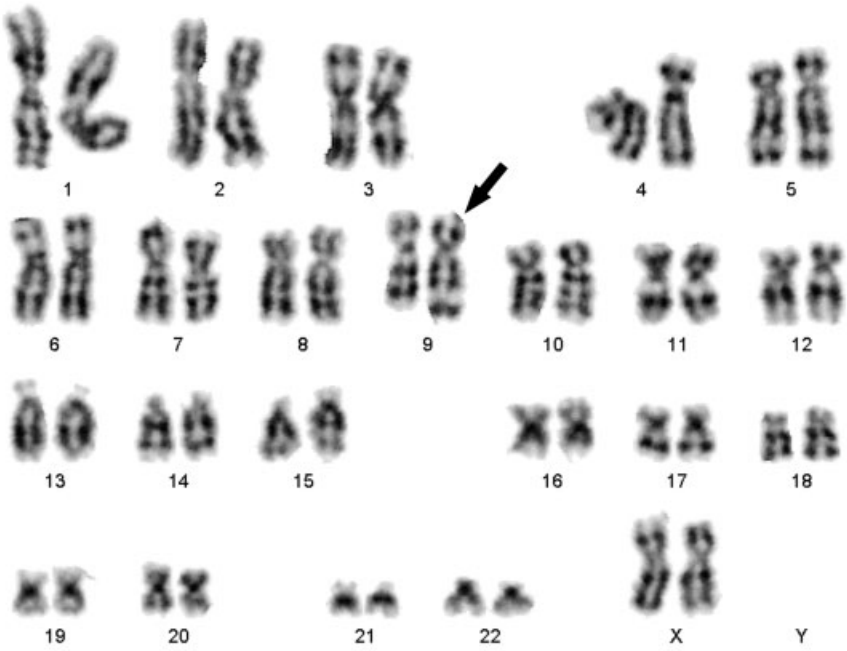

FIG. 2. Karyotype of G-banded chromosomes, showing the normal chromosome 9 and the derivative chromosome 9 (arrow).

9qter. Although the distal half of the short arm of chromosome 9 (9p13 → 9pter) is responsible for the major clinical features of trisomy 9p [Fryns et al., 1979; de Pater et al., 2002], CHDs, including conotruncal defects, are uncommon [Tennstedt et al., 1999; Morrissette et al., 2003]. Subtelomeric FISH demonstrated that there was a cryptic subtelomeric deletion at the insertion breakpoint

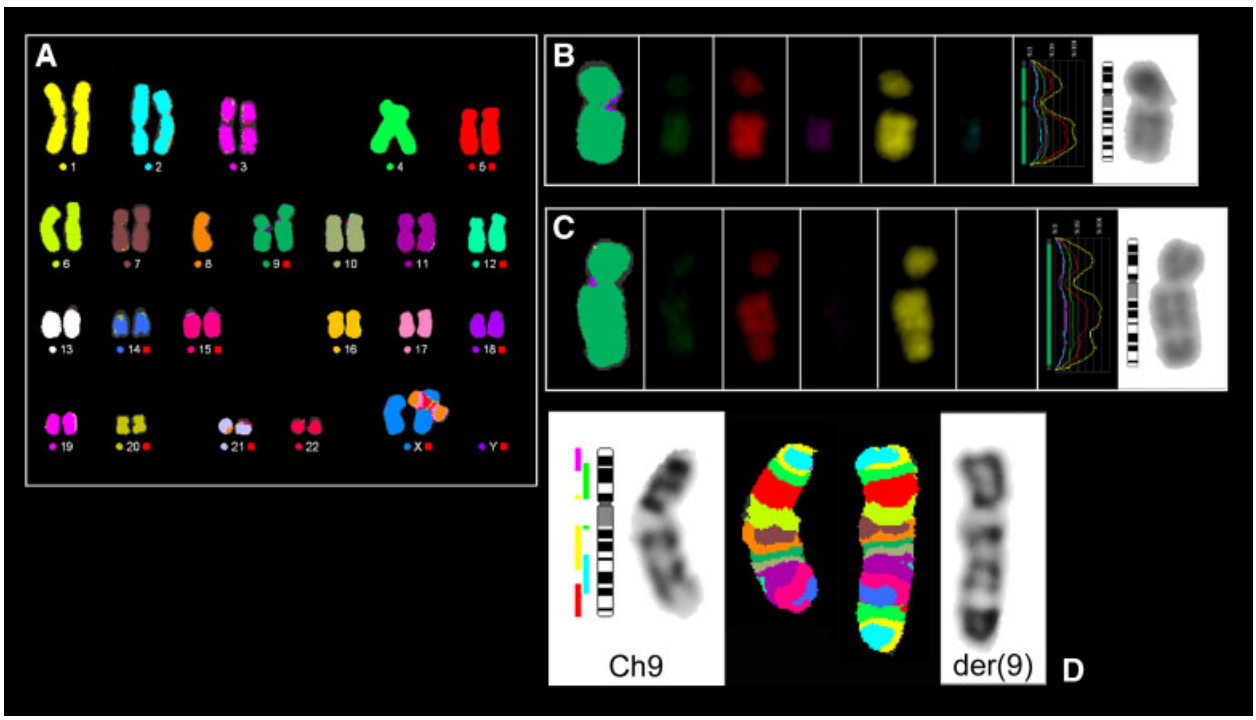

FIG. 3. **A**: Karyotype of mFISH confirmed that the extra material of the derivative chromosome 9 was all of chromosome 9 origin (**B** and **C**). **D**: mBAND showed the duplicated 9p13 → pter inserted to the 9qter. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

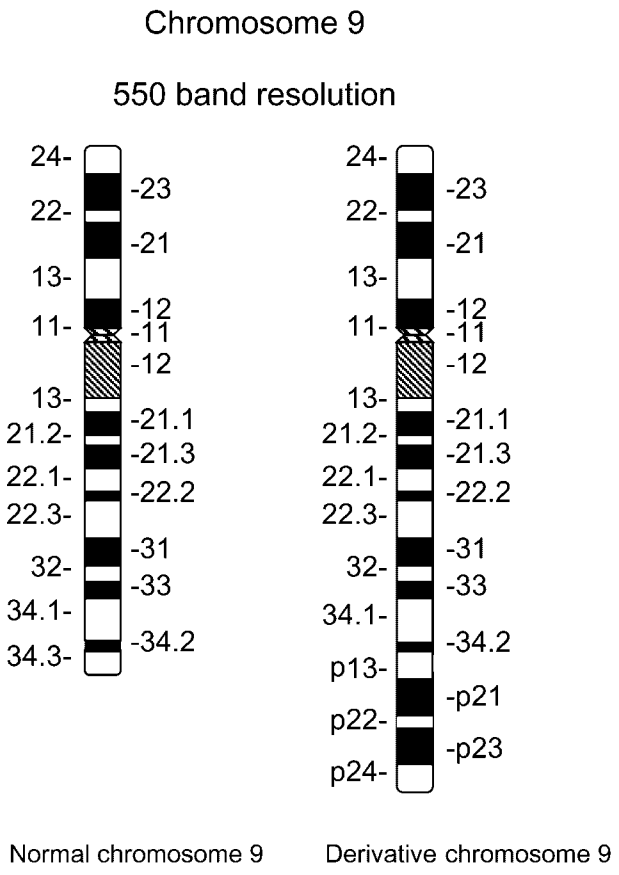

FIG. 4. Ideogram illustrates the duplicated segment of the short arm and the insertion breakpoint at the terminal end of the long arm of the derivative chromosome 9.

(9q34.3) on the 9qter, which might explain the CHDs in this patient.

Fine mapping of the 9q34.3 deletion breakpoint indicated that the deletion size was approximately 1.4 Mb, corresponding to the critical chromosomal region responsible for subtelomeric 9q34.3 deletion phenotypes [Stewart et al., 2004]. This minimum critical region is an approximately 1.2 Mb interval from the 9q telomere, encompassing at least 14 genes or transcripts, and it is suggested that haploinsufficiency of one or more genes within this region most probably accounts for the 9q- syndrome. Recent study suggested that *EHMT1* gene, which locates in this commonly deleted region, was most likely responsible for the larger part of 9q- phenotype [Kleefstra et al., 2005; Kleefstra et al., 2006]. A review of the literature showed that the phenotypic findings of the subtelomeric deletions of chromosome 9q34.3 generally consist of mental retardation, distinct facial features and CHDs particularly conotruncal heart defects [Iwakoshi et al., 2004; Stewart et al., 2004; Neas et al., 2005]. Table II summarizes the clinical features in 25 reported patients with subtelomeric 9q34.3 deletions compared to the clinical features of partial trisomy 9p patients. Our patient showed the characteristic face and hands of the trisomy 9p syndrome while the features commonly seen in 9q34.3 deletion patients such as mid-facial hypoplasia with synophrys and/or arched eyebrows,

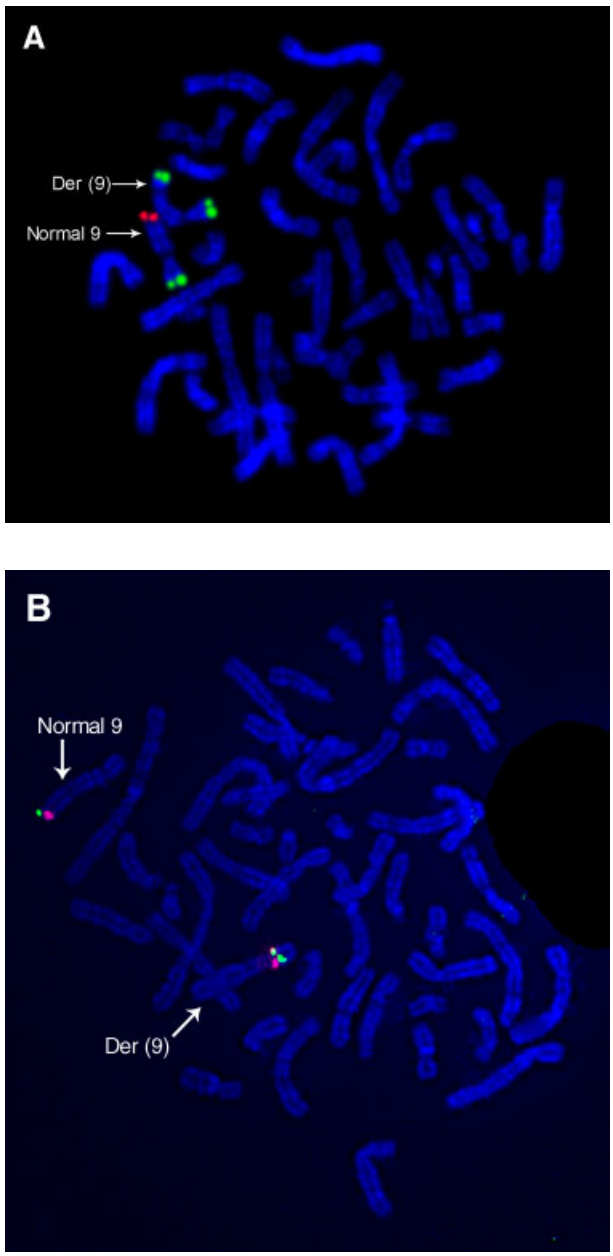

FIG. 5. FISH mapping of the 9q deletion and 9p duplication breakpoints. **A**: Metaphase spreads were analyzed for the presence of subtelomeric region of the long arm of the derivative chromosome 9 at the insertion breakpoint by FISH using subtelomeric probes. The green signals indicated the subtelomeric region of the 9p and the red signal was the subtelomeric region of the 9q. The 9q subtelomeric region of the der(9) was found to be deleted at the insertion breakpoint. **B**: Clone RP11–413M3 was labeled in green and RP11–374P20 was labeled in red. Both green and red signals could be detected on both chromosome 9 and der(9). **C**: Clone RP11–229P13 and RP11–399H11 were labeled in green and red, respectively. Red signals could be detected on both chromosome 9 and der(9), whereas green signal could not be detected on der(9). The results indicated that the deletion breakpoint lies between BAC clone RP11–229P13 and RP11–413M3. **D**: Clone RP 11–205M20 and RP11–395N21 were labeled in red and green, respectively. The result showed an additional red signal indicative of duplication on der(9) (arrow). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

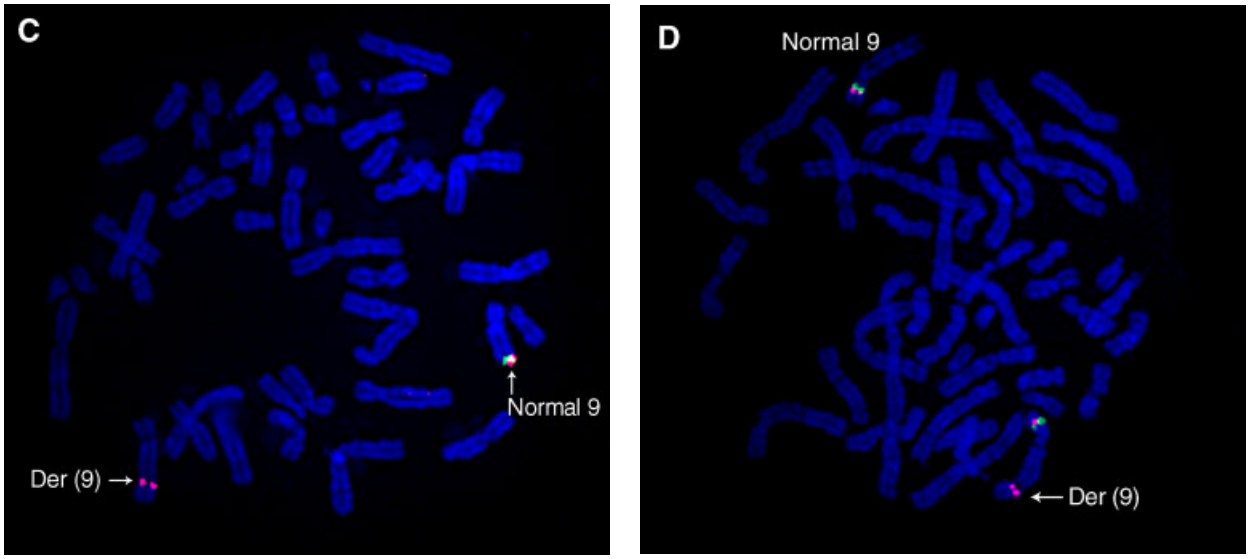

FIG. 5. (*Continued*)

TABLE II. Clinical Manifestations of Our Patient Compared With Partial Trisomy 9p and 9q34.3 Deletion

Clinical manifestation	Partial trisomy 9p[a] p13 → pter (%)	9q34.3 deletion[b] (%)	Our case
Craniofacial			
Microcephaly	8/24 (33)	23/25 (92)	+
Brachycephaly	7/24 (29)	12/25 (48)	+
Flat face		5/25 (20)	−
Hypoplastic midface		13/25 (52)	−
Arched eyebrows		8/25 (32)	−
Deep-set eyes	7/24 (29)		+
Hypertelorism	10/24 (42)	12/25 (48)	+
Down-slanting palpebral fissures	9/24 (37.5)	6/25 (24)	+
Bulbous nose	14/24 (58)		+
Short nose		14/25 (56)	−
Anteverted nostrils		9/25 (36)	−
Carp mouth		20/25 (80)	−
Thin upper lip		6/7 (86)	−
Thick lower lip		5/25 (20)	−
Down-turned corners of the mouth	13/24 (54)		+
Protruding tongue		12/25 (48)	−
Low-set ears	10/21 (48)	3/5 (60)	+
Malformed ears	19/24 (79)	12/25 (48)	+
Musculoskeletal			
Short or webbed neck	7/24 (29)		+
Clinodactyly	19/24 (79)		+
Single palmar transverse crease	16/24 (67)	5/6 (83)	+
Syndactyly	1/24 (4)	2/6 (33)	−
Hypoplasia of phalanges	20/24 (83)	4/22 (18)	+
Dysplasia or hypoplasia of nails	11/24 (46)		+
Congenital heart defects[c]			
Total	1/24 (4.2)	12/25 (48)	
Conotruncal defects		3/12 (25)	TOF with absent pulmonary valve
Left-sided obstruction		1/12 (8.3)	
Atrial and ventricular septal defects		8/12 (66.7)	
Valvular defects	1/1 (100)		

[a]Number positive/number informative. Frequencies are derived from cases in Lewandowski et al. [1976], Fryns et al. [1979], Bussani Mastellone et al. [1991], Phelan et al. [1993], Haddad et al. [1996], Fujimoto et al. [1998], Tsezou et al. [2000], and de Pater et al. [2002].

[b]Number positive/number informative. Frequencies are derived from cases in Iwakoshi et al. [2004], Stewart et al. [2004], Neas et al. [2005], and Kleefstra et al. [2006].

[c]Additional CHD types were not observed.

anteverted nares, carp shape mouth, thick lower lip, and tongue protrusion, were not present (Table II). TOF with absent pulmonary valve in our patient was more compatible with the conotruncal CHDs associated with 9q34.3 deletion [Stewart et al., 2004]. It is possible that the subtelomeric region of 9q might be critical for heart development, especially for conotruncal development.

In most reported cases, the partial trisomy 9p was the result of a parental reciprocal translocation between chromosome 9 and another chromosome; in a small number, it was due to the tandem duplications [Cuoco et al., 1982; Haddad et al., 1996; Fujimoto et al., 1998; Tsezou et al., 2000; Christina et al., 2003]. In this new patient, the duplicated 9p segment (9p13 → 9pter) was inserted at the terminal end of the long arm, and the subtelomeric region of 9q34.3 was found to be deleted. One possible mechanism to explain how this unusual cytogenetic finding occurred is the telomere capture, through which a terminally deleted chromosome is stabilized by acquiring a new telomeric sequence from another chromosomal location, which results in a derivative chromosome [Ballif et al., 2004]. However, such a chromosome abnormality was not typical of cases of de novo trisomy 9p and could not be confirmed by the G-banding technique alone.

ACKNOWLEDGMENTS

We gratefully acknowledge Dr. Ilse Chudoba for assistance with the analysis of the mBAND images and Miss Sukanya Meesa for her technical assistance. We are also grateful to Professor Dr. Apiwat Mutirangura for his useful advice and Professor Dr. David Ledbetter for critical reading of the manuscript.

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