3600del11 show homology (6 of 8 bp) to the chi-recombination-stimulating element GCTGGTGG (Smith 1983; Steinmetz et al. 1987). Whether the deletion-promoting influence of symmetric elements is a gene-specific (i.e., regional) phenomenon or whether it applies to genomic DNA in general is at present unclear, but the increasing number of disease-associated inherited deletions, within gene-coding regions,that are being reported in the literature (Krawczak and Cooper 1997) will soon allow this question to be addressed.

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Goosecoid-Like Sequences and the Smallest Region of Deletion Overlap in DiGeorge and Velocardiofacial Syndromes

To the Editor:

In the May 1997 issue of the *Journal,* Gottlieb et al. (1997) reported the identification of a homeobox-coding gene named "*GSCL*" (*Goosecoid*-like) from a 22q11.2 region deleted in DiGeorge syndrome (DGS) and velocardiofacial syndrome (VCFS). The gene is located within the so-called minimal DiGeorge critical region (MDGCR), as defined by Lindsay et al. (1993) and Gong et al. (1996). Figure 3 of that article (Gottlieb et al. 1997, p. 1199) shows the *GSCL* gene as localized in the smallest region of deletion overlap (SRDO, a subsegment of the MDGCR), because *GSCL* was presumed to be deleted in patient G. Patient G is affected by DGS and has an interstitial deletion the proximal breakpoint of which defines the proximal boundary of the SRDO (Levy et al. 1995). However, chromosomes from patient G were not tested with *GSCL* sequences. We decided to perform experiments ourselves to test whether patient G is in fact deleted for *GSCL*.

From our bacterial-artificial-chromosome contig covering the homologous mouse region (Botta et al., in press), we have subcloned a 3.7-kb *Sma*I DNA fragment corresponding to nt 27970–31642 of the genomic sequence MMU70231 (Galili et al. 1997) and containing the three coding exons of the murine *Gscl*. We have used this fragment to screen our contig of the human DGS critical region (Lindsay et al. 1996). A 12.2-kb *Hin*dIII DNA fragment (pHgscl) was identified and subcloned from fosmid 39g9. Partial sequencing confirmed that this fragment contains *GSCL* sequences and corresponds to nt 129598–141800 of the genomic sequence HSU30597 (Gottlieb et al. 1997); thus it includes the entire *GSCL* gene, as characterized by Gottlieb et al. (1997), with the caveat that the transcription initiation of this gene has not yet been experimentally determined but has only been deduced on the basis of sequence features. FISH experiments on patient G's chromosomes showed that pHgscl is not deleted (fig. 1*A*); no detectable difference was seen in the hybridization-signal intensities in the two chromosomes. The *Not*I-*Hin*dIII 3.7-kb fragment (containing most of the coding sequences) was hybridized to restriction-digested genomic DNA from patient G and normal controls. *Bam*HI, *Hin*dIII, *Taq*I, and *Xho*I were tested. With none of these enzymes could we detect a rearranged genomic fragment in patient G. In particular, with *Hin*dIII the expected 12.2-kb band, corresponding to the fragment cloned, was detected apparently intact. Fosmid 39g9, from which pHgscl was subcloned, contains 5′ sequences of gene *ES2,* alias *DGS-I* (the clone was termed "Fos39" in the article by Lindsay et al. [1996]) and, by FISH analysis, is partially deleted in patient G (fig. 1*B*); hence, *GSCL* is located between *ES2* and the deletion breakpoint in patient G, within ~20–30 kb of DNA (fig. 2). Of course, even though *GSCL* is not deleted in patient G, the deletion may still affect its

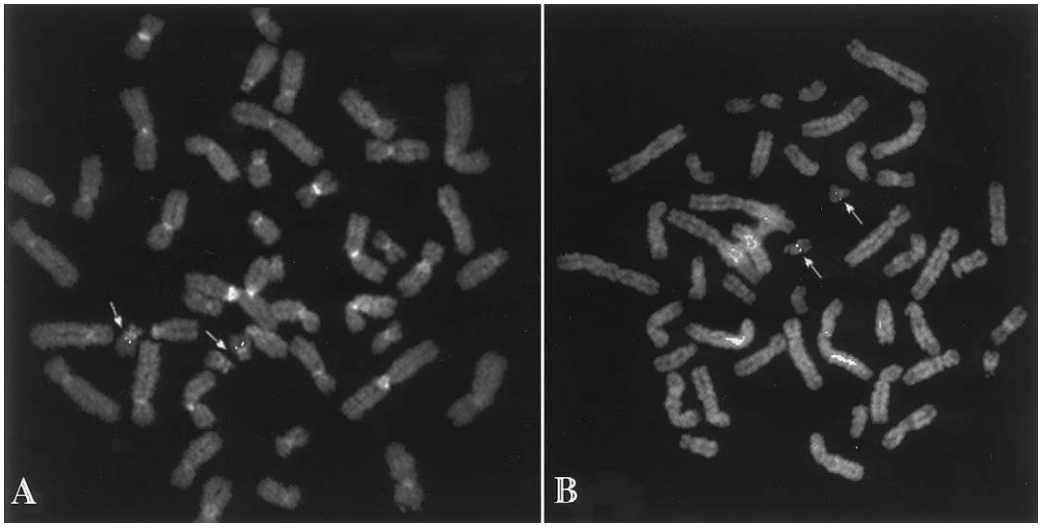

Figure 1 Examples of FISH experiments on chromosomes of patient G. *A,* Probe pHgscl, a *Hin*dIII 12.2-kb cloned fragment containing the GSCL gene, hybridizes to both chromosomes 22. *B,* Fosmid 39g9, which contains 5′ sequences of the gene ES2 and from which pHgscl was subcloned, consistently produced a lower-intensity signal on one of the chromosome 22 homologues, indicating that 39g9 sequences are partially deleted from this chromosome. The chromosome 22 homologues can be distinguished from each other because of the different morphology of the short arms, a normal cytogenetic variant.

normal expression/regulation. Unfortunately, this is impossible to test, because the function of *GSCL* is probably most significant for the phenotype during early embryogenesis. Even if it is outside the SRDO, *GSCL* remains an important candidate gene, because deletion breakpoints may affect this as well as other genes in the region. With the presence of nonoverlapping genetic lesions (e.g., those in ADU and in patient G; Levy et al. 1995) and with the recent proposal of a second, distal critical region (Kurahashi et al. 1996), the biological significance of "critical region(s)," MDGCR, and SRDOs (or other acronyms used by various research groups) remains to be understood. Suggestions have been made (Dallapiccola et al. 1996), but the hunt for the elusive "DiGeorge gene(s)"—or, why not, "DGGs"—is not over.

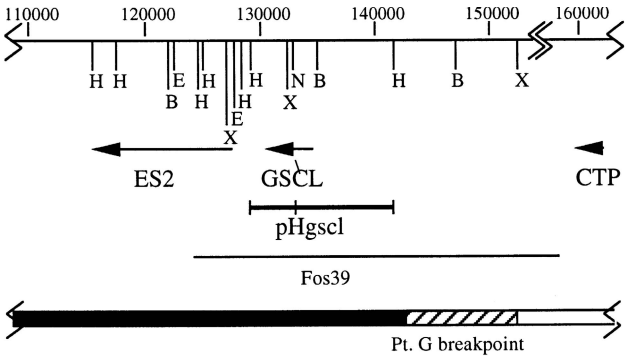

Figure 2 Schematic map showing both the position of the reagents used in the experiments presented, the interval within which the breakpoint in patient G is localized (*hatched portion of bar*), the region not deleted in this patient (*blackened portion of bar*), and the deleted region (*unblackened portion of bar*). Numbers on top indicate nucleotide number of the genomic sequence HSU30597. The restriction sites were mapped by use of sequence information. B = *Bam*HI; H = *Hin*dIII; E = *Eco*RI; N = *Not*I; and X = *Xho*I. The centromere is on the left.

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Reply to Pragliola et al.

To the Editor:

In their letter to the editor, Pragliola et al. (1997 [in this issue]) have noted that in our manuscript (Gottlieb et al. 1997) we assume that the *GSCL* gene is deleted in patient G (Levy et al. 1995), a patient with DiGeorge syndrome who has an atypical deletion boundary in chromosome 22. Their FISH analysis indicates that *GSCL* in fact is not deleted in this patient. Since patient G was unavailable to us for analysis, we were unable to verify the deletion status of *GSCL*. We based our estimate of the deletion boundary in patient G on the information provided in a previous paper published by the same senior investigator (Rizzu et al. 1996): in an article by Rizzu et al. (1996), the deletion endpoint of patient G is described as 100–150 kb telomeric to the ADU/VDU breakpoint, "close to the 5′ end" of the *DGSI/ES2* gene. Since we had the complete sequence of the ~120-kb region extending from the ADU breakpoint to *GSCL,* and since we knew that the 5′ end of *GSCL* is ~6 kb from *DGSI/ES2,* we felt that it was reasonable to assume that *GSCL* was disrupted or deleted in this patient. Pragliola et al. indicate in their letter that, although the deletion endpoint in patient G is within a fosmid containing *GSCL,* the breakpoint is distal to the 5′ end of the gene. However, as they note—and as we pointed out in our paper—a deletion or translocation breakpoint can easily affect the expression of genes in the vicinity. Therefore, the expression of *GSCL* could still be affected in patient G. Moreover, as we stated in our paper, the definition of a minimal critical region does not exclude a role for genes outside the region. Thus, although we consider *GSCL* to be a strong candidate gene for some of the abnormalities associated with DiGeorge syndrome/velocardiofacial syndrome, we have not excluded the possibility that genes outside the regions that we have called the "DiGeorge minimal critical region," or the smallest region of deletion, play a role in the disease phenotype. We appreciate the additional data from Pragliola et al., clarifying the location of the proximal breakpoint in patient G.

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