Am. J. Hum. Genet. 53:1239-1249, 1993

Physical Mapping by FISH of the DiGeorge Critical Region (DGCR): Involvement of the Region in Familial Cases

C. Desmaze,* M. Prieur,‡ F. Amblard,†† M. Aïkem,* F. LeDeist,§ S. Demczuk,* J. Zucman,* B. Plougastel,* O. Delattre,* M.-F. Croquette,‡‡ G.-M. Brevière,§§ C. Huon,† M. Le Merrer,|| M. Mathieu,|||| D. Sidi,# J.-L. Stephan,** and A. Aurias*

*Laboratoire de Génétique des Tumeurs INSERM CJF9201 and CNRS URA 620, Institut Curie, †Service de Médecine Néonatale, Hôpital Cochin, and ‡Laboratoire de Cytogénétique, §INSERM Unité 132, ||Clinique de Génétique Médicale, #Service de Cardiologie Infantile, **Service d'Immunohématologie, Hôpital des Enfants Malades, Paris; ††Laboratoire de Génétique, Histologie et Biologie de la Reproduction, Faculté de Médecine, Grenoble, France; ‡‡Laboratoire de Cytogénétique, Hôpital Saint-Antoine, and §§Service de Cardiologie Infantile, CHRU, Lille, France; and ||||Service de Pédiatrie, CHRU, Amiens, France

Summary

We describe the relative ordering, by fluorescence in situ hybridization, of cosmid loci and translocation breakpoints in the DiGeorge syndrome (DGS) critical region of chromosome 22. This physical map enables us to define a large region, commonly deleted in a majority of affected patients, and the smallest deleted region which, when lost, is sufficient to produce DGS. In four instances, a similar large deleted region is observed in a familial context. In these pedigrees, the deletion is encountered in one parent with mild features of the disease.

Introduction

DiGeorge syndrome (DGS) is a developmental field defect characterized by hypoplastic or absent thymus and parathyroids and by conotruncal heart defects (Lammer and Opitz 1986). A large variability in the expression of the malformations has been observed, and additional features such as facial dysmorphologies or mental retardation might be associated with DGS (Müller et al. 1988). The common embryological origin of the structures affected in DGS is the third and fourth branchial pouches, which suggests that DGS could be related to an abnormal interaction of the rhombencephalic neural crest cells (Couly et al. 1983; Kirby and Bockman 1984).

The DGS cases first reported were sporadic. Then, in 1972, Steele et al. (1972) described two affected children born to a mother who presented deficiencies in T cells and parathyroids. This familial case and subsequently others (Greenberg et al. 1984; Rohn et al. 1984; Keppen et al. 1988) suggested that DGS could be a genetic disorder with autosomal dominant transmission. However, autosomal recessive or X-linked inherited cases have also been reported (reviewed in Lammer and Opitz 1986). Up to now, both sporadic and familial cases have been encountered.

Cytogenetic studies have revealed chromosome abnormalities in 15%–20% of DGS cases. The first reported case associated with a chromosome abnormality was a monosomy 22 case (Rosenthal et al. 1972) which could have in fact been an unbalanced translocation of chromosome 22. Afterward, several unbalanced translocations involving this chromosome were observed with loss of the der(22) (Back et al. 1980; de la Chapelle et al. 1981; Kelley et al. 1982). Other chromosome abnormalities (reviewed in Greenberg et al. 1988) have been observed in association with DGS but are less frequent than chromosome 22 deletions. In that paper, Greenberg presented a series of 27 DGS patients studied by high-resolution cytogenetic analysis of peripheral blood lymphocytes, to search for chromosome 22 aberrations. Five patients had a chromosome abnormality, and three of them had a monosomy for the 22q11.2 region—two of them by unbalanced translocation, and one of them by interstitial microdeletion. Thus it seems that the main chromosome abnormality observed in

Received January 4, 1993; final revision received August 18, 1993.

Address for correspondence and reprints: A. Aurias, Institut Curie, Section de Biologie, 26 Rue d'Ulm, 75231 Paris Cedex 05, France.

0002-9297/93/5306-0010$02.00

DGS is a deletion in the 22q11.2 region, the consequence of which could be a deletion for a number of genes, leading some authors to propose that DGS would be a contiguous gene syndrome (Schmickel 1986; Emanuel 1988). In contrast, Augusseau et al. (1986) described a DGS patient with an apparently balanced translocation t(2;22)(q14.1;q11.2) and no additional chromosome abnormality. In the same way, no correlation has been established between the severity of the pathology and the size of the deletion (Scambler et al. 1991*a*). According to these observations, the hypothesis that DGS is a contiguous gene syndrome should be readdressed.

In a majority of DGS cases, no cytogenetic abnormalities are observed; therefore, further molecular investigations have been performed. The first reports with gene dosage analysis and/or RFLP studies have been published by Carey et al. (1990) and Fibison et al. (1990). Demonstration of loss of sequences in the 22q11.2 region has been first provided by Scambler et al. (1991*a*).

In a previous study, we performed fluorescence in situ hybridizations (FISH) on metaphases from peripheral blood lymphocytes of a series of 10 DGS patients (Desmaze et al. 1993). We probed this series with a cosmid clone, Sc11.1, corresponding to a locus, D22S139, lying in 22q11.2 (Carey et al. 1990), and demonstrated that all of the DGS patients studied were hemizygous at this locus. Among them, two had a microdeletion in 22q11.2 detectable by high-resolution chromosome analysis, and the eight others had a normal karyotype.

All these molecular results lead authors to consider that a genetic etiology for DGS would include a major sequence lying in the 22q11.2 region. In the present study, we report on the organization, by means of both unicolor FISH translocation breakpoint mapping and bicolor FISH, of three other loci that map near the Sc11.1 loci and that are always lost in the DGS patients we have studied. We also report on four new familial cases of deletions associated with DGS.

Patients, Material, and Methods

Patients and Cell Lines

The patients were essentially ascertained through their congenital heart defects. Of a large series of 50 DGS patients (authors' unpublished data), a familial context was suspected in five instances.

Family A.—The proband, a 1-mo-old female, has micrognathia and mild hypocalcemia. There is no thymic shadow, but T lymphocytes are normal. There is no congenital heart defect. Her brother died of typical DGS (interrupted aortic arch, absence of thymus, hypocalcemia, and low T-cell count) 1 year before. The mother has neonatal hypocalcemia, a mildly dysmorphic facial appearance (mild micrognathia) evoking DGS, and mild mental retardation. The father is also slightly dysmorphic and has mild mental retardation.

Family B.—The proband, a 2-year-old girl, has an interrupted aortic arch, mental retardation, no thymic shadow, a low T-lymphocyte count, and dysmorphic features, including micrognathia, bilateral epicanthus folds, and low-set ears. The mother is mildly retarded and has mildly dysmorphic features of DGS (i.e., micrognathia and low-set ears). She is now pregnant. The fetus is a male with an interrupted aortic arch and a low T-lymphocyte count. The father is normal.

Family C.—The proband is an 8-year-old male with a ventricular septal defect and pulmonary atresia. Calcemia is normal. The thymus is present, and T-cell counts are normal. The patient has typical DGS facial dysmorphies, mild mental retardation, and hypernasal speech. The older brother has undergone an operation for an aortic arch anomaly. This brother has mild mental retardation with dysmorphic features including retrognathia, ear anomalies, and short uvula. His calcemia is normal. The thymus is present. T-lymphocyte counts are normal. The two parents are normal, but the father is very slightly dysmorphic (prominent nose and discrete micrognathia), and a systematic dosage revealed mild hypocalcemia.

Family D.—The proband is a woman with dysmorphic features (micrognathia, prominent nose, and small auricles) closely resembling those observed in the velocardio-facial syndrome (VCFS) (Shprintzen et al. 1978) and with a cleft uvula but no congenital heart defect. Her two deceased children had typical DGS but were not analyzed in our study. The older child presented at birth with pulmonary atresia and ventricular septal defect, a hypoplastic thymus, microstomia, ear anomalies, and hypertelorism. The younger child presented with truncus arteriosus, ventricular septal defect, and absent thymus. Dysmorphic features are noted in the clinical data but without details. The husband is normal.

Family E.—The proband is a newborn male with mildly dysmorphic features (micrognathia, small auricles, and bulbous nose), hypocalcemia, and a heart murmur. Further investigations failed to confirm a congenital heart defect. The father appears normal. The mother presented at birth with hypocalcemia but without any other pathological sign.

Cell Lines

GM5401 (Greenberg et al. 1984) and GM5878 (Kelley et al. 1982; Cannizzaro and Emanuel 1985) are cell lines obtained from the NIGMS Human Genetic Mutant Cell Repository (Camden). The first cell line was established from a DGS patient with an unbalanced t(4;22)(q35.2;q11.2) translocation, and the second cell line was established from the father of a DGS patient with an unbalanced t(10;22)(q26;q11.2) translocation. This father carries the balanced translocation.

ADU and VDU are lymphoblastoid cell lines from a DGS proband and her mother, respectively. Except for hypernasal speech, the mother is normal. Both the mother and the daughter present the same reciprocal and apparently balanced t(2;22)(q14;q11.2) translocation (Augusseau et al. 1986). Karyotypes and clinical details for all the patients and all the cell lines are summarized in table 1.

Hybrid Cell Lines

Four hybrid cell lines were used as a somatic cell hybrid mapping panel. (1) EYE F3A6 (Van Keuren et al. 1987) retained the entire chromosome 22. (2) 33-11-Tg (Geurts van Kessel et al. 1980) was obtained from a human cell carrying a reciprocal t(X;22) translocation. The hybrid cell line retained the derivative X chromosome. (3) ALE has been derived from the lymphoblastoid cell line ICB100 which carries a recurrent t(11;22)(q23.1;q11.2) translocation (Zhang et al. 1990). The hybrid cell line kept the der(11). (4) AJO has been derived from the lymphoblastoid cell line ICB101 and retained the der(4) of a constitutional t(4;22)(p16.2;q11.2) translocation. The breakpoints of the three last hybrid cell lines allow us to define four subregions in the proximal part of band 22q11.2 (Delattre et al. 1991).

FISH

Cytogenetic preparations.—Metaphases and prometaphases were obtained from cell lines or phytohemagglutinin-stimulated lymphocytes by using standard methods. G0-G1 interphase nuclei were obtained from normal donors' unstimulated blood lymphocytes.

Molecular probes.—All probes used for FISH were cosmid clones. Sc11.1, described in Halford et al. (1993), was provided by P. Scambler. This cosmid clone probes two independent loci on chromosome 22 (Halford et al. 1993; Scambler 1993), designated in our results as "Sc11.1a" for the telomeric locus and as "Sc11.1b" for the centromeric locus. From earlier work, we had also demonstrated by FISH that these two loci are recurrently deleted on one of the two chromosomes 22 in DGS patients (Desmaze et al. 1993). We have localized the corresponding loci between the breakpoints of the t(X;22) translocation observed in the 33-11-Tg cell line and that of the recurrent t(11;22) translocation observed in the ALE cell line (Delattre et al. 1991). This localization is in good agreement with the one reported elsewhere by Sharkey et al. (1992) and Halford et al. (1993).

We have searched for additional sequences localized in the same subregion which corresponds to the DiGeorge critical region (DGCR) (Scambler et al. 1991*a*; Driscoll et al. 1992*a*; Scambler 1993). Using an extended panel of somatic cell hybrids which included the 33-11-Tg and ALE cell lines, we mapped three independent loci in the DGCR. They correspond to the KI429 locus (D22S138) (Carey et al. 1990), to a *Not*I linking clone, Not54 (provided by H. Vissing) isolated from a chromosome 22–specific cosmid library, and to the catechol-o-methyltransferase (COMT) locus. This gene has previously been located on chromosome 22 between the breakpoints of the GL5 cell line and that of the t(10;22) translocation observed in the GM5878 cell line (Grossman et al. 1992). Cosmid probes 48F8 and 100C10—containing D22S138 and the COMT loci, respectively—were obtained by the screening of the cosmid library LL22NC01 constructed in Livermore from flow-sorted chromosomes of the human lymphoblastoid cell line GM131. Their localization and that of the Not54 cosmid were confirmed to be in band 22q11.2 by means of FISH on control prometaphases (data not shown). A cosmid, 26H9, containing the locus D22S9, was obtained from the same library. This locus is localized in the subregion immediately centromeric to the t(X;22) breakpoint observed in the 33-11-Tg cell line. Another cosmid, cosDs, probes a locus known to be localized distal to the recurrent t(11;22) breakpoint (S. Demczuk, unpublished data) and was used because of its good hybridization signal in interphase nuclei. ZNF70 was previously localized in the region immediately distal to the AJO breakpoint (Aubry et al. 1992).

Hybridization and immunodetection.—Cosmid DNAs were extracted by using the alkaline lysis procedure (Sambrook et al. 1989). DNA was labeled either by biotin-14-dATP by using the Bionick kit (BRL, Gaithersburg) or by digoxygenin-11-dUTP by using a nick-translation kit (Boehringer Mannheim) according to the recommendations of the suppliers. A total of 40–100 ng of labeled DNA was mixed with about 100-fold sonicated human DNA in 20 μl of hybridization buffer (2 × SSC [1 × SSC = 0.15 M NaCl, 0.015 M Na citrate],

Table 1

Clinical and Cytogenetic Data of the Different Families and Cell Lines

Patients	Karyotype	Thymus	T-Cell Level	Cardiac Malformations	Neonatal Hypocalcemia	Mental Retardation	Facial Dysmorphy	Other Features
Family								
AIII-2	46,XX, normal	−	Normal	None	+	+	+	Neurological disorders, swallowing difficulties
AII-1	46,XX, normal	ND	ND	None	+	+	+	None
AII-2	46,XY, normal	ND	ND	None	−	+	+	None
BIII-1	46,XX, normal	−	Decreased	IAA[a]	+	+	+	Swallowing difficulties, tapered fingers and toes
BIII-2	46,XY, normal	ND	Decreased	IAA[a]	ND	?	?	Not born
BII-1	46,XX, normal	ND	ND	NP[b]	ND	+	+	None
BII-2	46,XY, normal	ND	ND	NP[b]	ND	−	−	None
CIII-2	46,XY, normal	+	Normal	AAA[c]	−	+	+	Hypernasal speech
CIII-3	46,XY, normal	+	Normal	PA,[d] VSD[e]	−	+	+	Hypernasal speech, partial hearing loss
CII-2	46,XY, normal	ND	Normal	None	+	−	+	None
CII-1	46,XX, normal	ND	Normal	None	−	−	−	None
DII-1	46,XX, normal	ND	ND	None	ND	−	+	Cleft uvula
DIII-1	ND	Hypoplastic	ND	PA,[d] VSD[e]	ND	?	+	None
DIII-2	ND	−	ND	TA[f], VSD[e]	ND	?	+	None
EIII-1	46,XY, normal	ND	ND	None	+	?	+	None
EII-1	46,XX, normal	ND	ND	None	+	−	−	None
Cell lines								
ADU	46,XX,t(2;22)(q14.1;q11.2)	ND	Decreased	IAA[b]	+	−	+	None
VDU	46,XX,t(2;22)(q14.1;q11.2)	ND	Normal	None	−	−	−	Hypernasal speech
GM5401	45,XY,−4,−22,+der(4) t(4;22)(q35.2;q11.2)	ND	Decreased	TA[f]	+	+	+	None
GM5878	46,XY,t(10;22)(q26;q11.2)	ND	ND	ND	ND	ND	ND	None

NOTE.—ND = not determined. A plus sign (+) denotes presence, and a minus sign (−) denotes absence.

[a] IAA = interrupted aortic arch.

[b] NP = no patent.

[c] AAA = aortic arch anomaly.

[d] PA = pulmonary atresia.

[e] VSD = ventricular septal defect.

[f] TA = truncus arteriosus.

40 mM NaH_2PO_4, 50% deionized formamide, 10% dextran sulphate, 0.1% SDS, 1% Denhardt's solution). Slides were pretreated with 100 μg RNase A/ml (Sigma, St. Louis) for 1 h at 37°C, then dehydrated through a series of ethanol baths (50%, 75%, and 100%) and denatured for 2–3 min at 70°C in 2 × SSC 70% formamide.

The hybridization mixture was denatured by boiling for 10 min and was ice cooled and spotted onto the cytogenetic spreads. The slides were incubated overnight at 42°C in a moist chamber under a plastic coverslip. They were rinsed twice in 50% formamide 2 × SSC and then in 2 baths of 2 × SSC, at 42°C each. After a preincubation in PBT (PBS, 0.1% Tween 20, 0.1% BSA), the immunodetection was performed in two steps for unicolor FISH or in four steps for bicolor FISH. The biotinylated probes were revealed by a goat anti-biotin antibody, dilution 1/100 (Vector, Burlingame) and a by fluorescein-conjugated anti-goat antibody, dilution 1/400 (Biosys, Compiègne), and the digoxygenin-labeled probes were revealed by a mouse anti-digoxygenin antibody and a rhodamine-conjugated anti-mouse antibody, dilutions 1/100 and 1/10, respectively (Boehringer Mannheim). The slides were counterstained with propidium iodide and were mounted in an antifade solution (Johnson and Nogueira 1981).

Microscopic observations and data analyses.—The slides were observed on an Aristoplan Leitz microscope with a standard FITC filter combination for unicolor experiments and a double-band pass filter (Omega Optical, Brattleboro) for bicolor experiments. Photographs were taken with Kodak Ektachrome 400 film. For bicolor experiments, each cosmid probe was tested on interphase nuclei from a normal donor in order to estimate the hybridization efficiencies, defined as the ratio of observed number of labeled loci:theoretical number of probed loci. These efficiencies are 0.83, 0.87, 0.79, 0.68, and 0.94 for Sc11.1, 100C10, Not54, 48F8, and cosDs cosmid probes, respectively. All nuclei with three discrete single signals were scored, and the distribution was compared with a random distribution (χ^2 test).

Results

Physical Mapping of the DGCR

Two different approaches were developed—translocation breakpoint mapping and loci ordering by bicolor FISH. With regard to the former, we have performed unicolor FISH on a series of translocations involving band 22q11.2. We have confirmed by FISH that the breakpoint of the t(2;22) observed in the ADU cell line lies between the two loci probed by Sc11.1. As a first step, we have performed FISH on this cell line with the three other cosmid probes 48F8, 100C10, and Not54. With these three cosmid probes, only the derivative chromosome 2 is labeled, thus demonstrating that the three loci probed are telomeric to this breakpoint. As expected, the cosmid 26H9 containing the D22S9 sequence labels the derivative chromosome 22. In a second step, the cosmid probes Sc11.1, 48F8, 100C10, and Not54 were hybridized to metaphases obtained from the GM5878 and the GM5401 cell lines. With Sc11.1, the two derivative chromosomes 10 and 22 of GM5878 are labeled (fig. 1*a*). The breakpoint on chromosome 22 is thus localized between Sc11.1a and Sc11.1b. With the same probe, the derivative chromosome 4 of GM5401 is labeled, demonstrating that at least Sc11.1a is distal to the breakpoint. Because this cell line carries an unbalanced karyotype with loss of the derivative chromosome 22, this single experiment cannot demonstrate the relative position of the breakpoint with respect to the two loci. The two cosmid probes 48F8 and 100C10 do not label the derivative chromosome 4 of GM5401 as Not54 does, strongly suggesting that the breakpoint of this cell line is telomeric to the D22S138 and COMT loci and centromeric to the Not54 locus. Finally, we found that D22S138 is centromeric to the breakpoint of the reciprocal t(10;22) observed in GM5878 and that the COMT locus is telomeric to this breakpoint (fig. 1*b* and 1*c*). These results and those observed with the GM5401 cell line allow us to assign the Not54 locus distal to the GM5878 cell line breakpoint. Since we know that cosDs locus and D22S9 are respectively telomeric and centromeric to the other loci, we are able to propose a relative order for translocation breakpoints and loci, as summarized in figure 2. The bicolor FISH experiments were performed simultaneously and were in good agreement with the order deduced from the translocation breakpoint mapping.

Relative order of the loci probed by 100C10 (COMT locus) and Sc11.1.—The cosmid 100C10 was revealed by a rhodamine-conjugated antibody (red spots), and the cosmid Sc11.1 was revealed by an FITC-conjugated antibody (green spots). Among the 100 triplets observed, 76 were of the green-red-green type, and 24 were of the green-green-red type (fig. 3*a*). This distribution is clearly not random ($\chi^2 = 14.5$, $P = .0001$) and confirms that the COMT locus is between Sc11.1a and Sc11.1b.

Relative order of the loci probed by 100C10 (COMT locus), 48F8 (D22S138), and cosDs.—100C10 and cosDs were revealed in green, and 48F8 was revealed in

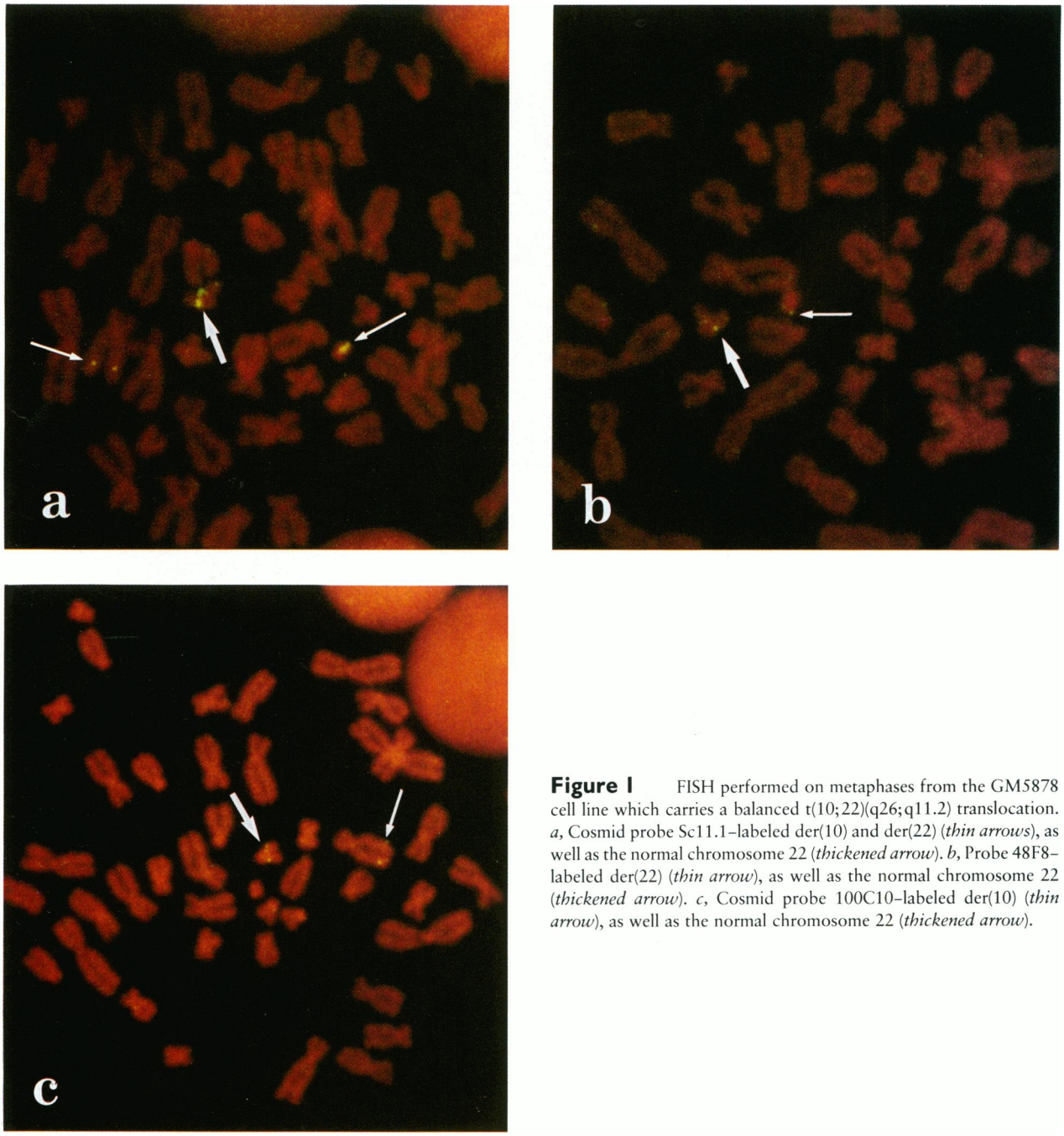

Figure 1 FISH performed on metaphases from the GM5878 cell line which carries a balanced t(10;22)(q26;q11.2) translocation. *a*, Cosmid probe Sc11.1–labeled der(10) and der(22) (*thin arrows*), as well as the normal chromosome 22 (*thickened arrow*). *b*, Probe 48F8–labeled der(22) (*thin arrow*), as well as the normal chromosome 22 (*thickened arrow*). *c*, Cosmid probe 100C10–labeled der(10) (*thin arrow*), as well as the normal chromosome 22 (*thickened arrow*).

red. Among the 100 triplets analyzed, 85 were green-green-red, and 15 were green-red-green (χ^2 = 28, P = .0001), confirming the localization of D22S138 outside the interval COMT-cosDs loci (fig. 3*b*). Probably because of either a peculiar configuration of the chromatin or a polymorphism in the population, the same technique performed for the loci probed by Not54 and Sc11.1 on four independent donors gave clear-cut different ordering from one to the other.

Analysis of the Patients

All the probands were studied with high-resolution R-banding (850-bands stage). No deletion was suspected in any of these patients.

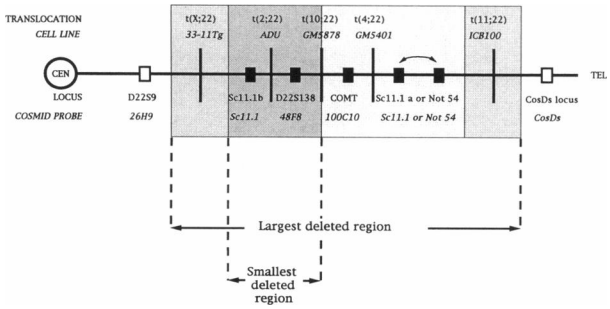

Figure 2 Physical map of the DGCR, with the relative positions of loci and translocation breakpoints. In our series, the largest deleted region is between loci D22S9 and ZNF70. The distal border of this region could be proximal or distal to the ICB100 breakpoint. The smallest deleted region is bounded distally by the GM5878 breakpoint and proximally by the locus pH11, which is distal to the 33-11-Tg breakpoint.

All the probands and all available members of their families, with or without features of DGS, were first studied with the cosmid probe Sc11.1. Deletions for the two loci thus probed were found in all patients and in all members of the families with mild or severe features of DGS, with the exception of the mother of family E.

In all patients deleted for these loci, the five probes 26H9, 48F8, 100C10, Not54, and ZNF70 were then tested (because of a lack of cytogenetic preparations, we could not analyze the mother AII-1 with the probe ZNF70). All these patients and all members of their families with mild DGS features were found to have deletions for the three loci probed by 48F8, 100C10, and Not54 but not for the loci probed by 26H9 and ZNF70 (fig. 4).

Discussion

There Is No Correlation between the Phenotype and the Size of the Deletion

In this familial series, the deletion is bounded proximally by D22S9 and distally by the locus probed by ZNF70. These two loci are conserved in all our patients. These results are in good agreement with the DGCR described elsewhere (Scambler et al. 1991*a*; Driscoll et al. 1992*a*; Scambler 1993).

With the set of probes we have used, there is no correlation, among the patients, between the severity of the disorder and the size of the deletion. In addition, within a single family, even if we can reasonably assume that all the affected members carry the same deletion, a wide range of phenotypes is often observed. This variability in phenotype between parent and child could eventually be due to an ascertainment bias. However, this explanation does not account for the variability within a sibship. Therefore, this lack of correlation between the phenotype and the size of the deletion do not

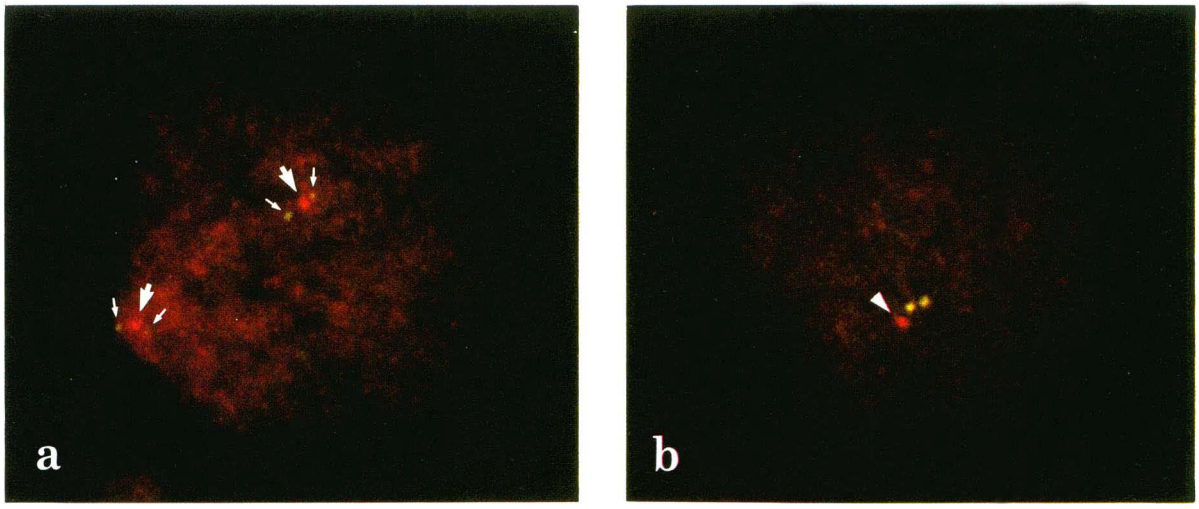

Figure 3 Bicolor FISH performed on G0 nuclei from normal donors. *a*, Sc11.1 and 100C10 probes were revealed in green (*thin arrows*) and red (*thickened arrows*), respectively. The COMT locus appears between Sc11.1a and Sc11.1b. *b*, cosDs and 100C10 probes were revealed in green, and the 48F8 probe was revealed in red (*arrowhead*). D22S138 lies outside the interval COMT–cosDs loci. A single triplet is visible in this nucleus.

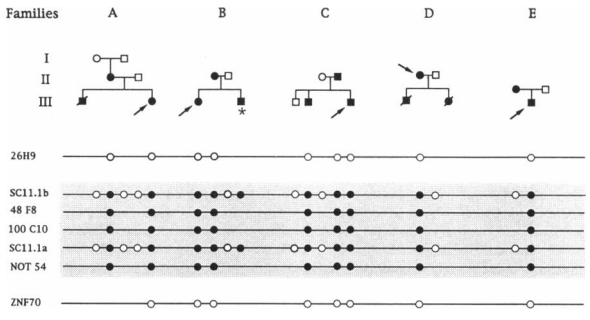

Figure 4 FISH results for the five pedigrees. Each column of circles corresponds to the patient immediately above, in the pedigree. Unblackened circles correspond to conserved loci, and blackened circles correspond to deleted loci. Arrows point to the probands. The asterisk (*) corresponds to an unborn affected male. Symbols with slashes through them correspond to deceased individuals. The shaded area designates the commonly deleted region.

support the hypothesis that the severity of the cardinal features of DGS could be related to the extent of the deletion. Similarly, the facts that all the affected structures have a common embryological origin and that the variability among the phenotypes essentially concerns the severity of the disorder but not the number of affected structures are not in favor of this hypothesis either.

The mother of family E who presented at birth with hypocalcemia was found dizygous for all loci probed by our set of cosmids. Even if we cannot rule out the possibility of a smaller rearrangement or of a germ-line mosaicism, a more probable hypothesis is that her hypocalcemia was not causally related to a DGS status.

If we propose that DGS is not a contiguous gene syndrome, in particular for its cardinal features, then it is striking that all patients exhibit such a large deletion in band 22q11.2. Therefore, we can assume that these recurrent events are related to the presence of specific bounding sequences allowing, and even furthering, the deletions as proposed elsewhere (Scambler et al. 1991*b;* Halford et al. 1993). The physical mapping of band 22q11.2 has now demonstrated that this region contains families of homologous sequences (Halford et al. 1993, and authors' unpublished data), suggesting that this region has undergone a series of duplications. Such an internal structure could lead to frequent recombination events. The cloning of the borders of the DGS deletions and the sequencing of this region will be of great importance to document these hypotheses. The fact that the locus D22S9 is always present on the two chromosomes 22 in our series rules out, as a general mechanism for DGS deletion, the hypothesis of an adjacent 2 malsegregation of a translocation between chromosome 22 and another acrocentric chromosome as proposed elsewhere (Driscoll et al. 1992*a*).

Nevertheless, it appears from our data and from results published elsewhere (Scambler et al. 1991*a;* Driscoll et al. 1992*a;* Scambler 1993) that almost all patients are hemizygous for a large region of sub-band 22q11.2. Moreover, we demonstrate here that the gene encoding the COMT is deleted in all the patients we have studied. As proposed elsewhere (Scambler 1993), deletion of this locus could eventually be relevant for some clinical minor aspects of DGS and related disorders, in particular when the conserved allele encodes a low-activity enzyme (MIM 212730). The same deleted region was previously shown to contain two potential promoter sequences: the *Not*I linking clone N25 and the locus pH160b which contains a CpG island (Driscoll et al. 1992*a*). We localize here another *Not*I linking clone, Not54, in the deleted region. It would be of great importance to demonstrate that these loci really contain coding sequences and to find the role they play in the disorder.

A Large Deletion Is Not Necessary to Express DGS

Two cell lines, ADU and VDU, carrying an apparently balanced translocation t(2;22), were obtained from a patient with DGS and her mother, respectively, both with minor clinical features. One hypothesis could be that this balanced translocation splits the major (or one of the major) genes involved in the syndrome. We have localized the breakpoint of this translocation in the proximal part of the DGCR, between the locus probed by 48F8 (D22S138) and the more proximal locus probed by Sc11.1 (Sc11.1b). Two of the unbalanced translocations we used for the physical mapping of the region were observed in patients with DGS. The more centromeric breakpoint is observed in the t(10;22) from GM5878. This breakpoint is thus the distal boundary of the minimal critical region which, when deleted, produces DGS. In our series, all the patients are dizygous at the D22S9 locus. Our minimal DGCR is thus bounded proximally by the locus D22S9 and distally by the breakpoint of GM5878. It is noticeable that the chromosome 22 breakpoint of ADU and VDU lies within this small region. Driscoll et al. (1992*a*) reported that the locus pH11 is not constantly deleted in their series. This locus is distal to the breakpoint of the cell line 33-11-Tg and thus to D22S9, and the minimal critical region could be then bounded proximally by the locus pH11 and distally by the GM5878 breakpoint

(fig. 2). The loci corresponding to the COMT gene and to Not54 are not localized in this region and thus are probably not involved in the appearance of the main clinical signs of DGS.

Parental Origin of the Deleted Chromosome

In our series, three of four deletions were inherited from the mother. In results published elsewhere (Wilson et al. 1991; Scambler et al. 1992), one case of maternal inheritance was demonstrated, and two cases of paternal inheritance were strongly suggested by clinical and preliminary molecular analyses. From these pooled data, it appears that there is no clear particular parental origin for the inheritance of the deleted chromosome 22 in familial DGS. Similar deletions have now been described in related disorders such as VCFS (Scambler et al. 1992; Driscoll et al. 1992*b*; Kelly et al. 1993) and familial congenital heart diseases (Wilson et al. 1992), and, in our series, the two children of family C, referred to us as DGS cases, and the mother of family D could eventually be classified as incomplete VCFS. If we consider that the familial congenital heart diseases and the VCFS correspond to an identical or close genetic determinism, we can add to these data five families with congenital heart defects as published by Wilson et al. (1992) and seven families with VCFS as published by Shprintzen et al (1981), Driscoll et al. (1992*b*), and Kelly et al. (1993). In the families with congenital heart diseases, three deletions were inherited from the mother, and two were inherited from the father. In the seven families with VCFS, three deletions were demonstrated to be inherited from the mother (Driscoll et al. 1992*b*; Kelly et al. 1993), and, in the pedigrees reported by Shprintzen et al. (1981), a maternal inheritance of the syndrome was strongly suggested by the clinical findings in the four instances. Assuming that a large proportion of, if not all, VCFS cases are related to 22q11.2 deletions (Driscoll et al. 1992*b*; Scambler et al. 1992; Kelly et al. 1993), we can suppose that such a deletion was inherited in these pedigrees. Thus, among the 13 demonstrated occurrences of inherited 22q11.2 deletions, 10 were maternal in origin, and 3 were paternal in origin. If we add to these data those strongly suggested by clinical or preliminary molecular analyses, it appears that, among the 19 pedigrees of inherited disorders, 14 could be maternal in origin, and 5 could be paternal in origin. This repartition, observed on that small sample, is statistically different from a random one ($\chi^2 = 4.2$) and evokes a preferential maternal transmission for inherited 22q11.2 deletions associated with congenital disorders, and with VCFS in particular. The definite demonstration of such a preferential maternal inheritance would be of great interest and clearly needs further investigation.

FISH as a Routine Technique for Diagnosis and Physical Mapping

FISH appears to be a particularly relevant technique for the screening of microdeletions and the rapid characterization of the size of these deletions. This technique does not require study of the parents' DNA to demonstrate that a patient is hemizygous at a locus and thus appears as the most convenient technique for the routine diagnosis of DGS. As shown elsewhere by Trask et al. (1991), bicolor FISH is an efficient tool for ordering loci from small chromosome subregions. Combined with translocation breakpoint mapping, it allowed us to obtain a precise physical map of the 22q11.2 region.

Acknowledgments

We thank P. Scambler, M. Aubry, and H. Vissing for providing us with the probes Sc11.1, ZNF70, and Not54, respectively, and we thank Dr. P. Jalbert for the cell lines of ADU and VDU patients. This work was supported by the Association Française contre les Myopathies. C.D. is a recipient of a fellowship from the Ligue Nationale Contre le Cancer. S.D. is a recipient of a scholarship from the Fonds pour la Formation des Chercheurs et l'Aide à la Recherche du Québec. J.Z. is a recipient of a fellowship from the Fondation pour la Recherche Médicale. B.P. is a recipient of a fellowship from the Ministère de la Recherche et de la Technologie.

References

Aubry M, Marineau C, Zhang FR, Zahed L, Figlewicz D, Delattre O, Thomas G, et al (1992) Cloning of six new genes with zinc finger motifs mapping to short and long arms of human acrocentric chromosome 22 (p and q11.2). Genomics 13:641–648

Augusseau S, Jouk S, Jalbert P, Prieur M (1986) DiGeorge syndrome and 22q11 rearrangements. Hum Genet 74:206

Back E, Stier R, Bohn N, Adlung A, Hameister H (1980) Partial monosomy 22pter→q11 in a newborn with the clinical features of trisomy 13 syndrome. Ann Genet 23:244–248

Cannizzaro LA, Emanuel BS (1985) In situ hybridization and translocation breakpoint mapping. III. DiGeorge syndrome with partial monosomy of chromosome 22. Cytogenet Cell Genet 39:179–183

Carey AH, Roach S, Williamson R, Dumanski JP, Nordenskjold M, Collins VP, Rouleau G, et al (1990) Localization of 27 DNA markers to the region of human chromosome 22q11-pter deleted in patients with the DiGeorge syndrome and duplicated in the der22 syndrome. Genomics 7:299–306

Couly G, Lagrue A, Griscelli C (1983) Le syndrome de DiGeorge: neurocristopathie rhombencéphalique exemplaire. Rev Stomatol Chir Maxillofac 84:103–108

de la Chapelle A, Herva R, Koivisto M, Aula P (1981) A deletion in chromosome 22 can cause DiGeorge syndrome. Hum Genet 57:253–256

Delattre O, Azambuja CJ, Aurias A, Zucman J, Peter M, Zhang F, Hors-Cayla MC, et al (1991) Mapping of human chromosome 22 with a panel of somatic cell hybrids. Genomics 9:721–727

Desmaze C, Scambler P, Prieur M, Halford S, Sidi D, Le Deist F, Aurias A (1993) Routine diagnosis of DiGeorge syndrome by fluorescent in situ hybridization. Hum Genet 90:663–665

Driscoll DA, Budarf ML, Emanuel BS (1992*a*) A genetic etiology for DiGeorge syndrome: consistent deletions and microdeletions of 22q11. Am J Hum Genet 50:924–933

Driscoll DA, Spinner NB, Budarf ML, McDonald-McGinn DM, Zackai EH, Goldberg RB, Shprintzen RJ, et al (1992*b*) Deletions and microdeletions of 22q11.2 in velo-cardio-facial syndrome. Am J Med Genet 44:261–268

Emanuel BS (1988) Molecular cytogenetics: toward dissection of the contiguous gene syndromes. Am J Hum Genet 43:575–578

Fibison WJ, Budarf M, McDermid H, Greenberg F, Emanuel BS (1990) Molecular studies of DiGeorge syndrome. Am J Hum Genet 46:888–895

Geurts van Kessel AHM, Westerveld A, DeGroot PG, Meera KP, Hagemeijer A (1980) Regional localisation of genes coding for human ACO2, ARSA and NAGA on chromosome 22. Cytogenet Cell Genet 28:169–172

Greenberg F, Crowder WE, Paschall V, Colon-Linares J, Lubianski B, Ledbetter DH (1984) Familial DiGeorge syndrome and associated partial monosomy of chromosome 22. Hum Genet 65:317–319

Greenberg F, Elder FFB, Haffner P, Northrup H, Ledbetter DH (1988) Cytogenetic findings in a prospective series of patients with DiGeorge anomaly. Am J Hum Genet 43:605–611

Grossman MH, Emanuel BS, Budarf ML (1992) Chromosomal mapping of the human catechol-o-methyltransferase gene to 22q11.1→q11.2. Genomics 12:822–825

Halford S, Lindsay E, Nayudu M, Carey AH, Baldini A, Scambler P (1993) Low-copy-number repeat sequences flank the DiGeorge/velo-cardio-facial syndrome loci at 22q11. Hum Mol Genet 2:191–196

Johnson GD, Nogueira Araujo GM de C (1981) A simple method of reducing the fading of immunofluorescence during microscopy. J Immunol Method 43:349–350

Kelley RI, Zackai EH, Emanuel BS, Kistenmacher M, Greenberg F, Punnett HH (1982) The association of the DiGeorge anomaly with partial monosomy of chromosome 22. J Pediatr 101:197–200

Kelly D, Goldberg R, Wilson D, Lindsay E, Carey A, Goodship J, Burn J, et al (1993) Confirmation that the velo-cardio-facial syndrome is associated with haplo-insufficiency of genes at chromosome 22q11. Am J Med Genet 45:308–312

Keppen LD, Fasules JW, Burks AW, Gollin SM, Sawyer JR, Miller CH (1988) Confirmation of autosomal dominant transmission of the DiGeorge malformation complex. J Pediatr 113:506–508

Kirby ML, Bockman DE (1984) Neural crest and normal development: a new perspective. Anat Rec 209:1–6

Lammer EJ, Opitz JM (1986) The DiGeorge anomaly as a developmental field defect. Am J Med Genet, Suppl 2:113–127

Müller W, Peter HH, Wilken M, Jüppner H, Kallfelz HC, Krohn HP, Miller K, et al (1988) The DiGeorge syndrome. I. Clinical evaluation and course of partial and complete forms of the syndrome. Eur J Pediatr 147:496–502

Rohn RD, Leffell MS, Leadem P, Johnson D, Rubio T, Emanuel BS (1984) Familial third-fourth pharyngeal pouch syndrome with apparent autosomal dominant transmission. J Pediatr 105:47–51

Rosenthal IM, Bocian M, Krmpotic E (1972) Multiple anomalies including thymic aplasia associated with monosomy 22. Pedriatr Res 6:358

Sambrook J, Fritsch EF, Maniatis T (eds) (1989) Molecular cloning: a laboratory manual, 2d ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Scambler PJ (1993) Deletions of human chromosome 22 and associated birth defects. Curr Opin Genet Dev 3:432–437

Scambler PJ, Carey AH, Wyse RKH, Roach S, Dumanski JP, Nordenskjold M, Williamson R (1991*a*) Microdeletions within 22q11 associated with sporadic and familial DiGeorge syndrome. Genomics 10:201–206

Scambler P, Halford S, Wadey R, Lindsay E, Kelly D, Dumanski J, Nordenskjold M, et al (1991*b*) Duplicated sequences within 22q11 and their relationship to gene deletion syndromes. Second International Workshop on the Mapping of Human Chromosome 22, Montebello, Canada, September 10–13

Scambler PJ, Kelly D, Lindsay E, Williamson R, Goldberg R, Shprintzen R, Wilson DI, et al (1992) Velo-cardio-facial syndrome associated with chromosome 22 deletions encompassing the DiGeorge locus. Lancet 339:1138–1139

Schmickel RD (1986) Contiguous gene syndromes: a component of recognizable syndromes. J Pediatr 109:231–241

Sharkey AM, McLaren L, Carroll M, Fantes J, Green D, Wilson D, Scambler PJ, et al (1992) Isolation of anonymous DNA markers for human chromosome 22q11 from a flow-sorted library, and mapping using hybrids from patients with DiGeorge syndrome. Hum Genet 89:73–78

Shprintzen RJ, Goldberg RB, Levin ML, Sidoti EJ, Berkman MD, Argamaso RV, Young D (1978) A new syndrome involving cleft palate, cardiac anomalies, typical facies, and learning disabilities: velo-cardio-facial syndrome. Cleft Palate J 15:56–62

Shprintzen RJ, Goldberg RB, Young D, Wolford L (1981)

The velo-cardio-facial syndrome: a clinical and genetic analysis. Pediatrics 67:167–172

Steele RW, Limas C, Thurman GB, Schuelein M, Bauer H, Bellanti JA (1972) Familial thymic aplasia: attempted reconstitution with fetal thymus in a millipore diffusion chamber. N Engl J Med 287:787–791

Trask BJ, Massa H, Kenwrick S, Gitschier J (1991) Mapping of human chromosome Xq28 by two-color fluorescence in situ hybridization of DNA sequences to interphase cell nuclei. Am J Hum Genet 48:1–15

Van Keuren ML, Hart IM, Kao FT, Neve RL, Bruns GAP, Kurnit DM, Patterson D (1987) A somatic cell hybrid with a single human chromosome 22 corrects the defect in the CHO mutant (Ade$^-$I) lacking adenylosuccinate activity. Cytogenet Cell Genet 44:142–147

Wilson DI, Cross IE, Goodship JA, Coulthard S, Carey AH, Scambler PJ, Bain HH, et al (1991) DiGeorge syndrome with isolated aortic coarctation and isolated ventricular septal defect in three sibs with a 22q11 deletion of maternal origin. Br Heart J 66:308–312

Wilson DI, Goodship JA, Burn J, Cross IE, Scambler PJ (1992) Deletions within chromosome 22q11 in familial congenital heart disease. Lancet 340:573–575

Zhang FR, Aurias A, Delattre O, Stern MH, Benitez J, Rouleau G, Thomas G (1990) Mapping of human chromosome 22 by in situ hybridization. Genomics 7:319–324