

SHORT COMMUNICATION

Prenatal Detection and Mapping of a Distal 8p Deletion Associated with Congenital Heart Disease

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We report the prenatal diagnosis, at 18 weeks' gestational age of a del(8)(p23.1→pter) in a fetus with an atrio-ventricular canal, persistent left superior vena cava and hypoplastic right ventricle detected by sonographic imaging. We further refine the breakpoints associated with this defect using fluorescent *in situ* hybridization analysis (FISH). Our findings correlate with recent reports of the localization and importance of GATA4 (a zinc finger transcription factor) in cardiac development. Though microcephaly, mental retardation and typical behavioural features are well described in various deletions in 8p, the absence of notable microcephaly in this case raises the possibility for a separate genetic aetiology for some of these features. Indeed, primary autosomal recessive microcephaly (MCPH1) was recently mapped to a nearby region and may be the cause for this frequent observation in some cases of 8p deletions. These observations illustrate the role of FISH in prenatal diagnosis and refinement of chromosomal breakpoints. In addition, mappings of loci significant for cardiac development are presented. Our findings suggest that some features of the 8p deletion syndrome may ultimately be uncoupled from one another, and underscore the need for further study of this region of chromosome 8, in order to achieve adequate information for genetic counselling. Copyright © 1999 John Wiley & Sons, Ltd.

KEY WORDS: 8p deletion; prenatal diagnosis; FISH; atrio-ventricular canal

INTRODUCTION

Prior reports of 8p deletion syndrome have included children evaluated for developmental delay and pregnancies late in the third trimester with fetal anomalies. Some of the common features reported with partial monosomy of 8p include growth and mental retardation, impulsive and aggressive behaviour, congenital cardiac defects, and in males, genital abnormalities (Blennow and Brondum-Nielsen, 1990; Claeys *et al.*, 1997; Hutchinson *et al.*, 1992; Ostergaard and Tommerup, 1989; Wu *et al.*, 1996). Recently a case of diaphragmatic hernia diagnosed at 22 weeks' gestation was reported with an 8p23.1 deletion (Faivre *et al.*, 1998). Here, we describe the earliest reported prenatal diagnosis of this chromosome anomaly and further refine the chromosomal breakpoints with fluorescent *in situ* hybridization (FISH) technology.

CASE REPORT

The patient, a 31-year-old G3 P1 TAB1, was referred for diagnostic examination of severe congenital heart defects noted on level 2 ultrasound at 18 weeks' gestation. The fetus was the desired product of a healthy, non-consanguineous, Nigerian couple. This pregnancy had been uneventful. There was no family history of mental retardation or heart defects. Their first child was a healthy male of birth weight 7 lb 3 oz.

The antenatal ultrasound revealed normal cerebral lateral ventricles, posterior fossa and cerebellum. The stomach, kidneys, urinary bladder, fetal cord insertion site, face and extremities were also reported as normal. The biparietal diameter and femur length were consistent with estimated gestational age of 17 and 17.9 weeks, respectively. Amniotic fluid volume and fetal activity were reported as within normal limits.

Fetal echocardiogram confirmed significant cardiac abnormalities with a baseline heart rate of 120–140 beats per minute. The report described a single functional ventricle, likely left, with good qualitative function and a double outlet with anterior, rightward aorta. A malaligned complete atrio-ventricular canal defect was present. In addition, pulmonary atresia was present and the right ventricle hypoplastic. Aortic valve size (0.3 cm), ascending aorta diameter (0.37 cm),

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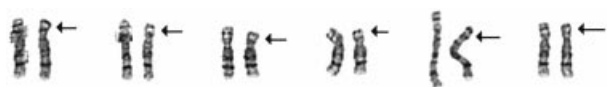


Fig. 1—Partial G-banded karyotype showing chromosome 8 pairs with deleted (8) (p23.1-pter) on the right

aortic isthmus diameter (0.2 cm), right (0.12 cm) and left (0.13 cm) pulmonary artery diameters were all within normal limits for gestational age of 18.4 weeks.

Amniocentesis was performed and cytogenetic analysis revealed an abnormal karyotype, 46,XX, del(8)(p23.1→pter). An unbalanced chromosome complement, consistent with a deletion of the short arm of one chromosome number 8 distal to band p22 (Fig. 1). The karyotype of the mother was normal and the paternal karyotype was not available.

The patient and her 35-year-old husband were referred for genetic counselling. They were informed of previous reports of severe heart defects, mental retardation, microcephaly, aggressive behaviour and hyperactivity through adolescence, associated with this chromosome abnormality (Blennow and Brondum-Nielsen, 1990; Bresson *et al.*, 1977; Brocker-Vriends *et al.*, 1986; Dobyns *et al.*, 1985; Fryns *et al.*, 1989; Hutchinson *et al.*, 1992; Marino *et al.*, 1992; Morrison *et al.*, 1992; Orye and Craen, 1976; Patil and Hanson, 1980; Pecile *et al.*, 1990; Reiss *et al.*, 1979; Wu *et al.*, 1996). They were uncertain about termination of this pregnancy initially, but ultimately chose not to continue the pregnancy citing their concerns about the potential for limited cognitive development and impact of severe cardiac disease and surgery on future development. The pregnancy was terminated at 20 weeks' gestation.

Fetopsy revealed a mildly dysmorphic female fetus with anteverted nares, posteriorly rotated left ear, thin upper vermilion border, long philtrum and nuchal oedema. The thorax, abdomen, external genitalia and limbs were normal. The abdomino-thoracic cavity revealed normal viscerotransverse situs, two intact diaphragms, and grossly and microscopically intact gastro-intestinal and neurological organs. The heart was confirmed to be morphologically abnormal. The cardiac pathology showed persistent left superior vena cava draining into an enlarged and aneurysmal coronary sinus, tricuspid atresia and pulmonary atresia, consistent with the fetal echocardiogram. The following organs were identified and were appropriate for gestational age: adrenal glands, kidneys, gastro-intestinal tract, liver, thymus, ovary, uterus, fallopian tubes and three vessel cord.

Fluorescence *in situ* hybridization (FISH) performed with a whole chromosome 8 painting probe (Oncor, Inc., Gaithersburg, MD, U.S.A.) showed complete hybridization with both normal and abnormal 8 chromosomes, with no outlying areas of visible hybridization (Fig. 2(a)). A subtelomeric probe, D8S596, for the short arm of chromosome 8 (Oncor, Inc.) and the probe D8S11, that maps proximal to the subtelomeric probe (Genzyme Genetics, Frammingham, MA, U.S.A.), were both found to be deleted from the

abnormal chromosome 8 (Figs 2(b), (c)). Two other probes from the 8p23 band that map more proximally in that region, D8S252 and D8S574 (Genzyme Genetics) (Figs 2(d), (e)), were present on both 8 chromosomes. These findings along with the G banding pattern confirmed a terminal deletion in the short arm distal to the probe D8S252.

DISCUSSION

In this study we report the earliest diagnosis of a deletion 8p. Antenatal level 2 ultrasound demonstrated cardiac abnormalities, and cytogenetic analysis revealed a 8p23→pter deletion which was later mapped with FISH to a region near 8p23.1. These results were discussed with reported phenotype correlations and resulted in elective termination at 20 weeks' gestation. Though parental karyotypes were recommended, only the maternal karyotype has been obtained to date and this is normal. Prior reports of 8p deletions have shown most to be *de novo* mutations.

The cardiac abnormalities observed, persistent left superior vena cava (Marino *et al.*, 1992; Hutchinson *et al.*, 1992), atrio-ventricular canal, hypoplastic right ventricle and pulmonary atresia, are consistent with previous published reports of deletions from 8p21→pter. While some reports describe instead an atrio-ventricular septal defect with or without pulmonary atresia, all previous studies report defects in cardiac chamber formation (see Digilio *et al.* (1993, 1998)). Our mapping of the cardiac abnormalities to a very small region (p23.1→pter) suggests that the region of 8p23.1 harbours an important gene for the development of the heart.

Human genes in this region of chromosome 8 include: progressive epilepsy with mental retardation (EPMR), autosomal recessive primary microcephaly 1 (MCPH1), at 8p22-pter, defensin α 5 and 6, and Paneth cell-specific (DEFA 5 and 6) at 8p21-pter, coagulation factor VII regulator (F7R), defensin α 1 myeloid-related sequence (DEFA1), and defensin β 1 (DEFB1) at 8p23.1→p23.2, arylamine N-acetyltransferase-1 and 2 (NAT1 and 2) at 8p23.1→21.3, defensin β 2 (DEFB2), squalene synthetase (FDFT1), and GATA-binding protein-4 (GATA4) at 8p22→p23.1, cryptidin-related sequence-1C (CRS1C) at 8p23, BLK non-receptor tyrosine kinase (BLKP), carboxypeptidase N polypeptide 2 (CPN2), and keratolytic winter erythema (KWE) at 8p22→p23.

Given this list of candidates to date, the only gene known to be implicated in cardiac development is GATA4. A member of the GATA-binding family, this zinc finger transcription factor is expressed in cardiogenic splanchnic mesoderm and associated endoderm during fetal development (Arceci *et al.*, 1993; Huang *et al.*, 1995). Indeed, GATA4 null mice have marked defects in cardiac tube formation. In particular, a failure of fusion of the lateral myocardial primordia and ventral morphogenesis (Kuo *et al.*, 1997; Narita *et al.*, 1997; Molkenin *et al.*, 1997). In knockout mice, this defect is fatal by 9.5 days post-coitum.

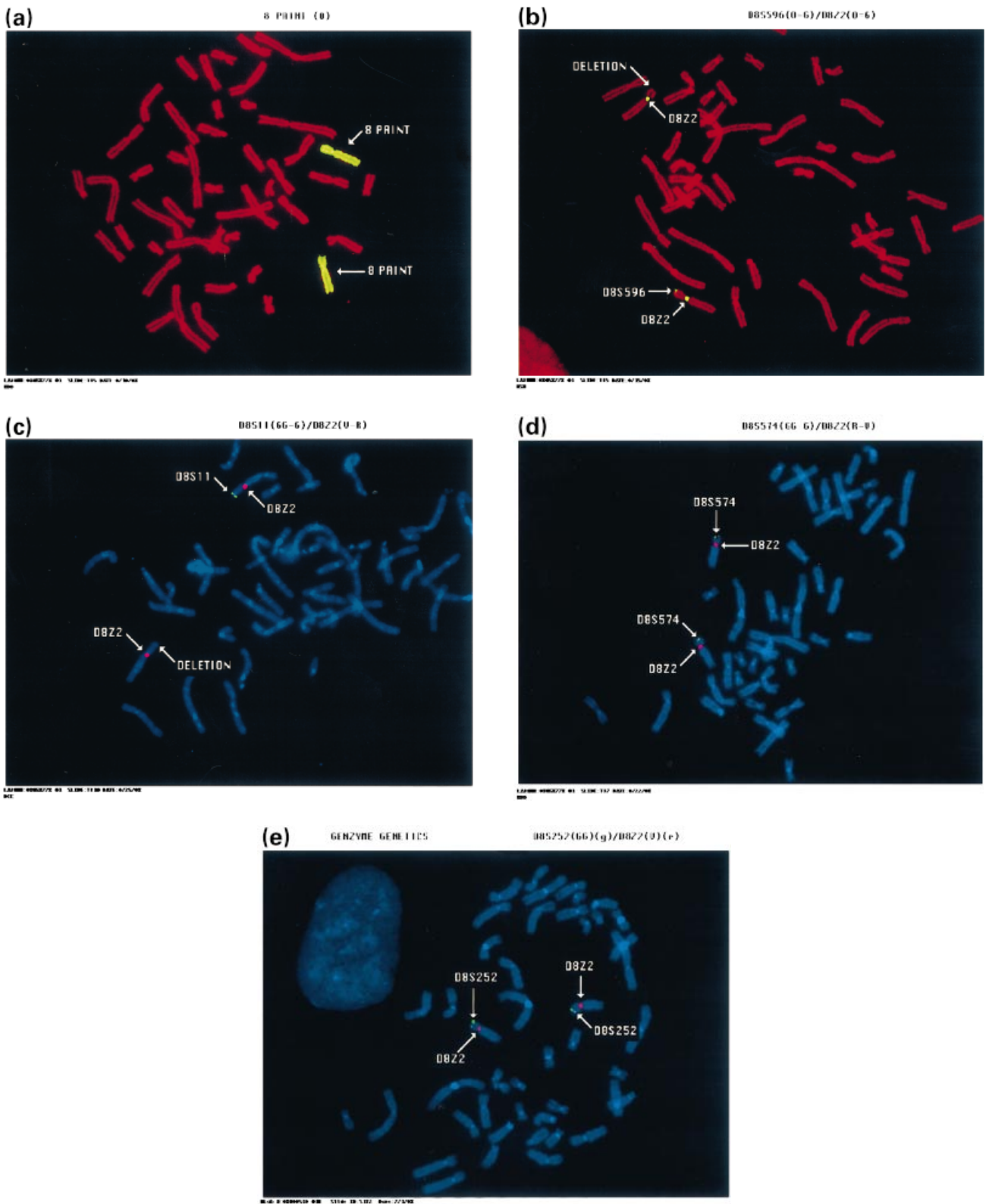


Fig. 2—Fluorescence *in situ* hybridization (FISH) analysis. (a) Chromosome 8 specific painting probe shows that each chromosome 8 is entirely painted with no outlying staining. (b and c) Probes D8S596 and D8S11 with centromeric controls show deletion of the distal region of chromosome 8. (d and e) Probes D8S574 and D8S252 are shown with centromeric controls, indicating that the 8p deletion is distal to these sites

Furthermore, GATA4 has been implicated in the regulation of genes critical for myocardial differentiation and function, including troponin C, cardiac α -myosin heavy chain and brain-type natriuretic factor, suggesting defects in this gene may have many implications on cardiac development (Molkentin *et al.*, 1994).

The mapping of GATA4 to (8p→p23.1) correlates well with the mapping of the 8p deletion associated with fetal cardiac abnormalities in our case report (Huang *et al.*, 1996). FISH analysis indicated the likely deletion to be distal to the 8p23 microsatellite probes D8S252 and D8S574, supporting the possibility of a defect in GATA4 in the observed phenotype. In addition, Marynen *et al.* (1998) recently reported a series of nine patients with a *de novo* deletion in distal chromosome 8p. Six patients presented with the characteristic findings for terminal 8p deletions: cardiac defects (i.e. AVSD or ASD plus pulmonary stenosis), microcephaly and mental retardation, all with deletions between YAC's 770G9 and 918C8 in the region of GATA4. Of the remaining three patients without cardiac defects, none had deletions in the GATA4 gene by FISH analysis, strengthening its putative role in cardiac development.

Interestingly, we and others have not observed microcephaly prior to 30 weeks *in utero*. The biparietal diameter in our study was within normal limits at 18 weeks' gestation. Devriendt *et al.* (1998) propose that perhaps microcephaly associated with 8p deletion occurs later in development or is mediated by a separate gene. Of note, Jackson *et al.* (1998) recently reported that the gene for primary autosomal recessive microcephaly (MCPH1) maps to chromosome 8p22→pter. In addition, at least one patient had normal intelligence despite microcephaly (Rizzo and Pavone, 1995). Thus, perhaps microcephaly, mental retardation and cardiac malformations have independent underlying genetic defects in a contiguous region on 8p similar to other well-known contiguous gene syndromes such as: Langer-Giedion (del8q24.1), DiGeorge/Velo-cardiofacial (del22q11.2), Williams (del7q11.23) and Smith-Magenis (del17p11.2) (Dallapiccola *et al.*, 1995; Ligon *et al.*, 1997).

With the progression of mapping the human genome, the number of genes identified in this segment of chromosome 8 will continue to increase. Further clarification of genotype/phenotype correlations in fetal development will enable more precise genetic counselling. This type of information will be critical to decisions made about a pregnancy as we continue to acquire more refined sonographic and chromosomal data earlier in pregnancy.

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