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Delineation of the Critical Deletion Region for Congenital Heart Defects, on Chromosome 8p23.1

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Summary

Deletions in the distal region of chromosome 8p (del8p) are associated with congenital heart malformations. Other major manifestations include microcephaly, intrauterine growth retardation, mental retardation, and a characteristic hyperactive, impulsive behavior. We studied genotype-phenotype correlations in nine unrelated patients with a de novo del8p, by using the combination of classic cytogenetics, FISH, and the analysis of polymorphic DNA markers. With the exception of one large terminal deletion, all deletions were interstitial. In five patients, a commonly deleted region of ~6 Mb was present, with breakpoints clustering in the same regions. One patient without a heart defect or microcephaly but with mild mental retardation and characteristic behavior had a smaller deletion within this commonly deleted region. Two patients without a heart defect had a more proximal interstitial deletion that did not overlap with the commonly deleted region. Taken together, these data allowed us to define the critical deletion regions for the major features of a del8p.

Introduction

There is increasing evidence that genetic factors play a major role in the pathogenesis of specific types of congenital heart defect (CHD) (Payne et al. 1995; Burn et al. 1998). At present, very few genes for CHD have been identified, partly because of the lack of large pedigrees segregating a well-defined type of CHD. Alternatively, much interest is paid to the association between certain chromosomal aberrations and a specific type of CHD. The heart defects are thought to result from an abnormal dosage of one or more gene(s) within these chromosomal fragments. Examples include deletions in chromosome 22q11 and conotruncal heart malformations (Wilson et al. 1993), as well as trisomy 21 and atrioventricular septal defect (AVSD) (Korenberg et al. 1992).

It has been recognized that, in particular, deletions in the distal region of chromosome 8p (del8p) are associated with CHD, typically in the form of AVSD (Marino et al. 1992; Digilio et al. 1998). Besides a CHD, frequent phenotypic manifestations of del8p include intrauterine growth retardation, microcephaly, and mild mental retardation (MR) (Dobyns et al. 1985; Hutchinson et al. 1992). Features that have been recognized more recently are a characteristic behavioral phenotype (Claeys et al. 1997), hypospadias, and seizures (Digilio et al. 1998). The del8p phenotype often is relatively mild, without associated facial dysmorphism or other major internal malformations (Fryns et al. 1989; Hutchinson et al. 1992; Wu et al. 1996).

Earlier phenotype-karyotype correlations have assigned the critical deletion region for heart defects to 8p23, and the severity of MR and the occurrence of microcephaly appear to relate to the extent of the deletion (Hutchinson et al. 1992; Digilio et al. 1993). To define more precisely both the del8p critical region for CHD (HDCR8p) and the other features of the del8p phenotype, we have conducted clinical, cytogenetic, and molecular studies in nine patients with a de novo del8p.

Patients and Methods

Patients

The clinical features of the patients reported in this study are summarized in table 1. Informed parental consent was obtained for all patients in this study. Patient number 1 (Pt1) (Devriendt et al. 1998) and patients 2, 3, 4, 7, and 8 (Pt2, Pt3, Pt4, Pt7, and Pt8, respectively)

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Table 1

Clinical Features of Patients with a del8p

	Pt1	Pt2	Pt3	Pt4	Pt5	Pt6	Pt7	Pt8	Pt9
Sex	Male	Male	Female	Male	Male	Male	Male	Male	Male
Gestational age (wk)	37	39	40	38	40	40	40	37	42
Birth data:									
Weight (g)	1,900 (<10%-ile)	2,400 (<10%-ile)	2,300 (<10%-ile)	2,250	2,500 (<10%-ile)	2,500 (<10%-ile)	3,150 (25%–50%-ile)	2,400 (10%–25%-ile)	3,480 (25%-ile)
Length (cm)[a]	40.5 (<10%-ile)	47 (10%–25%-ile)	45 (<10%-ile)	44 (10%-ile)	46 (<10%-ile)	…	55 (>97%-ile)	44 (10%–25%-ile)	51 (50%-ile)
OFC (cm)[b]	30 (<10%-ile)	…	…	32 (10%–25%-ile)	…	…	…	32 (25%-ile)	36 (50%–75%-ile)
Heart[c]	AVSD, DORV, HRV, PVS, left isomerism	AVSD, PVS, HRV, AVS, azygoscon-tinuation, left isomerism	ASDII, PVS	AVSD, HRV, DILV, PVS	ASDII, PVS	ASDII, PVS	Normal	Normal	Normal
Age at diagnosis	Prenatal	11 years	8 years	6 mo	17 years	4 years	9 years	Neonate	6 mo
Head circumference (cm)	38.8 (3%-ile = 41.5)	50 (3%-ile = 50.5)	48 (3%-ile = 49.8)	38.4 (3%-ile = 42)	51.8 (3%-ile = 52.2)	49.5 (3%–10%-ile [relative microcephaly])	57.5 (25%–50%-ile)	41.5 (3%-ile = 43)	41 (3%-ile = 42)
Development	Delayed	Mild MR (IQ 64)	Mild MR (IQ 66)	Delayed	Mild MR (IQ 73)	Mildly delayed	Mild MR (IQ 62)	Delayed	Delayed
Behavioral problems[d]	NA	+++	+++	NA	+++	+++	+++	NA	NA
Other	Hypospadias, deceased	Epilepsy, cryptor-chidism, gut malrotation	Hypernasal speech	Deceased	External ear anomaly		Epilepsy, cryptorchidism	Facial dysmorphism	Facial dysmorphism, undescended tes-tis, inguinal hernia
Karyotype	del(8)(p21.3→ter)	del(8)(p23.1→ter)	del(8)(p23.1→ter)	del(8)(p23.1→ter)	del(8)(p23.1→ter)	del(8)(p23.1→ter)	del(8)(p23.1→ter)	del(8)(p22.1→ter)	del(8)(p12→22)

[a] An ellipsis (…) denotes that data were not recorded.

[b] Occipito-frontal circumference. An ellipsis (…) denotes that data were not recorded.

[c] DORV = double-outlet right ventricle, AVS = aortic-valve stenosis, and DILV = double-inlet left ventricle; other abbreviations are as defined in the text.

[d] NA = not assessable.

(Fryns et al. 1989; Devriendt et al. 1995; Claeys et al. 1997) have been reported elsewhere, whereas Pt5, Pt6, and Pt9 are being reported for the first time.

Pt5 is the second child of healthy, unrelated parents. He was born after a normal-term pregnancy, with birth weight 2.5 kg, and length 46 cm. He underwent surgery for an atrial septal defect, ostium secundum type (ASDII), with pulmonary valve stenosis (PVS). He has a smaller right ear with a narrow external ear canal, resulting in a conductive hearing loss of 40 db. During childhood, he displayed the characteristic behavioral disturbances seen in other children with a del8p, including extreme hyperactivity, impulsiveness, and aggressiveness, with destructive episodes. Sleep was severely disturbed—he awakened consistently at 4–5 o'clock in the morning. Motor development was normal. Speech was delayed, with his first words being spoken at age 3 years. Full-scale IQ at age 12 years was 73, with verbal IQ of 83 and performance IQ of 69, as assessed by Wechsler's Intelligence Scale for Children–Revised. Behavioral difficulties ameliorated from puberty onward. Now, at age 17 years, his head circumference is 51.8 cm (3%-ile = 52.2 cm), his height is 163 cm (3%–10%-ile), and his weight is 51.5 kg (3%–10%-ile).

Pt6 was the third of four siblings with normal parents. He was born at term, with birth weight 2.5 kg (<3%-ile). He had both a CHD (ASDII) that required surgery and mild PVS. During infancy there was failure to thrive. After the 1st year of life, behavioral problems—including hyperactivity and concentration problems—became evident, and he was very impulsive. Social skills were impaired. Development at age 4 years was mildly delayed, by 1 year. At age 5 years, physical examination showed only a relative microcephaly, with head circumference 49.5 cm (3%–10%-ile), height 119 cm (25%–50%-ile), and weight 22 kg (75%–97%-ile).

Pt9 was the second child of healthy unrelated parents. The pregnancy was uncomplicated, and delivery was at 42 wk gestation, with birth weight 3,480 g (25%-ile), length 51 cm (50%-ile), and head circumference 36 cm (50%-75%-ile). During infancy there were feeding problems and failure to thrive. Facial features showed hypotelorism, nystagmus, a small nose, and long philtrum. The ears were low set. There was a simian crease in one hand, as well as long slender fingers. A left inguinal hernia with undescended testis was present. No heart defect was detected by either cardiac ultrasound or electrocardiogrphy. Magnetic-resonance imaging of the brain showed agenesis of the corpus callosum and dilated ventricles. At age 6 mo, contact was poor, and the child was hypotonic. Head circumference was 41 cm (3%-ile = 42 cm), length 62.5 cm (25%-ile), and weight 5.6 kg (3%-ile = 6.2 kg).

Cytogenetic Studies

High-resolution G-banded chromosomes were prepared from peripheral white blood cells, according to standard procedures.

Probe Isolation.—The subtelomeric probe for 8p used in this study is the P1 artificial chromosome (PAC) 63M14 (National Institutes of Health and Institute of Molecular Medicine 1996). A set of YACs from 8p23 was selected on the basis of mapping information from the YAC contig WC8.1 (Genome Database). YAC DNA was isolated according to standard protocols (Sambrook et al. 1989). The human inserts were amplified by inter-*Alu* PCR as described elsewhere (Lengauer et al. 1992). A DNA probe for GATA4 was made by PCR by means of genomic DNA, used as a template, and sequence-tagged site (STS) primers SHGC-33472 contained in GATA4 (Genome Database). The PCR fragment was labeled and was used to screen the RPCI5 human genomic PAC library from Pieter de Jong. Positive colonies were selected, and PAC DNA was prepared by means of the Quiagen plasmid-purification kit.

To confirm the presence of the GATA4 gene within PAC 1043K1, a PCR amplifying the 3′ end of the GATA4 gene was developed. The primer set 5-CTAGACCGT-GGGTTTTGCAT-3′ and 5′-TGGGTTAAGTGCCCC-TGTAG-3′ was used to amplify a fragment in the 3′ end of the GATA4 gene, by standard PCR conditions.

FISH.—Metaphase spreads were prepared from phytohemagglutinin-stimulated human peripheral blood lymphocytes cultured for 72 h. Then 1 μg of DNA was labeled with either biotin-16-dUTP (Boehringer Mannheim) or Lissamine-5-dUTP (DuPont/NEN), by the Nick Translation System (Gibco-BRL). Unincorporated nucleotides were removed through Sephadex G-50 columns (Nick columns; Pharmacia Biotech). Prior to FISH, the slides were treated with RNAse A and pepsin, as described elsewhere (Wiegant et al. 1992). Human Cot1 DNA (Life Technologies) was used as a competitor. Denaturation of the slides and probes, as well as hybridization and subsequent cytochemical detection of the hybridization signals, were as described elsewhere (Vermeesch et al. 1995). Posthybridization treatment included three 5-min washes in 50% formamide, 2 × SSC, followed by three 5-min washes in 2 × SSC at 42°C. Chromosomes were counterstained with 4,6-diamidino-2-phenylindole, and the slides were mounted in Vectashield mounting medium (Vector Laboratories). The signal was visualized by means of digital imaging microscopy, by means of a cooled charge-coupled–device camera (Photometrics). Merging and pseudocoloring were performed by Smart Capture software (Vysis).

Microsatellite Analysis

A panel of microsatellite markers (CA repeats) on distal 8p were analyzed in DNA extracted from peripheral

white blood cells from the patients and parents. The primer sequences were obtained from Dib et al. (1996). One primer of each pair was fluorescently labeled with fluorescein isothiocyanate (FITC). Genomic DNA (300 ng) was used for 30 cycles in an amplification in 50 μl of PCR mix containing 200 μM of each dNTP, 0.5 μM of each primer, and 1 U of *Taq* polymerase (Perkin-Elmer). Cycling conditions were as follows: 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. The PCR products were electrophoresed on an ALF DNA sequencer (Pharmacia Biotech) and were analyzed by Fragment Manager software (Pharmacia Biotech).

Results

Cytogenetic Analysis

The results of the cytogenetic analyses are shown in table 1. In the majority of patients (Pt2–Pt7), a small, apparently terminal deletion was detected, encompassing bands 8p23.1-pter. In Pt1, a large terminal deletion, del(8)(p21.3–pter), was present. In Pt8, the deletion was tentatively identified as del(8)(p22.1–pter), and, in Pt9, an interstitial deletion, del(8)(p12–p22), was present. In all patients, the deletion was de novo, with normal parental karyotypes.

FISH

YACs covering chromosome bands 8p22-8p23.1 were isolated and were used as probes to determine the extent and location of the deletions in all patients (table 2). FISH analysis using a subtelomere probe for 8p showed a normal signal on both chromosomes 8, indicating the presence of an interstitial deletion in all patients except Pt1, who has a large terminal deletion.

In five patients (Pt2–Pt6) YACs 843-E1 and 770-E9 were deleted. A commonly deleted region on distal 8p was thus identified in these five patients. In Pt7, only YAC 843-E1 was deleted. In the two other patients without CHD but with microcephaly (Pt8 and Pt9), a deletion was detectable with YAC 859-A7, which is located on 8p22, 14 cM centromeric from YAC 770-E9. Therefore, the position of the deletion in these two patients was more centromeric and did not overlap with the commonly deleted region.

Screening a PAC library by means of a probe recognizing GATA4-gene sequences yielded five clones. After FISH on metaphase spreads from normal individuals, four clones were localized to the long arm of chromosome 8, whereas one PAC, 1043K1, was localized to 8p23. This is in agreement with the observation that the STS SHGC-33472 amplifies two loci on chromosome 8 (Genome Database). A PCR specific for the GATA4 gene (see the Patients and Methods section) yielded the expected-size product from PAC 1043K1 but not from the four other PACs. FISH showed that PAC 1043K1 was deleted in all five patients (Pt2–Pt6) with a deletion of the commonly deleted region but not in Pt7.

Microsatellite Analysis

DNA from patients and parents was available for Pt2, Pt3, Pt5, and Pt6. DNA was available from Pt7 but not from his parents. No DNA was available from Pt1, Pt4, Pt8, and Pt9. The results of microsatellite analysis are summarized in table 3. In all four patients for whom parental DNA was available, absence of inheritance of a maternal allele could be shown for markers within the deleted region, providing evidence for a deletion on the maternally derived chromosome ($P = .06$, one-tailed binomial test assuming an equal parental origin of the deletions).

The results confirmed the presence of a commonly deleted region on distal 8p. Combining these data with the FISH results allowed us to map this region more precisely, with the distal breakpoint between markers D8S1825 and D8S1706 and with the proximal break-

Table 2

Deletion Sizes Detected by FISH

	Status of YACs and PAC 1043K1[a]								
	Subtelomere	918-C6	1043K1	843-E1	770-E9	773-G4	799-B1	752-D3	859-A7
Pt1	Deleted	Deleted	…	Deleted	Deleted	Deleted	Deleted	Deleted	Deleted
Pt2	Present	Present	Deleted	Deleted	Deleted	Present	Present	Present	Present
Pt3	Present	Present	Deleted	Deleted	Deleted	Present	Present	Present	Present
Pt4	Present	Present	Deleted	Deleted	Deleted	Present	Present	Present	Present
Pt5	Present	Present	Deleted	Deleted	Deleted	Present	Present	Present	Present
Pt6	Present	Present	Deleted	Deleted	Deleted	Present	Present	Present	Present
Pt7	Present	Present	Present	Deleted	Present	Present	Present	Present	Present
Pt8	Present	Present	…	Present	Present	Present	Present	Present	Deleted
Pt9	Present	Present	…	Present	Present	Present	Present	Present	Deleted

[a] The order is telomeric to centromeric and is derived from the Whitehead Institute for Biomedical Research/MIT Center for Genome Research radiation-hybrid map for chromosome 8. An ellipsis (…) denotes that the patient was not tested for the marker.

Table 3

Deletion Sizes Detected by Microsatellite Analysis

Marker[a]	Estimated Maximum/Minimum Distance from Telomere (Mb)	Status in[b]				
		Pt2	Pt3	Pt5	Pt6	Pt7
D8S277	7.51/7.72	Present	Present	Present	Present	NI
D8S1706	8.36/8.72	Present	Present	Present	NI	NI
D8S1825	11.46/11.46	NI	Deleted	Deleted	…	NI
SHGCD-33472	11.6/11.6					
D8S503	11.69/11.92	Deleted	Deleted	NI	…	NI
D8S516	11.77/11.99	NI	Deleted	Deleted	Deleted	NI
D8S542	11.69/11.99	Deleted	NI	Deleted	…	NI
D8S1721	11.69/11.99	Deleted	Deleted	Deleted	NI	NI
D8S520	12.30/12.64	Deleted	NI	Deleted	…	NI
D8S1755	12.64/13.44	Deleted	Deleted	Deleted	NI	NI
D8S550	12.98/12.98	NI	Deleted	Deleted	…	NI
D8S265	13.13/13.28	Deleted	NI	Deleted	…	NI
D8S1695	12.64/13.28	NI	NI	NI	…	Present
D8S1759	12.39/13.28	NI	NI	NI	Deleted	Present
D8S552	14.48/14.48	Present	Present	NI	Present	Present

[a] The order is telomeric to centromeric and is derived from the Whitehead Institute for Biomedical Research/MIT Center for Genome Research radiation-hybrid map. SHGC-33472 indicates the position of the marker detecting the GATA4 gene.

[b] NI = not informative (including possibly deleted); an ellipsis (…) denotes that the patient was not tested for the marker.

point around the distal end of YAC 773-G4, near D8S1759 and D8S1695 (fig. 1). This corresponds to an estimated region of 5.76–6.12 Mb (Genome Database). In Pt7, a smaller deletion was present that minimally includes the 580-kb YAC 843-E1 and that maximally spans the region between SHGC-33472 and markers in YAC770-E9, corresponding to ~2 Mb (fig. 1).

Discussion

We have performed genotype-phenotype correlations in nine unrelated patients with a de novo del8p. In five patients, a uniform interstitial deletion of ~6 Mb in 8p23.1 was detected. One patient carried a large terminal deletion encompassing this commonly deleted region. All these patients have a similar phenotype, with a CHD, microcephaly, mild developmental delay, intrauterine growth retardation, and a characteristic behavioral phenotype. Interestingly, one additional patient with a partial phenotype (i.e., MR and behavioral disturbances but neither CHD nor microcephaly) carried a deletion of only part of the commonly deleted region. This suggests that the different clinical features are probably caused by the deletion of contiguous genes within the commonly deleted region. Alternatively, we cannot exclude the possibility that the absence of a CHD and microcephaly in this patient are the result of nonpenetrance. However, clinical studies indicate a high penetrance of both cardiac defects and microcephaly in patients with cytogenetically large terminal 8p deletions (which almost certainly include this commonly deleted region) (Dobyns et al. 1985; Ostergaard and Tommerup 1989). Therefore, the critical deletion region for microcephaly and heart defects, HDCR8p, corresponds to the commonly deleted region, most likely with the exclusion of the region deleted in the patient (Pt7) with a smaller deletion. It is probable that haploinsufficiency for one or more genes in the HDCR8p will lead to disrupted heart development. The homologue of the human GATA4 gene is involved in cardiac morphogenesis in the mouse, and GATA4 maps to human 8p23.1–pter (Huang et al. 1996; Kuo et al. 1997; Molkentin et al. 1997). We have shown that this gene is deleted in all of our patients with a heart defect but not in our patient without a heart defect. The GATA4 gene is therefore a candidate gene for CHD.

Another chromosomal aberration involving distal 8p, San Luis Valley recombinant chromosome (8) (SLVRec[8]), is associated with CHD in >90% of cases (Gelb et al. 1991). This chromosomal aberration is the unbalanced product of a pericentric inversion, inv(8)(p23;q22), with both a deletion of distal bands 8p23-pter and a duplication of bands 8q22-qter (Gelb et al. 1991). The breakpoint in 8p resides in or near the squalene synthase gene (FDFT1) (Patterson et al. 1995). Interestingly, this gene maps to the commonly deleted region in 8p, defined here as being between microsatellite markers D8S520 and D8S550 (Genome Database). Therefore, the del8p in patients with SLVRec(8) involves part of the commonly deleted region. However, the CHDs in SLVRec(8) differ from those seen in patients with del8p and typically are conotruncal heart defects

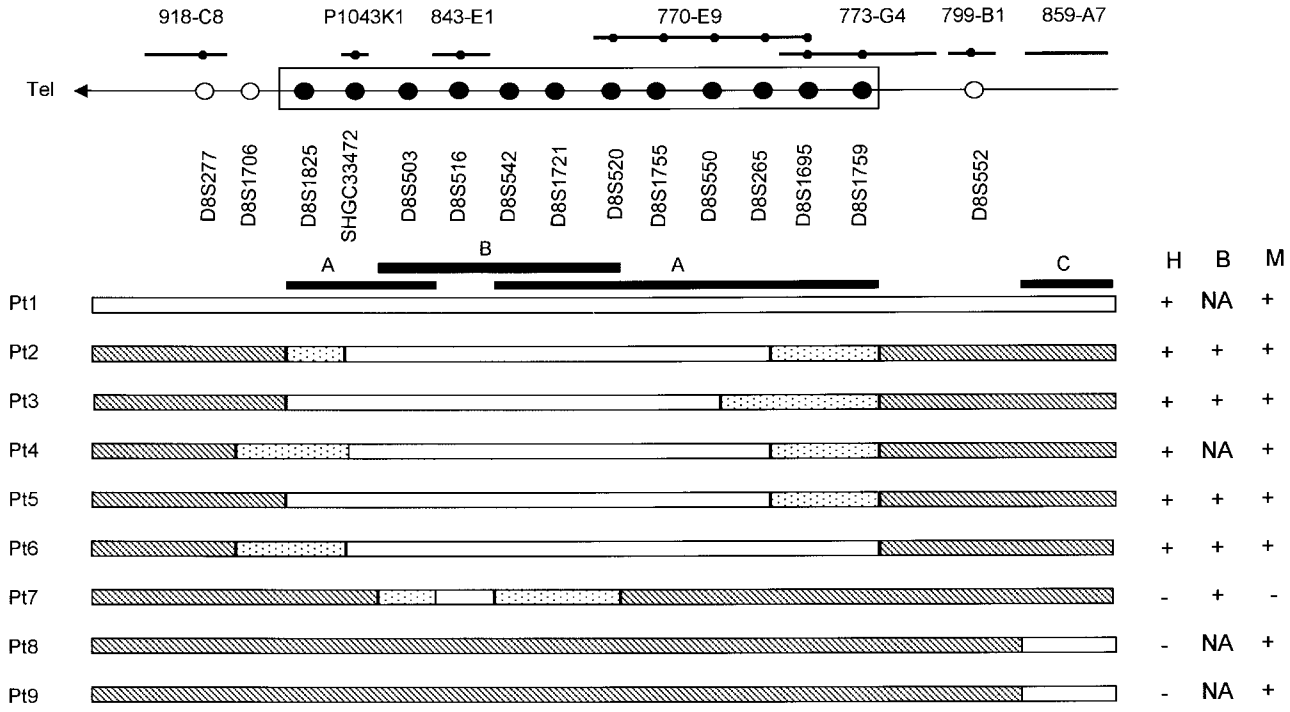

Figure 1 Position of the YACs (*top*) and microsatellites (*bottom*). The extent of del8p in the patients is shown, and the normal chromosomal regions (cross-hatched boxes), maximal limits of the deleted regions (dotted boxes), and minimal deleted regions ("open" boxes) are indicated. The clinical features are summarized: H = heart defect; B = characteristic behavior; M = microcephaly; NA = not assessable. The commonly deleted region of the microsatellites is boxed. The two separate critical deletion regions, for heart defects (A) and microcephaly (B), which are interrupted by the critical deletion region for the characteristic behavior, are indicated, as are the more proximal deletions (C) that are also associated with microcephaly.

such as tetralogy of Fallot (Gelb et al. 1991). AVSD has not been observed in SLVRec(8) (Gelb et al. 1991). One possible explanation could be that the gene(s) responsible for the CHD in del8p is centromeric from the SLVRec(8) breakpoint. Alternatively, the CHDs in SLVRec(8) may result from the combined effects of the 8q duplication/8p deficiency associated with this chromosomal aberration, as has been suggested by Gelb et al. (1991).

The characteristic heart defect associated with a del8p is AVSD. Interestingly, in all three patients with an AVSD who have been described here, as well as in several other published cases, this heart defect was associated with right-sided lesions, such as hypoplastic right ventricle (HRV) and PVS (Digilio et al. 1998). This type of AVSD can be distinguished from the AVSD seen in Down syndrome and resembles the AVSD associated with Ivemark syndrome (Carmi et al. 1992). The AVSD in trisomy 21 and Ivemark syndrome therefore probably have a different pathogenesis, the latter resulting from a primary defect in left-right situs determination (Carmi et al. 1992). Interestingly, in two of the patients studied here, isomerism of the atria was present as well and, in one of them, was associated with azygoscontinuation and malrotation of the gut. This further extends the similarity with the Ivemark syndrome and suggests that the CHD observed in del8p may also be part of a lateralization defect.

A characteristic behavioral phenotype, with early-onset hyperactive, impulsive behavior has been described in children with a del8p (Claeys et al. 1997). Two previously unreported patients included in the present study had the same behavioral phenotype, thus confirming the findings by Claeys et al. (1997). Genotype-phenotype correlations in the present study suggest that the locus for this behavior is within the subregion of the commonly deleted region, defined by the patient carrying a smaller deletion who also had the characteristic behavior. Since this patient also has mild developmental delay, which could not otherwise be explained, this subregion also contains one or more loci for developmental delay. Whether the behavioral phenotype and developmental delay are caused by haploinsufficiency for the same gene(s) is not known.

In most cases, the apparently terminal deletions in 8p that have been studied here were, in fact, interstitial de-

letions. One possible explanation is that the deletion of the subtelomeric region of 8p would result in a prenatal lethal phenotype. However, this is not supported by our observation of a patient with a large terminal deletion but without either additional congenital malformations or a more severe phenotype. Two other patients with a terminal deletion of 8p also had no additional clinical features (Wu et al. 1996). Alternatively, the finding that the breakpoints cluster within the same chromosomal regions may suggest a common underlying mechanism predisposing to the deletion of this interstitial fragment. This is reminiscent of the microdeletions found in Williams and DiGeorge syndromes, in which breakpoints also cluster within the same region and in which flanking repetitive sequences may predispose to unequal crossing-over during meiosis (Baumer et al. 1998). In all four informative patients, the deletion was of maternal origin. Although more observations are needed to confirm this, it suggests a bias in the parental origin of the common deletion in 8p, most likely because the deletion preferentially occurs during formation of maternal germ cells. Sex-dependent mechanisms in the origin of interstitial deletions have also been described in both Charcot-Marie-Tooth 1A and hereditary neuropathy with liability to pressure palsies (Lopes et al. 1997). In summary, the commonly deleted 8p23.1 region delineated in this study will be the starting point for a further molecular characterization of this region—and, ultimately, identification of genes implicated in CHD, MR, microcephaly, and the characteristic behavioral phenotype.

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Electronic-Database Information

Accession numbers and the URLs for data in this study are as follows:

Genome Database, http://www.gdb.org/ (for YAC contig WC8.1 [5672347] and STS primers SHGC-33472 within GATA4 [6454751])

Whitehead Institute for Biomedical Research/MIT Center for Genome Research, http://www-genome.wi.mit.edu (for radiation-hybrid map for chromosome 8)

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