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Characterization of 10p Deletions Suggests Two Nonoverlapping Regions Contribute to the DiGeorge Syndrome Phenotype

To the Editor:

DiGeorge syndrome (DGS; MIM 188400 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?188400]) is a developmental-field defect characterized by abnormalities of structures derived from the pharyngeal arches and pouches (for a review, see Driscoll and Emanuel 1996). The vast majority of individuals with DGS have been found to have deletions of chromosomal region 22q11.2. However, a small number of patients have been shown to have deletions of chromosome 10p, with normal chromosome 22s (for a review, see Greenberg et al. 1988). We report here the location and extent of the deletion on chromosome 10, determined by means of a combination of heterozygosity tests and FISH analysis, in five DGS patients. Our results do not support the existence of a single, commonly deleted region on 10p in these five patients. Rather, they suggest that deletion of more than one region on chromosome 10p can be associated with the DGS phenotype.

We examined the extent of the chromosome-10p deletions in five patients. Phenotypic characterizations of three (GM6936, CH92-304, and CH95-199) of the five patients were reported elsewhere, by Greenberg et al. (1986), by Monaco et al. (1991) and Pignata et al. (1996), and by Lipson et al. (1996), respectively. Molecular characterizations of the deletions present in GM6936 and CH95-199 were also reported by Daw et al. (1996). Patient CH95-199 is designated as "P3" in Daw et al. (1996). The remaining two patients were referred to us after their deletions were detected by means of high-resolution cytogenetic analysis. All of the patients exhibit at least one of the classic features of DGS (cardiac defect, hypocalcemia, and/or immune defect). Clinical findings are summarized in table 1.

We first performed heterozygosity mapping by use of markers between loci D10S249 and D10S213. These sequence-tagged sites (STSs) correspond roughly to the cytogenetic location 10p15-10p12 (Chumakov et al. 1995). Three of the five patients had a single allele for the three most distal markers, D10S249, D10S591, and D10S189; this finding is consistent with a terminal deletion of 10p (table 2). The remaining two patients were heterozygous, at loci D10S249, D10S591, and D10S189, but had single alleles for a series of more centromeric markers (table 2); this finding is consistent with the presence of an interstitial deletion. As shown in table 2, there is no marker for which all patients have a single allele, which suggests that there is no common region-of-deletion overlap among these five patients.

To confirm the heterozygosity-mapping results, we performed FISH analysis on these five patients, using YACs from the region. All YACs were from the CEPH/Genethon megaYAC library, except 194G1, which was isolated from the smaller-insert CEPH library. FISH analysis confirmed and extended the results obtained from the heterozygosity tests: there is no common region of overlap among all five patients (fig. 1). Four of the five patients have a common region of deletion that includes the shortest region-of-deletion overlap (SRO) described in Daw et al. (1996). Two of these four patients, GM6936 and CH95-199 ("P3"), were included in Daw et al. (1996), and the other two do not narrow the SRO further. Patient CH92-092, although without a deletion for this SRO, does share an extensive region of deletion with the other two patients with terminal deletions. Further, it is possible that there is a small region-of-deletion overlap between CH92-092 and CH92-304. The endpoints of the deletions in these two patients could not be defined precisely because the YAC contig is not continuous from D10S1431 to D10S226. On the basis of the results presented here, it is not possible to attribute

Table 1

Clinical Features of Five Patients with 10p Deletions

Patient	Sex	Cardiac Defect	Hypocalcemia	Immune Defect	Cleft Palate	Facial Dysmorphia	Developmental Delay	Other[a]
CH92-092	F	−	+	+	−	−	+	Right renal hypoplasia, strabismus, high arched palate
CH92-304[b]	M	−	+	+	−	+	+	. . .
CH92-319	F	−	+	−	−	+	. . .[c]	Hearing loss, short neck, syndactyly (digits 3 and 4), long fingers
CH95-199[d,e]	F	+	−	+	+	+	+	GE reflux, finger contractures
GM6936[f]	F	−[g]	+	+	−	+	+	Ureteral reflux, recurrent UTI, short neck, mild pectus excavatum

NOTE.—Plus sign (+) = presence of the condition, and minus sign (−) = absence of the condition.
[a] GE = gastroesophageal; UTI = urinary tract infection.
[b] Reported by Monaco et al. (1991).
[c] Patient too young to be evaluated.
[d] Reported by Lipson et al. (1996).
[e] This patient is referred to as "P3" in Daw et al. (1996).
[f] Reported by Greenberg et al. (1986).
[g] Probable pulmonic branch stenosis.

the DGS phenotype to deletions of only one interval on chromosome 10p. Rather, these results suggest that there are at least two regions on chromosome 10p that, when deleted, can result in features seen in DGS.

After identification of two nonoverlapping regions of 10p loss, we screened cytogenetically normal DGS patients for submicroscopic deletions of 10p. These patients had been the subjects of molecular study and had been shown not to have deletions of 22q11.2. Samples for cytogenetic analysis were available from 11 patients who had the diagnosis of DGS and no detectable deletion of 22q11. These patients appear to have normal chromosome 10s by analysis with high-resolution G-banding. We performed FISH analysis on these 11 patients, using YACs from two deleted regions on chromosome 10p. YACs 916D6 and 959B9 map to the SRO defined by Daw et al. (1996), and YAC 944D12 maps to a second, more telomeric locus (see fig. 1). None of the patients we examined appeared to have deletions. However, because the YACs are large, it is possible that a small deletion within these regions would not have been detected. When a smaller deletion interval is defined, perhaps these patients can be reexamined.

In conclusion, we examined chromosome 10p deletions in five patients diagnosed with DGS. Our results indicate that there is no apparent common region of overlap between these five patients. Instead, these results suggest that, in addition to the SRO reported by Daw et al. (1996), there is a second, more telomeric region on chromosome 10p that, when deleted, can produce a

Table 2

Results of Heterozygosity Tests

	MARKER (POSITION)									
PATIENT	D10S249 (0 cM)	D10S591 (12.3 cM)	D10S189 (17.3 cM)	D10S1431	D10S226 (26.2 cM)	D10S465	D10S585 (28.9 cM)	D10S570 (32.1 cM)	D10S611	D10S213 (56.9 cM)
CH92-92	1	1	1	1	2	2	2	2	2	2
CH92-304	2	2	2	1	1	1	1	1	1	2
CH92-319	1	1	1	1	1	1	1	1	1	2
CH95-199	2	2	2	2	2	2	1	1	2	2
GM6936	1	1	1	1	1	1	1	2	2	2

NOTE.—Markers are named in order; the most distal markers are on the left. Map positions are given for those markers currently available from the Whitehead Institute/MIT Center for Genome Research. The numbers "1" and "2" indicate the number of alleles present at a particular locus. Markers that have been underlined are contained within the YACs shown in figure 1.

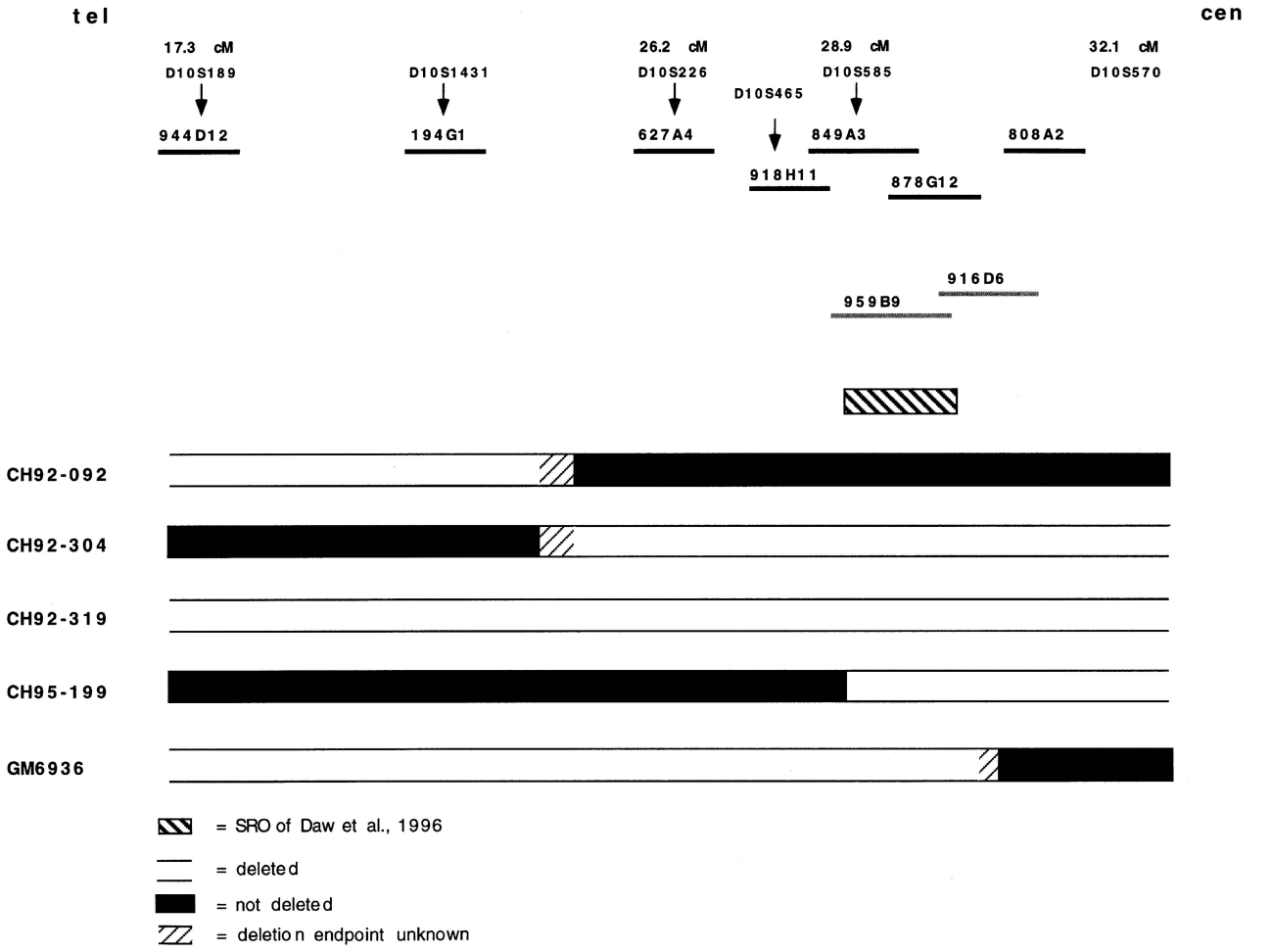

Figure 1 Diagram showing the regions-of-deletion overlap, as determined by the combination of heterozygosity tests and FISH analysis. At the top of the figure are YACs that were used for FISH analysis. Two of the three YACs used for the analysis of chromosomally normal patients, 959B9 and 916D6, are stippled, because the 10p-deletion patients were not tested with these two YACs. The STSs that are positive for a given YAC are shown. Map positions for several of the markers are also shown (taken from Hudson et al. [1995], with supplementary data from the Whitehead Institute/MIT Center for Genome Research, Human Genetic Mapping Project, data release 12 [July 1997]).

DGS phenotype. Alternatively, it is possible that either CH92-092 or CH95-199, the two patients who appear to have no region-of-deletion overlap, has a small internal deletion, rearrangement, or point mutation that maps within the region that is deleted in the other patients. Further characterization of these patients, as well as analysis of additional DGS patients with deletions on chromosome 10p, will enable us to distinguish between these possibilities. These findings also suggest that it is premature to screen for microdeletions of 10p in DGS patients in whom no 22q11 deletions have yet been identified.

One interesting feature of the patients examined here is that there is no obvious correlation between the phenotypic traits of the patients and the extent of the deletion. In particular, the patient (CH92-319) with the largest deletion exhibits one of the less severe phenotypes. Because of this observed variability, it is not possible to associate deletions in one region of the chromosome with a particular phenotype. The lack of a correlation between the size of a deletion and the phenotype is also observed in patients with deletions on chromosome 22, and may be a characteristic of haploinsufficiency disorders.

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