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A Genetic Etiology for DiGeorge Syndrome: Consistent Deletions and Microdeletions of 22q11

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Summary

DiGeorge syndrome (DGS), a developmental field defect of the third and fourth pharyngeal pouches, is characterized by aplasia or hypoplasia of the thymus and parathyroid glands and by conotruncal cardiac malformations. Cytogenetic studies support the presence of a DGS critical region in band 22q11. In the present study, we report the results of clinical, cytogenetic, and molecular studies of 14 patients with DGS. Chromosome analysis, utilizing high-resolution banding techniques, detected interstitial deletions in five probands and was inconclusive for a deletion in three probands. The remaining six patients had normal karyotypes. In contrast, molecular analysis detected DNA deletions in all 14 probands. Two of 10 loci tested, D22S75 and D22S259, are deleted in all 14 patients. A third locus, D22S66, is deleted in the eight DGS probands tested. Physical mapping using somatic cell hybrids places D22S66 between D22S75 and D22S259, suggesting that it should be deleted in the remaining six cases. Parent-of-origin studies were performed in five families. Four probands failed to inherit a maternal allele, and one failed to inherit a paternal allele. On the basis of these families, and of six maternally and five paternally derived unbalanced-translocation DGS probands in the literature, parent of origin or imprinting does not appear to play an important role in the pathogenesis of DGS. Deletion of the same three loci in all 14 DGS probands begins to delineate the region of chromosome 22 critical for DGS and confirms the hypothesis that submicroscopic deletions of 22q11 are etiologic in the vast majority of cases.

Introduction

DiGeorge syndrome (DGS) is a developmental field defect of the third and fourth pharyngeal pouches, characterized by thymic aplasia or hypoplasia, absent or hypoplastic parathyroid glands, and conotruncal cardiac malformations. The etiology is presumed to be heterogeneous, with reported cases demonstrating autosomal dominant, autosomal recessive, X-linked, and chromosomal modes of inheritance (Lammer and Opitz 1986). Approximately 15%–20% of patients with DGS have chromosomal abnormalities (Greenberg et al. 1988). Most cytogenetically abnormal cases involve chromosome 22. They are either unbalanced translocations with monosomy 22pter→q11 (Back et al. 1980; de la Chapelle et al. 1981; Kelley et al. 1982; Greenberg et al. 1984, 1988; Augusseau et al. 1986; Bowen et al. 1986; Faed et al. 1987) or interstitial deletions, del(22)(q11.21q11.23) (Greenberg et al. 1988; Mascarello et al. 1989). On the basis of these cytogenetic findings, it has been hypothesized that the DGS critical region (DGCR) lies within 22q11.

Previous molecular studies of the two aforementioned interstitial deletion DGS probands demonstrated that loci D22S9 (p22/34) and D22S43 (pH32) flank the critical region proximally. Loss of an allele at a more distal locus, BCRL2, in one of these probands suggested that the distal boundary for the DGCR might be in proximity to the BCRL2 locus (Fibison et al. 1990*a*). Recently, DNA microdeletions were detected in probands whose karyotypes were reportedly normal on 450-band routine cytogenetic analysis. However, since none of the probes (KI-506, KI-197, and KI-716) were deleted in all patients studied, these

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markers were proposed as DGS flanking markers (Scambler et al. 1991).

We have examined 14 probands in detail, using molecular and high-resolution cytogenetic techniques (850-band stage). In the present study, we describe the results of our molecular analysis of these 14 probands, using RFLP and/or dosage analysis for 10 probes previously mapped to 22q11. The results of these studies support a genetic etiology for DGS and begin to delineate a DGCR. Furthermore, the identification of submicroscopic deletions of 22q11 in cytogenetically normal probands supports the use of molecular studies as a requisite adjunct to high-resolution cytogenetic analysis for the diagnosis and clinical management of patients with DGS.

Material and Methods

Cell Lines

Three DGS cell lines (GM07215, GM07939, and GM05876) were obtained from the Coriell Cell Repository (CCR; Coriell Institute for Medical Research, Camden, NJ). Two additional DGS cell lines have been described elsewhere: 7248 (Greenberg et al. 1988) and KM4987 (Mascarello et al. 1989). The remaining nine patients were obtained from our hospital and from referring physicians. Blood or skin was obtained to establish lymphoblastoid or fibroblast cell lines. Lymphoblastoid cell lines were established from the parents whenever possible, and our analysis includes a total of five families.

A chromosome 22 regional somatic cell hybrid mapping panel was used to determine the order of several of the probes used in the present study. The hybrid panel included (1) GM10888, a human/hamster hybrid containing a normal chromosome 22 as its only intact human chromosome; (2) GM11220, a human × Chinese hamster hybrid cell line made from a constitutional t(X;22)(q21;q11) which retains the der(X); (3) cl-9/GM05878, a human × hamster hybrid cell line made from a constitutional t(10;22)(q26.3;q11.2) which was selected for adenylosuccinate lyase and which retains the der(10) as the only relevant human chromosome; (4) Rad-110a, a radiation-reduced hybrid that appears to contain a single large fragment of chromosome 22 with proximal breakpoint between D22S66 (pH160b) and D22S259 (pR32); (5) GL5, a human × mouse hybrid made from a constitutional der(17)t(17;22)(p13;q11) which retains the der(17) as the only relevant human chromosome; (6) GB/cl-4, a human × hamster hybrid cell line, made from a patient with a constitutional t(11;22)(q23;q11) which retains the der(22) as the only relevant human chromosome; and (7) $514AA_2$-A_2, a human × mouse hybrid made from tumor cells of a patient with t(9;22)(q34;q11) acute lymphocytic leukemia and containing the der(22) as the only relevant human chromosome. Parts of this panel have been described elsewhere (McDermid et al. 1989; Budarf et al. 1991).

Probes

The probes utilized in the present study include anonymous markers pH98 (D22S57), pH11 (D22S36), pR32 (D22S259), pH160b (D22S66), and pH162 (D22S68) that have been isolated from flow-sorted chromosome 22 libraries (Budarf et al. 1991). We also used clone N25 (D22S75), isolated from a *Not*I linking library (McDermid et al. 1989); probe p22/34 (D22S9), isolated from a chromosome 22–enriched library and localized by in situ hybridization to 22q11 (McDermid et al. 1986); and probe W21G (D22S24), derived from a flow-sorted chromosome 22 library (Rouleau et al. 1989). The probe used for the BCR-related genes is a 162-bp cDNA *Sau*3A fragment from the 3′ end of the BCR gene (Croce et al. 1987; Budarf et al. 1988). Probe 22C1-18 (D22S10) was obtained from ATCC. Probes βIVS_2 and CRI-R365 (D11S129) were used as control probes for dosage studies. Both map to chromosome 11, an autosome not involved in DGS. βIVS_2 is a 920-bp unique fragment derived from the second intervening sequence of the β-globin gene. CRI-R365 is a unique 2-kb *Hin*dIII fragment (Donis-Keller et al. 1987).

Clinical and Cytogenetic Evaluation

Clinical information was obtained either from the referring physicians or from the literature for published cases. High-resolution cytogenetic analysis at the 800–850-band stage was performed in our laboratory using standard techniques or was performed by the referring hospital's clinical cytogenetics laboratory.

DNA Studies

DNA was extracted from the DGS and parental cell lines by routine methods and was digested with restriction enzymes as recommended by the manufacturer (New England BioLabs). Digested DNA was separated by agarose gel electrophoresis and was transferred to either Immobilon (Millipore) or Gene Screen Plus (DuPont) by using the method of Southern (1975). A depurination step (0.25 N HCl; 15 min) to

fragment the DNA for efficient transfer was performed. DNA probes were digested with the appropriate restriction enzymes and were purified in low-melt agarose by gel electrophoresis. DNA probes were labeled with [α-^{32}P]dCTP by using the random-primer method (Feinberg and Vogelstein 1984). Labeled probes N25, pR32, and p160b were preannealed with sonicated placental DNA (Litt and White 1985). Hybridization was either at 42°C with 50% formamide, 5 × Denhardt's solution, 3 × SSC, 1% SDS, 5% dextran sulfate, denatured herring sperm DNA (100 μg/ml) or at 65°C with 1% SDS, 1 M NaCl, and 10% dextran sulfate. Filters were washed twice with 0.2 × SSC, 0.1% SDS at 65°C and were exposed to Kodak XAR-5 film at −70°C for varying lengths of time.

Dosage Analysis

Quantitative analysis of Southern blots was performed to determine copy number. A data set consisted of genomic DNA from three DGS probands and from a normal control. Each set was repeated in triplicate on a single gel. Resulting filters were simultaneously hybridized both to a test probe and to one of two control probes (βIVS_2 or CRI-R365) that map to chromosome 11. Hybridization signal was quantitated by an imaging system that directly measured counts per minute (cpm) (AMBIS). Direct quantitation (in cpm) was performed to avoid nonlinearity problems encountered in densitometric measurements of autoradiographs. Standard hybridization signals were calculated by taking the ratio of cpm of the test probe to cpm of the control probe. Copy number is designated as two times the geometric mean of the triplicate ratios of standard signals from a DGS patient relative to a normal individual. It is assumed that one or two copies of a locus are present; values less than 1.50 are consistent with a deletion, whereas ratios greater than this are not.

Results

Clinical and Cytogenetic Studies

All of the patients and cell lines included in the present study were referred with the diagnosis of DGS on the basis of the presence of the cardinal features: conotruncal cardiac malformations, thymic aplasia or hypoplasia, and hypocalcemia. Cytogenetic analysis of the three cell lines obtained from CCR were initially reported as normal at the 400–450-band stage of resolution. Repeat analysis utilizing high-resolution banding techniques demonstrated a visible interstitial deletion of 22q11 in GM07215 and GM07939, while GM05876 had a possible deletion (B. Hoffman, personal communication). Patients 7248, KM4987, and DGS-4 have del (22)(q11.21q11.23). Patients DGS-2 and DGS-3 have possible cytogenetic deletions within 22q11. Patients DGS-1, DGS-5, DGS-6, DGS-7, DGS-8, and DGS9 were determined to have normal karyotypes by utilizing high-resolution banding techniques. Table 1 summarizes the cytogenetic and clinical findings for our 14 patients.

RFLP Analysis

DNA obtained from cell lines of five patients with DGS and from their parents was studied by RFLP analysis. Deletions were detected by demonstration of failure to inherit a parental allele. These DGS patient cell lines and those for whom parental DNA was unavailable were analyzed for the presence of two alternative RFLP alleles. Families who were uninformative by RFLP analysis, as well as probands who demonstrated a single allele at a test locus, were subsequently studied with dosage analysis. Three probes (pH11, N25, and pR32) detected deletions in the five DGS patients for whom parental DNA was available. Deletions were detected in two patients with normal karyotypes, in two with visible interstitial deletions, and in a fifth proband, whose cytogenetic study was inconclusive for a deletion.

Probe pR32 (D22S259) was informative in three of five families, those of probands 7248, DGS-4, and DGS-5. Figure 1 shows an autoradiogram of a Southern blot of genomic DNA digested with *Taq*I and probed with pR32. This probe detects a 10.1- and a 9.4-kb allele. The father is heterozygous in each family. The mothers of both 7248 (interstitial deletion) and DGS-5 (cytogenetically normal) are homozygous for the 9.4-kb allele. The probands in these two families have a single 10.1-kb allele, inherited from the father. Thus, the child in both of these families failed to inherit a maternal allele. The mother of DGS-4 (interstitial deletion) is homozygous for the 10.1-kb allele. The proband has a single 9.4-kb allele shared by the father. In these three families, the data are consistent with deletion of a maternal allele for D22S259.

Probe N25 (D22S75) was informative in one of five families. DGS-4 (interstitial deletion) demonstrated loss of a maternal allele at D22S75 (N25) (fig. 2). The N25 probe detects a *Taq*I polymorphism, producing alleles of either 3.3 or 2.3 and 0.96 kb, in addition to a 1.6-kb constant band (Fibison et al., submitted). The father of DGS-4 is homozygous for the 3.3-kb

Table 1

Summary of Clinical Findings and Cytogenetic Analysis of DGS Patients

Patient	Heart Defect[a]	Thymic Aplasia[b] or Hypoplasia	Hypocalcemia	Other Symptoms
Interstitial del 22(q11.21q11.23):				
7248	Truncus arteriosus, VSD, and right-sided aortic arch	+	+	Dysmorphic facies
KM4987	Interrupted aortic arch and VSD	+	−	Dysmorphic facies
DGS-4	Tetralogy of Fallot	−	+	
GM07215 ...	Interrupted aortic arch, ASD, and VSD	+	−	
GM07939 ...	Truncus arteriosus	+[c]	+	Dysmorphic facies
Possible interstitial deletion:				
GM05876 ...	Interrupted aortic arch and VSD	+	+	Dysmorphic facies
DGS-2	VSD	+[c]	+	Dysmorphic facies
DGS-3	PDA	+[c]	+	Dysmorphic facies
Normal karyotype:				
DGS-1	Truncus arteriosus, VSD, hypoplastic left heart	+	+	Dysmorphic facies, biliary cirrhosis, and abnormally lobed lungs
DGS-5	Interrupted aortic arch, hypoplastic aorta and valve, and VSD	+	+	Dysmorphic facies
DGS-6	Interrupted aortic arch, subaortic stenosis, ASD, VSD, and PDA	+[c]	+[d]	Dysmorphic facies
DGS-7	Truncus arteriosus and right-sided aortic arch	+	+[d]	MZ twin with double-outlet RV, thymic aplasia, and hypocalcemia
DGS-8	Overriding aorta, VSD, and infundibular stenosis	+[c]	+	Dysmorphic facies
DGS-9	Interrupted aortic arch and VSD	+	+	Dysmorphic facies and multicystic kidney

NOTE: — A plus sign (+) indicates presence of clinical feature; a minus sign (−) indicates absence of clinical feature.

[a] VSD = ventricular septal defect; ASD = atrial septal defect; PDA = patent ductus arteriosus.

[b] Includes both radiographic absence of thymic shadow and absent or small thymus either at surgery or on autopsy.

[c] Documented cellular immune deficiency.

[d] Transient decrease in serum calcium.

allele; the mother is homozygous for the alternative allele. DGS-4 has a single 3.3-kb, paternally inherited allele. This is consistent with the observed loss of the maternal allele at D22S259 (pR32).

Two of the five families were informative at locus D22S36 (pH11): DGS-3, with a possible cytogenetic deletion, and DGS-9, with a normal karyotype (fig. 3). Probe pH11 detects a *Msp*I polymorphism consisting of two alleles, 3.3 and 1.6 kb, and two constant bands, 3.7 and 2.3 kb. The parents of DGS-3 are homozygous for different alleles. DGS-3 has a 3.3-kb shared by his father, and he failed to inherit a maternal allele (1.6 kb). The father of the proband DGS-9 is homozygous for the 1.6-kb allele; the mother is heterozygous at this locus. Proband DGS-9 demonstrated a single 3.3-kb allele shared with her mother, failing to inherit a paternal allele.

An additional nine individual DGS probands, for whom parental DNA was not available, were examined for the presence of heterozygosity at these three loci. All probands demonstrated a single RFLP allele when hybridized with probes N25 and pR32, necessitating dosage studies to determine zygosity. Three of these probands (KM4987, GM07215, and GM07939) were heterozygous at D22S36 (pH11), placing D22S36 outside the DGCR.

Table 2 summarizes the results of RFLP analysis of DGS cell lines by utilizing the three aforementioned probes plus five additional polymorphic probes from 22q11. Patients designated as "hemizygous" are those who failed to inherit a parental allele, as determined by RFLP analysis. By RFLP-based family studies, deletions were detected in all five probands but at only three of the eight loci; D22S36 (pH11), D22S75 (N25), and D22S259 (pR32). RFLP analysis at proximal loci D22S24 (W21G), D22S9 (p22/34), and D22S57 (pH98) and at the more distal loci D22S10 (22C1-18) and D22S68 (pH162) failed to detect dele-

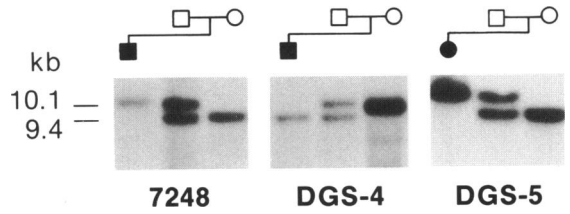

Figure 1 Autoradiogram of Southern blot of *Taq*I-digested DNA from three informative families of DGS probands (blackened circle or square) hybridized with probe pR32 (D22S259). The fathers (unblackened squares) are heterozygous for a 9.4- and a 10.1-kb allele. The mothers of probands 7248 and cytogenetically normal DGS-5 are homozygous for the 10.1-kb allele. The probands have the 9.4-kb allele in common with their fathers and failed to inherit a maternal allele. Proband DGS-4 inherited the paternal 9.4-kb allele and did not inherit a maternal allele.

tions in the five families studied, and 30%–40% of the probands tested were heterozygous at these loci.

Dosage Analysis

Probands were analyzed for copy number at loci D22S75 (N25), D22S259 (pR32), D22S66 (pH160b), and BCRL2. Southern blots of restriction enzyme–digested DNA were analyzed by the AMBIS radioanalytic imaging system to determine the number of alleles present. The results of the quantitation experiments are summarized in table 3 and are consistent with loss of an allele at both D22S75 (N25) and D22S259 (pR32) in all 14 patients.

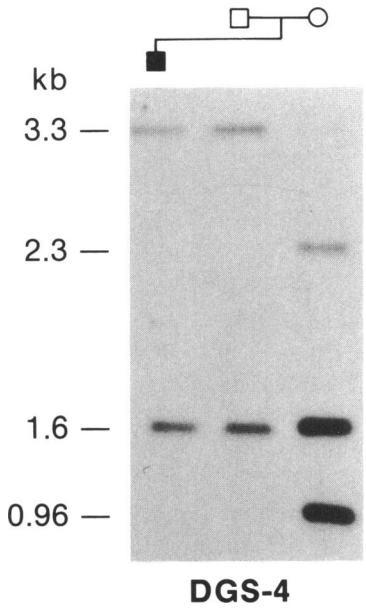

Figure 2 Hybridization of N25 (D22S75) to *Taq*I-digested DNA from proband DGS-4 and his parents. Probe N25 recognizes a 3.3- or 2.3- and .96-kb allele, as well as a constant band of 1.6 kb. The proband (blackened square) shares a single 3.3-kb band with his father (unblackened square). The mother (unblackened circle) is homozygous for the 2.3- and the 0.96-kb allele. The proband failed to inherit a maternal allele.

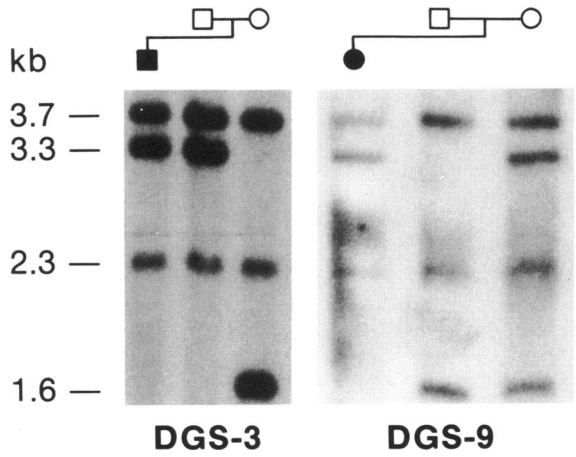

Figure 3 Autoradiogram of Southern blot of *Msp*I-digested DNA from two informative families hybridized with pH11 (D22S36). Probe pH11 recognizes 3.3- and 1.6-kb alleles and two constant bands, 2.3 and 3.7 kb. Proband DGS-3 (blackened square) shares the 3.3-kb allele with his father (unblackened square). His mother (unblackened circle) is homozygous for the 1.6-kb allele. Proband DGS-3 failed to inherit a maternal allele. The mother of proband DGS-9 is heterozygous for the 3.3- and 1.6-kb alleles. Her father is homozygous for the 1.6-kb allele. Proband DGS-9 has a single band at 3.3 kb; she failed to inherit a paternal allele.

Probe pH160b (D22S66) is a nonpolymorphic probe that has been sublocalized in 22q11 by hybridization to the somatic cell hybrid mapping panel (fig. 4). On the basis of results obtained with the mapping panel, this locus appears to lie between D22S75 (N25) and D22S259 (pR32). Loss of an allele for D22S66 was demonstrated by dosage analysis in eight of eight patients studied (tab. 3).

The 3′ BCR probe detects four loci: BCR, BCRL2, BCRL3, and BCRL4. These loci map in distinct and separate regions of 22q11, with BCRL2 as the most proximal of these four loci (Budarf et al. 1988). As shown in figure 4, BCRL2 is the closest distal flanking marker to D22S259. Hence, dosage analysis was performed to determine whether it lies within the DGCR. The results of these experiments are consistent with a deletion of BCRL2 in two of the DGS cell lines studied. However, an additional seven probands had two copies of BCRL2, placing it outside the DGCR.

Table 2

Summary of RFLP Status of DGS Cell Lines Studied with Polymorphic Probes

RFLP Status	No. of Cell Lines by Probe							
	W21G	p22/34	pH98	pH11	N25	pR32	22C1-18	pH162
Hemizygous[a]	0	0	0	2	1	3	0	0
Heterozygous[b]	5	5	2	3	0	0	5	4
Uninformative[c]	1	8	5	9	13	11	7	3
Total no. of cell lines studied	6	13	7	14	14	14	12	7

[a] "Hemizygous" indicates number of probands demonstrating failure to inherit a parental allele (five families studied).
[b] "Heterozygous" indicates probands with two different alleles.
[c] "Uninformative" indicates probands with a single band that may be consistent with one or two alleles, requiring dosage analysis.

Discussion

Several studies suggest the presence of a DGCR within 22q11 (Driscoll et al. 1990, 1991*b*; Fibison et al. 1990*a*; Scambler et al. 1991). The data presented here confirm the presence of a DGCR by detection of overlapping deletions in all 14 DGS probands studied, regardless of cytogenetic status. Submicroscopic deletions were detected in six cytogenetically normal (850-band stage) patients, suggesting that microdeletions of 22q11 occur in the majority of cases without a visible cytogenetic abnormality. Molecular studies with probes from the DGCR are clearly more sensitive than the high-resolution cytogenetic analysis and strongly support a systematic approach which combines both methods of detecting deletions in DGS. Chromosomal analysis may detect either translocations and interstitial deletions involving chromosome 22 or cytogenetic abnormalities of chromosomes other than 22. However, even with high-resolution analysis, interstitial deletions within 22q11 are difficult to visualize in this primarily euchromatic chromosome. In addition, phytohemagglutinin stimulation of T-cells for cytogenetic analysis is often less than optimal in DGS patients with T-cell dysfunction.

Table 3

Summary of Dosage Analysis of DGS Cell Lines by Quantitative Hybridization

Patient	Probe			
	N25	pH160b	pR32	BCRL2
7248	.99[a]	. . .	. . .[b]	2.45
KM4987	1.13	.74	.92	1.77
DGS-4	.95[b]	.88	.49[b]	1.96
GM07215	.91	. . .	.82	1.65
GM07939	1.13	. . .	.78	1.68
GM05876	1.15	.69	.90	1.67
DGS-2	1.18	.68	.96	. . .
DGS-3	.94	.87	.97	. . .
DGS-1	1.30	.97	.79	. . .
DGS-5	1.02	.65	.61[b]	. . .
DGS-6	.66	. . .	.51	.79
DGS-7	1.25	.90	.67	. . .
DGS-8	.70	. . .	.62	1.02
DGS-9	.78	. . .	.56	1.95

Note.—These values represent locus copy number, standardized from quantitative analysis of the hybridization signals obtained with the test probe relative to those obtained with a standard probe. They were obtained by taking the geometric mean of three replicate ratios of patient to control. Values less than 1.50 are consistent with a deletion.

[a] Agrees with results found by Fibison et al. (1990*b*).
[b] Copy number was demonstrated by RFLP analysis (figs. 1 and 2).

This is the first study to employ families to demonstrate, by RFLP and dosage analysis, de novo deletion of loci in the DGCR. The origin of the de novo deletions was established by RFLP analysis of five informative families. Four of five probands failed to inherit a maternal allele; one proband did not inherit a paternal allele. Although we have examined only a small number of families, these data, as well as reports of both maternally (six cases) and paternally (five cases) inherited unbalanced translocations in DGS patients suggest that there is not a consistent parent of origin or imprinting effect (de la Chapelle et al. 1981; Kelley et al. 1982; Greenberg et al. 1984; Augusseau et al. 1986; Bowen et al. 1986; Pivnick et al. 1990; El-Fouly et al. 1991). This finding is in contrast to what has been observed in some other segmentally aneusomic syndromes—such as Prader-Willi and Angelman syndromes, where paternal and maternal deletions, respectively, are the rule (Knoll et al. 1989).

Heterogeneity of phenotype and various patterns of inheritance—including sporadic, autosomal dominant, and autosomal recessive—have been observed in association with DGS. As has been suggested for

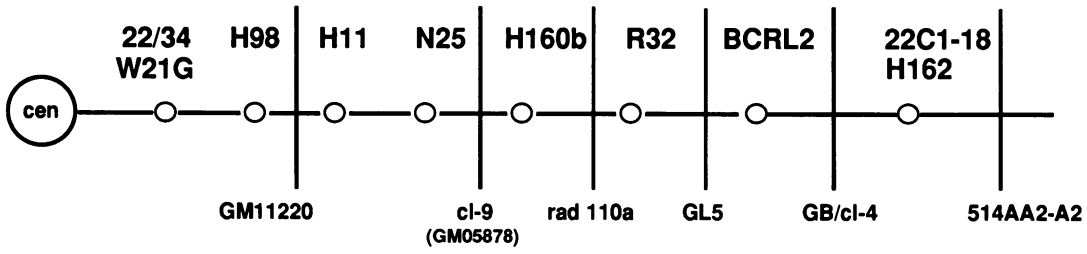

Figure 4 Schematic diagram of 22q11, showing relative order of probes used in these studies. The vertical lines represent somatic cell hybrid breakpoints used to determine the physical location of several of these probes. The order for 22/34, H98, H11, and N25 was established by genetic mapping in CEPH pedigrees (Fibison et al. 1990*a*).

deletions arising in the globin locus (Kan 1986), unequal crossing-over between multiple members of chromosome 22–specific gene families could produce de novo interstitial deletions, accounting for sporadic cases of DGS. Several such repeat families in 22q11 (3′BCR, GGT, IGL) have previously been described by Emanuel et al. (1991). Gonadal mosaicism for deletion-bearing chromosomes, although rare, could give rise to affected sibs. In addition, we have recently observed autosomal dominant segregations of DGS as inheritance of a deletion-bearing chromosome from a mildly affected parent (authors' unpublished results). Furthermore, cryptic balanced translocations between 22q11 and another acrocentric chromosome could arise during meiosis when all five pairs of acrocentric chromosomes coalesce around the nucleolus. Malsegregation of such a translocation could produce individuals with both monosomy for 22pter→q11 and trisomy for pter→q11 of the other involved acrocentric chromosome. Abnormalities of this type (*a*) would be difficult to detect with standard cytogenetic analysis, (*b*) could produce familial DGS, and (*c*) might explain the phenotypic variability seen among DGS patients. Further investigation of such malsegregation as a potential DGS-generating mechanism will require fluorescence in situ hybridization (FISH) analysis of DGS patients and their parents, using centromere-specific probes for each of the acrocentric chromosomes, together with hybridization of probes to the DGCR.

The detection of three commonly deleted loci begins to establish the minimal region of overlap for the DGCR and defines the proximal and distal flanking markers. Figure 5 summarizes our results. The order of the probes specified at the top of figure 5 is derived both from linkage data (Fibison et al. 1990*b*) and somatic cell hybrid mapping data (fig. 4). On the basis of RFLP analysis of DGS patients, proximal loci W21G (D22S24), p22/34 (D22S9), pH98 (D22S57), and pH11 (D22S36) and distal loci 22C1-18 (D22S10) and pH162 (D22S68) must lie outside the DGCR. Two loci, N25 (D22S75) and pR32 (D22S259), are deleted in 14 of 14 DGS probands. In addition, deletion of pH160b (D22S66) has been detected in eight of eight probands, including three with normal karyotypes. We presume that D22S66 is deleted in the remaining six probands, on the basis of its location between D22S75 and D22S259. The demonstration that there are both hemizygous and heterozygous probands at D22S36 excludes this locus from the DGCR. Similarly, dosage studies of DGS probands demonstrate that the more distal locus, BCRL2, is not consistently deleted. Therefore, we conclude that D22S36, proximally, and BCRL2, distally, flank the critical region.

Given the difficulties encountered with high-resolution banding for 22q11 in DGS patients, it is not surprising that the size of the molecular deletions detected with currently available probes does not reflect the ability to visualize the deletions cytogenetically. Accurate assessment of the size of the DGCR and the amount of material deleted in individual cases will require isolation of additional markers within the DGCR, between N25 and pH11 (the proximal flanking marker) and pR32 and BCRL2 (the distal flanking marker). On the basis of preliminary data from pulsed-field gel electrophoretic analysis of the three commonly deleted probes, we estimate a minimal size of 0.5 Mb for the DGCR. This is based on the size of pulsed-field gel fragments that only hybridize with pH160b (D22S66). Ultimately, it will be necessary to characterize the genes in the DGCR. In this regard, probes N25 and pH160b contain CpG islands, which suggest the presence of exon sequences in this region. Probe pH160b also recognizes conserved sequences in mouse, chicken, cow, monkey, and hamster (authors' unpublished results).

It is significant that all of the patients we have stud-

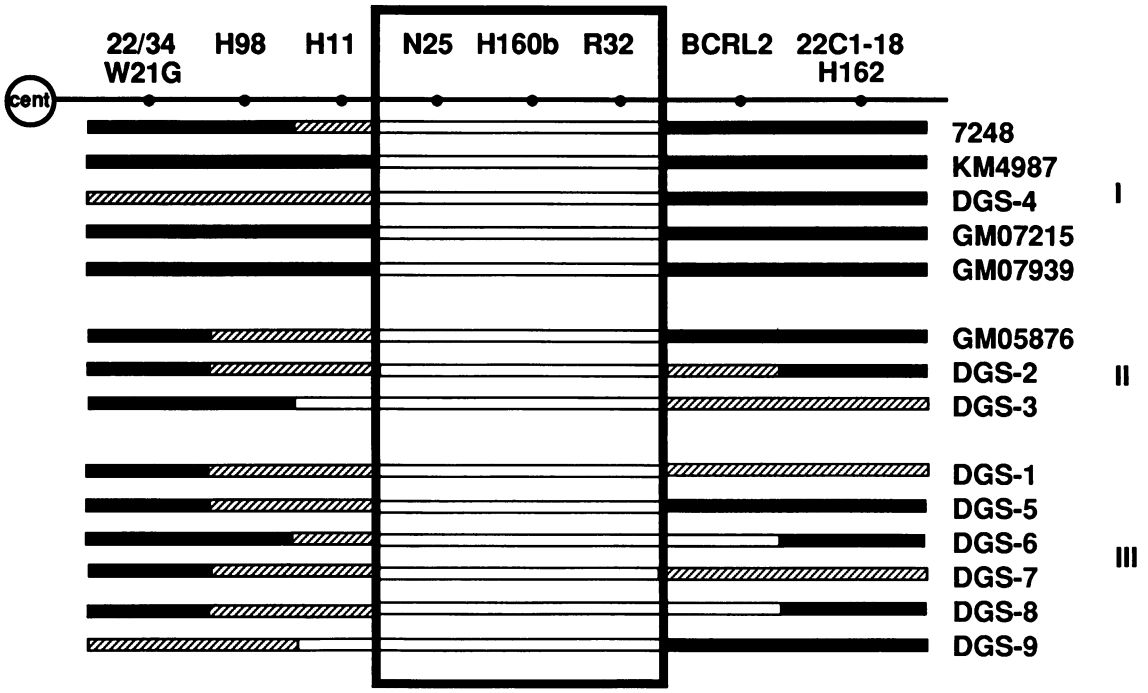

Figure 5 Summary of RFLP and dosage studies of 14 DGS probands, grouped according to cytogenetic findings. I = (del)22(q11.21q11.23); II = possible deletion of 22q11; III = normal karyotype. Probes are ordered from centromere (cent) to telomere (*right*). The bars represent results of hybridization studies; fully blackened bars represent the presence of two copies of the locus; hatched bars represent uninformative or nonpolymorphic loci for which dosage has not been performed to determine copy number; and unblackened bars represent deletions (i.e., single allele). The minimal region of overlap is indicated by the box and includes probes N25, pH160b, and pR32.

ied demonstrate deletions of multiple loci in 22q11. The detection of large deletions (greater than 0.5 Mb) suggests that the loss of function of more than a single gene is required for the pathogenesis of DGS. Thus, it is unlikely that a point mutation or a small deletion of a single locus is sufficient to produce DGS. Perhaps deletions of fewer loci might produce a less severe phenotype, e.g., the phenotype associated with the so-called partial DiGeorge syndrome. Furthermore, our observation of deletions of loci from within the DGCR in several patients with velo-cardio-facial (Shprintzen) syndrome (authors' unpublished results) may explain the overlapping phenotypic features observed in DGS and velo-cardio-facial syndrome (Goldberg et al. 1985; Shprintzen et al. 1985; Stevens et al. 1990). As more probes become available for use in this region, further characterization of the deletions in patients with these disorders may uncover differences in the size of the deletions, which may explain the variable phenotypes.

The finding of consistent deletions of multiple loci in patients with DGS supports the hypothesis that DGS is a contiguous-gene deletion syndrome. Furthermore, these studies confirm a genetic etiology for DGS, which dictates that molecular-diagnostic studies are required for the management of families concerned with recurrence risk (Driscoll et al. 1991*a*). Recently, FISH utilizing cosmid probes from the 17p13.3 region has been used to identify submicroscopic deletions and to define cryptic translocations in patients with another contiguous-gene deletion disorder, Miller-Dieker syndrome (Kuwano et al. 1991). In a similar fashion, chromosome 22–specific cosmids for the loci we have identified in the critical region should prove to be useful reagents for the rapid detection of microdeletions in the diagnosis of DGS.

Note added in proof. — We have demonstrated deletions of probe N25 (D22S75) by dosage analysis of five additional cell lines from DGS patients, including the mildly affected parent of proband DGS-2. Four of these patients are cytogenetically normal; the fifth has an interstitial deletion.

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