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Deletion mapping on chromosome 10p and definition of a critical region for the second DiGeorge syndrome locus (DGS2)

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DiGeorge syndrome (DGS) is a developmental field defect, characterised by absent/hypoplastic thymus and parathyroid, and conotruncal heart defects, with haploinsufficiency loci at 22q (DGS1) and 10p (DGS2). We performed fluorescence *in situ* hybridisations (FISH) and polymerase chain reaction (PCR) analyses in 12 patients with 10p deletions, nine of them with features of DGS, and in a familial translocation 10p;14q associated with midline defects. The critical DGS2 region is defined by two DGS patients, and maps within a 1 cM interval including D10S547 and D10S585. The other seven DGS patients are hemizygous for both loci. The breakpoint of the reciprocal translocation 10p;14q maps at a distance of at least 12 cM distal to the critical DGS2 region. Interstitial and terminal deletions described are in the range of 10–50 cM and enable the tentative mapping of loci for ptosis and hearing loss, features which are not part of the DGS clinical spectrum.

Keywords: chromosome 10p; deletion mapping; DiGeorge syndrome; DGS2; ptosis; hearing loss

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Introduction

DiGeorge syndrome (DGS, MIM 188400) is considered to be a developmental defect of the third and fourth pharyngeal pouches. It is characterised by an absent or hypoplastic thymus with T-cell deficiency, absent or hypoplastic parathyroid glands with hypoparathyroidism and hypocalcaemia, conotruncal heart defect, craniofacial dysmorphism, and mental retardation.[1,2] More than 90% of patients with DGS have a microdeletion of 22q11 (DGS1).[3,4] The associated phenotypes are variable, with a large proportion of patients exhibiting only a subset of the above mentioned traits.[5] The phenotypic spectrum includes the velocardiofacial syndrome (VCFS, MIM 19243), which shows a significant overlap with DGS, specifically hypoparathyroidism, and hypocalcaemia, hypoplastic thymus, T-cell abnormality and cardiac defects.[6,7] Several candidate genes from the 22q11 deletion region have been cloned, but no genotype–phenotype correlation has emerged so far.[8–12]

The etiology of DGS is heterogeneous and includes teratogens such as alcohol or retinoids[1] and chromosomal abnormalities, for instance partial monosomies,[13–17] partial trisomies,[18–22] and mosaic tetrasomy.[23] Most of these cases represent single reports of DGS associated with a specific chromosomal aberration, with the exception of partial monosomy 10p where ten cases have so far been described. Seven of these patients are hemizygous for the segment 10p13-pter,[15,24–29] one patient has a monosomy 10p11–13,[30] one patient has a monosomy 10p12–p13,[31] and one patient with an unbalanced *de novo* translocation 10p;14q was interpreted to be monosomic for 10p14-pter.[32] The association between partial monosomy 10p and DGS has prompted the definition of a second DGS locus (DGS2) on the short arm of chromosome 10.[33] Moreover, a patient with partial monosomy 10p has been described, who presents with features of VCFS.[31] This stresses the variability of the clinical spectrum of the developmental defect associated with partial monosomy 10p, which is similar to that of monosomy 22q11. In addition to the features of the DGS/VCFS spectrum, several other features can be observed in patients with monosomy 10p, including abnormally shaped skull, microcephaly, hand and foot abnormalities, genitourinary anomalies, hearing loss, and severe psychomotoric retardation (for review see).[15,34]

In an effort to localise the genes responsible for the features of the DGS/VCFS spectrum and other abnormalities observed in patients with partial monosomy 10p, we performed deletion analysis in 12 patients with partial monosomy 10p. Nine of these patients showed typical features. The phenotypes of the other three patients were considered not to belong to the DGS/VCFS spectrum. In addition, we include the analysis of an apparently balanced translocation 10p;14q. This translocation is associated with cleft lip in a male and ventricular septum defect in his son,[35] features which might be related to DGS2.

Recently, the critical region for DGS2 has been mapped to an interval flanked by the markers D10S1216 and D10S547.[36] The deletion map presented here is consistent with this result and further narrows the critical region to a 1 cM interval.

Materials and Methods

Clinical and Cytogenetic Evaluation

Epstein-Barr virus-transformed lymphoblast lines (LCL) or primary skin fibroblast lines (FCL) were studied from 12 patients (Table 1). Metaphase chromosomes from each cell line were karyotyped after GTG banding to confirm monosomy 10p. For one patient with partial monosomy 10p, only slides and DNA samples were available. Information about the sources of the cell lines, their cytogenetically determined deletions, and brief clinical descriptions are presented in Table 1. The phenotypic summaries are based on published reports. More detailed clinical descriptions of five patients, not reported previously, are given below. Cases with GM numbers have been obtained from the National Institute of Genetic Medical Sciences Human Mutant Cell Repository at the Coriell Institute, New Jersey.

Where available, parental DNA was prepared from EDTA-anticoagulated peripheral blood or from cell lines according to standard procedures.

DNA Probes

DNA markers, cosmid, P1 artificial chromosomes (PAC) and yeast artificial chromosomes (YAC) clones used for polymerase chain reactions (PCR) and fluorescence *in situ* hybridisation (FISH) are listed in Table 2. All YACs except 815c2 were selected from the Database of the Whitehead Institute (http://www-genome.wi.mit.edu)[49] and were obtained from the CEPH B-Mega YAC library. The YAC 815c2 was identified from the pooled CEPH B-Mega YAC library as a corresponding to the locus D10S570 by PCR using published microsatellite primers.[37] The size of the YAC 747h7 was determined by pulsed field electrophoresis as described elsewhere.[38]

PACs corresponding to loci D10S585, WI-2389 and D10S1720 were isolated from the library of P. DeJong (Sanger Center, Hinxton)[39] by PCR amplification from PAC pools using the primers reported[40] followed by Southern hybridisation on gridded filters.

FISH *studies*

Metaphase spreads were prepared from the cell lines according to standard procedures. FISH was performed with YACs, PACs or cosmids (Table 2) labelled by nick translation with

biotin-14-dUTP (Sigma) and preannealed with Cot-1 DNA (Gibco BRL). Detection and visualisation was achieved using the avidin–fluorescein isothiocyanate/antiavidin antibody system described elsewhere.[41,42] Chromosomes 10 were identified by DAPI counterstaining and in some experiments by simultaneous hybridisation with the nonchimeric YAC 821d2, which maps to 10q23.

To exclude partial monosomy 22q11, FISH was performed using the cosmid N25 (D10S75) from the DGS deletion region on 22q11 (Oncor Inc.).

PCR Analysis

PCR analysis of polymorphic (CA)n repeats was performed to complement the FISH analysis and to determine the parental origin of the deletions. Each (CA)n repeat was amplified from genomic DNA by PCR using published primers.[40] PCR reactions consisted of 300 ng of genomic DNA, 150 ng of each of the forward and reverse primers, 0.5 U Taq polymerase and 10 μCi alpha 32PdCTP. Conditions for PCR amplifications were 30 cycles of 94°C for 60 s, annealing for 60 s, and 72°C for 60 s. The final elongation cycle was 7 min at 72°C. The annealing temperature was between 54°C and 60°C depending on the loci investigated. The amplified products were resolved on 6% polyacrylamide denaturing gels and detected by autoradiography.

Results

Clinical analysis

Clinical data of 12 patients with partial monosomy 10p as well as of a father and son with a reciprocal translocation 10p;14q are presented in Table 1. Data of the previously reported cases were taken from the literature.

Features of the DGS/VCFS spectrum were observed in nine of the 12 patients, with marked variability of expression. Patients MAJ[15] and GM6936[26] had all typical traits including T-cell deficiency with recurrent infections, hypoparathyroidism/hypocalcaemia, and tetralogy of Fallot (MAJ) or pulmonary stenosis (GM6939). ROB showed an aortic valvular stenosis which is not a typical cardiac defect for the DGS/VCFS spectrum, whereas patients KAN, TAT,[28], HOV and LEM did not show a cardiac defect, although TAT had a murmur. The latter five patients (ROB, KAN, TAT, HOV, LEM) are affected by frequent infections and/or T-cell abnormality. They were classified as belonging to the DGS/VCFS spectrum due to the combination of hypoparathyroidism and immunodeficiency. Patient MAR had hypocalcaemia and hypoplastic thymus,[30] whereas MEG had hypoplastic thymus but no hypocalcaemia.[31] They presented with atrial septum and ventricular defects, respectively, heart defects more frequently observed in VCFS than in DGS. In addition, MEG has a cleft palate which is also frequent in VCFS.

Phenotype information for the cell line GM03470 is scanty and the patient is considered not to belong to the DGS/VCFS spectrum, although features of DGS or VCFS cannot be excluded. The diagnosis DGS was ruled out in patients BIH[34] and AMS (see case report). The bicuspid aortic valve found in BIH is not a typical cardiac defect of the DGS/VCFS spectrum, serum parathyroid hormone and calcium were in the normal range, and despite recurrent infections, the patient showed normal T-cells. AMS has no heart defect and calcium, parathyroid hormone and T-cell levels were also normal. Where data are available, nine out of 10 patients showed postnatal growth retardation and 11/11 patients showed psychomotoric/mental retardation. Other features present in a significant number of patients with 10p deletions are ptosis (MAR, LEM, MAJ, HOV, ROB, TAT, KAN), abnormality of the kidney and/or urinary tract (MAR, LEM, GM6936, BIH, MAJ, HOV, KAN), crypto-orchidism (MAR, LEM, HOV, ROB, TAT, KAN) and mild anomalies of distal limbs (AMS, MEG, MAR, GM6936, BIH, MAJ, HOV, ROB, TAT). Hernias were observed in MAR, ROB and TAT, hearing loss in BIH, MAJ and HOV, and seizures in GM6936, HOV, and TAT (Table 1).

Five patients, who have not been previously described, are presented below as case reports.

Case reports

Patient 1 The female patient AMS is the second child of healthy unrelated parents (mother 29 years old, father 42 years old). She was born in the 41st week of an uncomplicated pregnancy with a weight of 3470 g (p50–75), a length of 53 cm (p75–90), a head circumference of 35 cm (p75), and APGAR scores of 10 at 1 and at 5 min. There were no health problems during the first year of life, but psychomotoric development was delayed. She sat at the age of 12 months, walked at 2 years, and spoke only a few simple words at age 4. Chromosome analysis at age 4 revealed a partial monosomy 10p. Parental karyotypes were normal. Physical examination of the proposita revealed the following features (Figure 1a): brachycephaly, high nasal bridge, anteverted nares, small mouth, high arched palate, apparently low-set ears, clinodactyly 5 on both hands, and bilateral mild cutaneous syndactyly of toes 2 and 3. Ultrasound of the urinary tract was normal. Length (105 cm, p50–75), OFC (49 cm, p10), and weight (17 kg, p50) were within the normal range.

Table 1 Summary of clinical presentations and molecular analysis

Patient	*GM3470*	*AMS**	*MEG*	*MAR**	*LEM**	*GM6936*
Cell line	FCL	LCL	LCL	FCL	LCL	LCL
Reference	Coriell NGMS catalogue	This paper	[31]	[30]	This paper	[26]
10p aberration	del(10)(p12p13)	del(10)(p12p13)	del(10)(p12p13)	del(10)(p11p13)	del(10)(p13)	del(10)(p13)
Sex	f	f	f	m	m	f
Age at latest evaluation (years)		4 4/12	1 6/12	1/12	10 4/12	5
DGS features						
Hypoparathyroidism		–	–		+	+
Hypocalcaemia		–	–	+	+	+
Hypoplastic thymus		–	+	+	–	–
T-cell abnormality		–	+	+		+
Recurrent infections 1)		–	+	+	UT, OM	UT
Cardiac defect 2)		–	VSD, ASD	ASD	–	PS
Others						
Prenatal growth retardation		–	+	–	–	–
Postnatal growth retardation		–		+	+	+
Psychomotoric retardation	+	+	+		+	+
Muscular hypotonia		+			+	
Seizures		–	–		–	+
Renal defect 3)		–	–	HN	VR	VR
Crypto-orchidism				+	+	
Hernias		–	–	+(l)	–	
Hand/foot abnormalities 4)		K5, CS2/3	FC	K5, CS2/3	–	K
Craniofacial						
Abnormal skull 5)	MC	BC	–	DC	DC, MC	MC
Hypertelorism	–	–	–	–	+	+
Epicanthal folds		–	+	+	+	+
Downslanting palpebral fissures		–	–	+	+	–
Ptosis		–	–	+ (r)	+	–
Broad/flat nasal bridge		–/high	+/+	–/high	+/+	+/+
Short nose		–	+	–	+	
Anteverted nares		–	–	–	+	+
Choanal atresia		–	–	+	–	–
Short philtrum		+	–	+	+	
High arched palate		+	+	/	?	–
Cleft palate or lip		–	+	–	–	–
Microretrognathia		–	+	+	+	+
Low set malformed ears		+	+/+	+/+	+/–	+/+
Hearing loss		–	–		–	
Short neck		+	–		–	–
DGS/VCFS spectrum	no 7)	no	yes	yes	yes	yes
Molecular findings						
Parental origin of deletion		mat			pat	
Deleted loci	D10S570–D10S586	D10S585–D10S191	D10S585–D10S203	D10S189–D10S595	pter-D10S585	pter-D10S1216
Deletion size (in cM) 6)	15	10	17	29	30	30

A blank space indicates that this feature was not mentioned (is unknown); mat - maternal; pat - paternal; f - female; m - male; available; **systolic murmur and cardiac enlargement.

1) GT - gastrointestinal tract; OM - otitis media; RT - respiratory tract; UT - urinary tract; 2) ASD - atrial septal defect; AVS - defect; 3) DP - dichotomic pyelon; HK - hypoplastic kidney; HN - hydronephrosis; VR - vesicoureteral reflux; 4) CS 2/3 - asymmetric skull; BC - brachycephaly; DC - dolichocephaly; MC - microcephaly; 6) The size of terminal deletions is given as the given as the distance between the most distal and the most proximal deleted locus (minimal deletion size); 7) patient 3470 was otherwise healthy, his son carries the same translocation and has an isolated VSD.

BIH	*MAJ**	*HOV**	*ROB**	*TAT*	*KAN**	*GM10207***
FCL [34]	LCL [15]	– This paper	LCL This paper	LCL [28]	LCL This paper	LCL [35]
del(10)(p13) m 4	del(10)(p13) f 2 8/12	del(10)(p13) m 4 10/12	del(10)(p12) m 9 6/12	del(10)(p13) m 1 3/12	del(10)(p13) m 3	t(10;14)(p13;p24) m
–	+	+		–	+	–
–	+	+	+	+	+	–
	+	–	–		–	–
–	+	+	–	–	+	–
RT, diarrhea	RT, OM	UT	RT, GT	RT	RT	–
BAV	TOF	–	AVS	–	–	(VSD) 8)
–	–	?		–	+	–
+	+	+	+	+	+	–
+	+	+	+	+	+	–
	+	+	+	+	+	–
		+	–	+		–
HK (r)	HK (r), DP (l), VR	VR	–	–	HK (l), VR (l)	–
		+	+	+	+	–
–	–	–	+	+	–	–
K5	PE	K	K5	CS2/3	–	–
MC		MC	–	AS	AS	–
	+	–	–	–	+	
+	+	+	+	+	+	
+	–	–	+	–	+	
–	+(r)	+	+	+	+	
+/+	+/+	+/high	+/high	+/+	+/+	
	+	–	–	–	+	
+	+	+	+	+	+	
–	–	–	–	–	–	
	+	–	–	+		
+	+	–		+	+	
–	–	–	–	–	–	+
+	+	+	+	+	+	
+/	+/+	+/+	+	+/+	+/+	
+	+	+	–		–	–
+	+	+	+	+	+	
no	yes	yes	yes	yes	yes	no
pter-D10S585	mat pter-D10S570	pat pter D10S191	pat pter- D10S548	pter- D10S203	pat pter D10S203	breakpoint interval D10S552- D10S189
30	34	38	46	47	47	–

r - right side; l - left side; LCL - lymphoblastoid cell line; FCL - fibroblastoid cell line; *indicates cases where parental DNA was

aortic valve stenosis; BAV - bicuspid aortic valve; PS - pulmonary stenosis; TOF - tetralogy of Fallot; VSD - ventricular septum cutaneous syndaktyly of toes 2 and 3; FC - finger contractures; KS - klinodaktyly of the fifth finger; PE - pes equinovarus; 5) AS - genetic distance between the most distal deleted 10p locus and the most proximal deleted locus. The size of interstitial deletions is defined as ‘‘no DGS’’, due to sparse clinical information, DGS cannot be excluded; 8) GM10207 has bilateral cleft lip, but is

Hearing and vision appeared normal. Laboratory investigations revealed a normal serum total calcium level (2.4 mmol/l, normal range 2.0–2.6 mmol/l), and a normal parathyroid hormone level (69 pg/ml). Immunologic parameters (T-cells, B-cells, immunoglobulins) were also normal.

Patient 2 The male patient LEM is the second child of healthy unrelated parents (mother 25 years old, father 33 years old). He was born at term (38th week) after an uncomplicated pregnancy with a weight of 2800 g (p10–25), a length of 51 cm (p50–75), a head circumference of 34 cm (p50–70), and APGAR scores of 6/9/10. Physical examination of the patient at birth revealed crypto-orchidism, small penis, muscular hypotonia, and facial dysmorphisms (Table 1). Laboratory investigations revealed hypocalcaemia (serum total calcium level was 1.6 mmol/l; normal > 2.1 mmol/l) and hypoparathyroidism. At 4 months, hypocalcaemia and hypoparathyroidism had persisted, and the proband

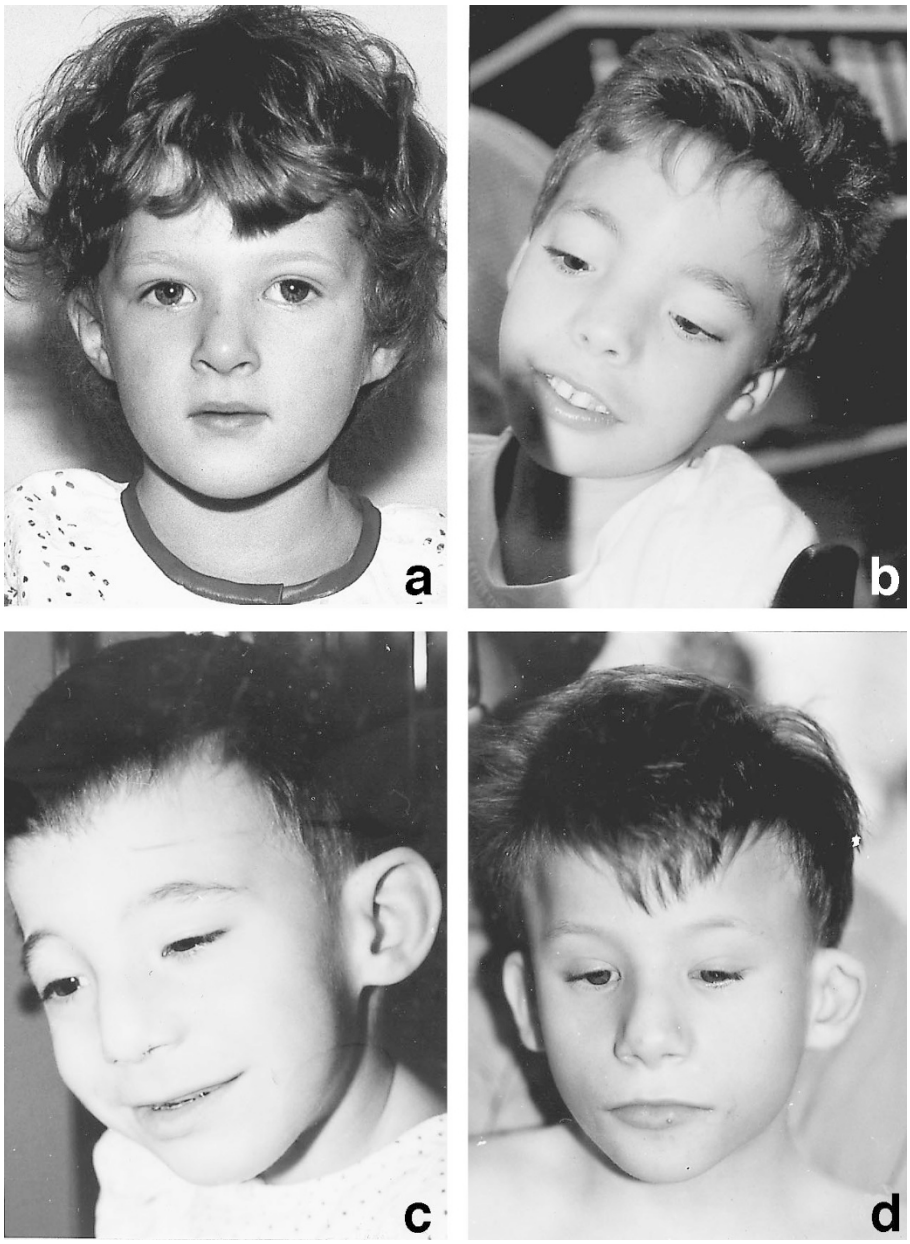

Figure 1 *Patients with 10p deletions. (***a***) patient AMS at age 4 4/12 years, (***b***) patient LEM at age 10 4/12 years, (***c***) patient HOV at age 4 10/12 years, and (***d***) patient ROB at age 9 6/12 years*

was treated with calcium and vitamin D. The postnatal period was characterised by frequent infections of the genitourinary tract, and a bilateral pyelocaliectasis with vesicoureteral reflux was diagnosed. A chromosome analysis at 7 months revealed a *de novo* deletion (10)(p13). At the age of 6 years, hypocalcaemia persisted and parathyroid hormone levels were at the lower normal range. He suffered from frequent infections. At age 10 he had a height of 126 cm (p50–75), a weight of 25 kg (p90) and a head circumference of 51 cm (p10–25). He showed generalised muscular hypotonia, a spastic diplegia and mental retardation. Until age 6, he showed autism, which was partially resolved at the age of 9 6/12 years. He sat at 18 months, walked at 3 years, and was able to speak two words at the age of 10 4/12 years.

Patient 3 The male patient HOV is the second child of healthy unrelated parents (mother 29 years old, father 27 years old). He was born in the 36th week after an uncomplicated pregnancy with a weight of 2300 g (p10–25), a length of 45 cm (p10–25), and APGAR scores of 7 and 9 at 1 and 5 min, respectively. Physical examination of the patient at birth revealed cryptoorchidism on the right side, hypospadia, two accessory nipples, muscular hypotonia, and facial dysmorphisms, including blepharophimosis and preauricular pits on the right side (Table 1). A chromosomal analysis was performed, and a *de novo* deletion (10)(p13) was diagnosed. Laboratory investigations revealed hypocalcaemia with a serum total calcium level of 1.5 mmol/l (normal range 2.5–2.8 mmol/l) and an ionised calcium of 0.5 mmol/l (normal range 0.9–1.4 mmol/l). Parathyroid hormone was in the lower normal range. The postnatal period was characterised by frequent genitourinary tract infections and muscular hypertonia. A reduced number of T-lymphocytes was found. Ultrasound of heart and thymus, and electrocardiogram were normal. At 4 10/12 years (Figure 1c) he had a length of 92 cm (< p3), a weight of 11.4 kg (< p3) and a head circumference of 47.5 cm (< p3). At this age hypocalcaemia persisted. He had sensorineural hearing loss of moderate degree on the right side and sensorineural and conductive hearing loss on the left side. Thoracic scoliosis was present. He sat at 13 months, stood with support at 2 years, and began to walk at 4 10/12 years. His psychomotoric development corresponded to a 9–10 month-old child.

Patient 4 The male patient ROB was the third child born to a healthy 24 year-old mother and 28 year-old father. The mother smoked heavily during pregnancy. The boy was born after an uncomplicated pregnancy in the 35th week of gestation with a weight of 2140 g (p10–25), a length of 42 cm (p3–10) and a head circumference of 31 cm (p25–50). The child was hospitalised after birth for nine weeks for transient hypoglycaemia, hypokalaemia and hypocalcaemia. In the first eight months he was hospitalised twice for pneumonia and enteritis. At 9 months a right-sided herniotomy and orchidopexy was performed. Repeated electrocardiograms and echocardiograms revealed a low-grade valvular stenosis of the aorta. His reaction to sound seemed present but reduced. Except for a predisposition to herpes labialis infections, there were no indications of immunological abnormalities. Cytogenetic analysis revealed a *de novo* deletion (10)(p12). Data of phenotypic analysis at age 11 months are presented in Table 1. Ptosis of both eyes was corrected surgically. When seen last at the age of 9 6/12 years (Figure 1d), he showed severe physical and mental retardation, and was unable to stand or walk.

Patient 5 The male patient KAN was born in Osaka as the first child of a healthy 32 year-old Japanese mother and a 53 year-old Indian father. Two paternal half-brothers are healthy. He was born at term after an uncomplicated pregnancy with a weight of 2700 g (p3–10) and a length of 47 cm (p3–10). The child was hospitalised as a neonate for five weeks because of respiratory difficulties, and failure to thrive. During the first year of life he suffered from recurrent respiratory infections. At 11 months the B- and T-cell counts were within the normal age-specific range. However, the T-cell subpopulations showed an inverse ratio of helper and suppressor T-cells, and we were unable to stimulate the T-cells with phytohaemagglutinin and concavalin A. IgG and IgM levels were increased (IgG 2063 mg/100 ml, normal range 350–1180 mg/100 ml; IgM 612 mg/100 ml, range 36–104 mg/100 ml), with a normal distribution of IgG subclasses, X-rays confirmed that the thymus was present. Laboratory investigation revealed hypocalcaemia (1.72 mmol/l; normal range 2.05–2.70 mmol/l). The parathyroid hormone level was very low (0.08 ng/ml, normal range 0.24–1.15 ng/ml). Cytogenetic analysis revealed a *de novo* deletion (10)(p13). At the age of 3 years (Table 1) his weight was 10.3 kg (< p3), his length was 87 cm (p3) and his head circumference was 47 cm (p3). A nephroureterectomy of the left kidney, an extirpation of the left testis and an orchidopexy on the right side was performed. The boy

was severely retarded, but otherwise in good physical condition.

Deletion analysis

A microdeletion 22q11 was excluded in all patients of this study by the demonstration of signals on both homologs with cosmid N25 (Oncor) corresponding to the locus D22S75, which maps within the DGS/VCFS microdeletion region at 22q11.

Of the 12 10p deletions, eight were terminal and four were interstitial (Figure 2). All patients who were defined as having a terminal deletion were hemizygous for the cosmid cTBQ14.16 (D10S33), which was the most distal locus tested (Table 2).

The largest deletions were found in patients ROB, TAT and KAN. The breakpoints in KAN, TAT and ROB map within the 2 cM interval between D10S548 (deleted) and D10S211 (present), and the deletions encompass at least 46 cM according to the Whitehead database. The second largest terminal deletion was found in HOV (38 cM). The breakpoints of the terminal deletions in patients MAJ, BIH and GM6936 were mapped within a 4 cM interval between D10S585 and D10S223. Fine mapping demonstrated that the breakpoints do not cluster (Table 2).

The smallest terminal deletion was found in patient LEM and encompasses 30 cM. YAC 918h11 (D10S547) was hemizygous and YAC 773c3 (D10S585) was dizygous in this patient. YAC 747h7 overlaps with YACs 918h11 and 773c3, and was found to be dizygous in LEM, suggesting that the breakpoint maps within this YAC. However, we did not identify a weaker signal of this YAC on the deleted chromosome 10 in comparison to the normal chromosome 10. Because no information about the size of YAC 747h7 was available from the database, we performed pulsed field gel electrophoresis (PFGE) and determined a size of 1.4 Mbp. PCR analysis showed that the YAC 747h7 includes the loci

Table 2 Loci and probes used for FISH analysis

Locus	*Genetic map position (cM)*	*Probe*	*GM3470*	*AMS*	*MEG*	*MAR*	*LEM*	*GM6936*	*BIH*	*MAJ*	*HOV*	*ROB*	*TAT*	*KAN*
D10S33	0–8	cTBQ14. 16	+	+	+	+	–	–	–	–	–	–	–	–
D10S552	14	809f9		+		+								–
D10S189	18	876f3		+	+	–	–*	–	–	–*	–*			
D10S226	27	627a4	+	+*		–*			–	–*			–	
D10S1720	27	PAC 123l2		+*	+		–				–*			
WI-600		PAC 414o17			+		–							
D10S547	29	918h11	+		+		–		–			–	–	–
WI-2389		PAC 204F19		–	–		–							
D10S585	30	PAC 323N1	+	–	–		–							
D10S585	30	773c3	+	–*	–		+	–	–		–*	–	–	–
D10S1216		916d6		–*	–			–		–	–			–
D10S1705	33		+*	–*			+*		+*	–*	–*			
D10S570	34	815c2	(+)							–*	–*			
D10S223	34	808a2	–		–			+	+/+*	+		–	–	–
D10S191	38	855d10	–	–*	–	–*	+*	+		+/+*	–/+*	–/–*	–	
D10S548	46	817e11	–			–					+*	–	–	–
D10S203	47	696f5			–			+					–	–
D10S595	47	764g1	–	+*		–								
D10S211	48	807b3				+						+	+	+
D10S586	49	934e11	–		+			+				+		
D10S1747	49	805e6	(+)											
D10S572	52	875b4	+					+					+	
D10S197	53			+*		+*				+*				+*

The order of deletion patients from left to right in the table corresponds to their order from bottom to top in Figure 2. The order of loci and their map position is taken from the Whitehead Institute.

The map position of locus D10S33 was taken from the EUROGEM map [50]. In that study D10S33 was mapped distally to D10S32, which was given a map position of 8 cM.

+Presence of the locus/corresponding YAC on both chromosomes 10; (+) weaker FISH signal of the YAC on the deleted chromosome 10; - absence of the locus/corresponding YAC on the deleted chromosome 10; *results of PCR analysis of polymorphic loci, which were informative in the patient or family.

The locus D10S570 maps to the same recombination interval as D10S223 in the Généthon linkage map (May 1997). PCR analysis demonstrated that for D10S570 only the YAC 815c2 was positive (overlapping YAC 916d6 and distal YAC 808a2 were negative). The finding that MAJ with a terminal 10p deletion is hemizygous for D10S570 but dizygous for the YAC 808a2, suggests that D10S570 maps distal to D10S223.

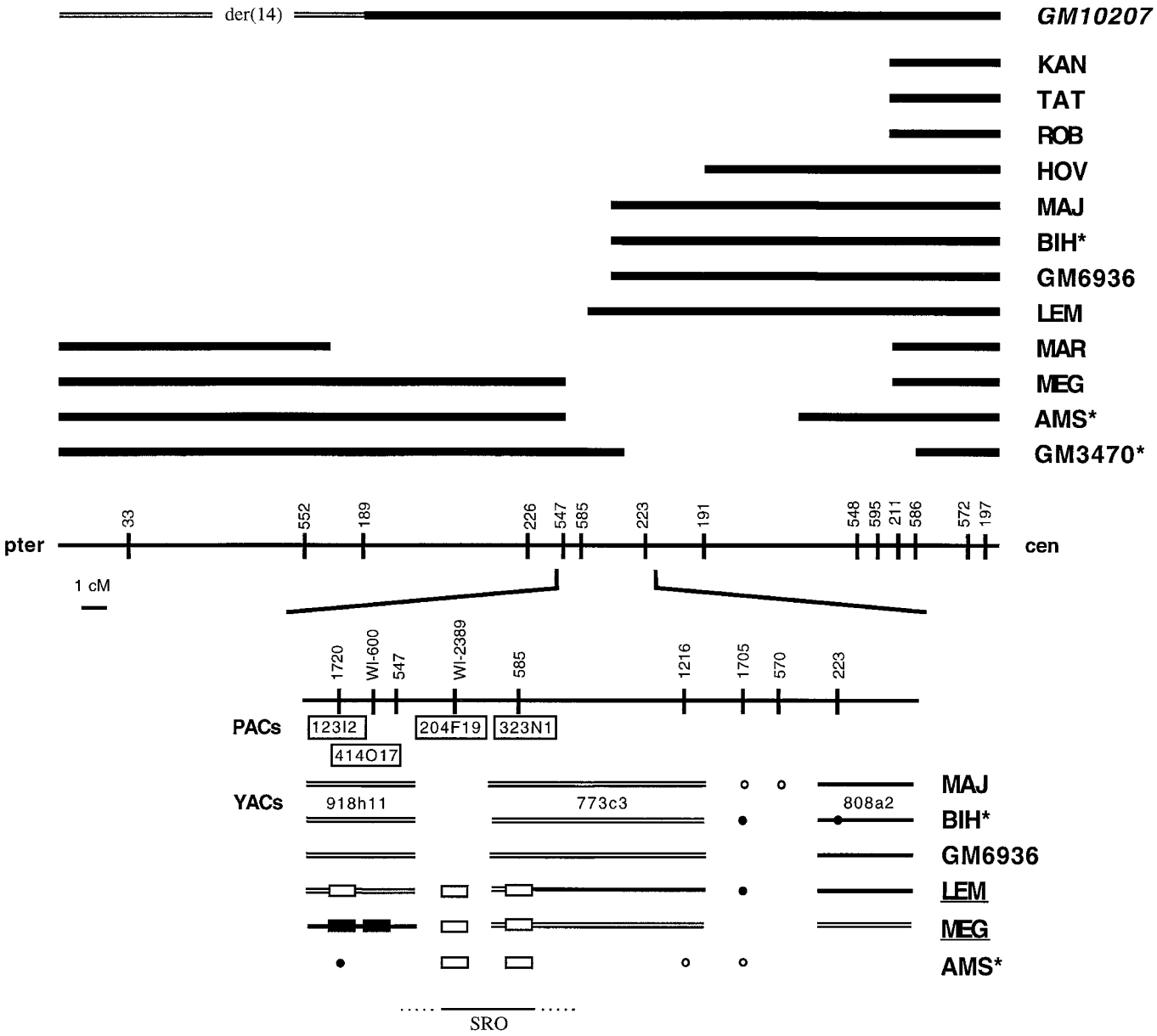

Figure 2 *Deletion map of patients with chromosome 10 aberrations. Solid horizontal bars indicate regions not deleted in the patients. Patients with an asterisk do not show a typical DGS phenotype. GM10207 has a reciprocal translocation (10p; 14q). The genetic map between markers D10S33 and D10S197 is drawn approximately to scale. At the bottom, the DGS2/VCFS2 region is shown in detail. YACs are drawn as bars, PACs as boxes, and the results of microsatellite analyses as dots. Open symbols represent the hemizygous (deleted) state; filled symbols represent the dizygous (not deleted) state. Underlined patients LEM and MEG define the proximal and distal boundaries of the DGS2/VCFS2 critical region (SRO, smallest region of overlap).*

D10S547 and D10S585, and carries a deletion. According to the Whitehead database the YAC 747h7 does not contain the marker D10S585. Further FISH analysis in patient LEM with several PAC clones demonstrated hemizygosity for the PAC 323N1 (D10S585). D10S585 maps to the distal segment of the YAC 773c3, for which LEM is dizygous. Thus, it can be concluded that the breakpoint in LEM maps within 773c3.

The interstitial deletions are ordered in Figure 2 from distal to proximal deletion intervals. Patient MAR shows the most distal and largest interstitial deletion. The distal breakpoint maps within the 4 cM interval between D10S552 and D10S189 and the proximal breakpoint maps within the 1 cM interval between the loci D10S595 and D10S211. Thus the deletion encompasses 29–32 cM.

The interstitial deletion of patient MEG encompasses about 18 cM, and includes the loci from D10S585 distally to D10S203 proximally. The distal breakpoint maps between D10S547 (YAC 918h11 dizygous) and D10S585 (YAC 773c3 hemizygous). YAC 747h7, which overlaps with the YACs 918h11 and 773c3 was dizygous in the patient, suggesting that the breakpoint maps within YAC 747h7. As in patient LEM, we did not find a weaker signal of this YAC on the deleted chromosome 10 in comparison with the normal chromosome 10 to support this assumption. Further analysis with PAC clones demonstrated hemizygosity for the PAC 204F19 (WI-2389) and dizygosity for the PAC 414017 (WI-600). Thus, the distal breakpoint of MEG maps between STS WI-600 (intact) and STS WI-2389 (deleted) within YAC747h7. The proximal breakpoint in MEG maps within the 1 cM interval between D10S595 and D10S563.

The distal breakpoint in patient AMS maps between D10S1720 and WI-2389 and the proximal breakpoint maps between D10S191 and D10S595 giving a deletion size of 10–18 cM.

The most proximal deletion was found in GM3470. The distal breakpoint in this cell line was mapped by FISH analysis with the YAC 815c2, which was isolated from the CEPH-Mega YAC library, and corresponds to D10S570. This YAC showed only a weak signal on the deleted chromosome 10 compared to the normal chromosome 10, and a signal at 5q on both homologues. This suggests that the breakpoint in GM3470 maps within the YAC 815c2 and demonstrates a chimera of this YAC. PCR analysis of the locus D10S1705 showed heterozygosity in the patient. D10S1705 maps distal to YAC 815c2 according to the database of the Whitehead Institute. These apparently contrasting results suggest a complex chimera of YAC 815c2. In addition to a chromosome 5q sequence, the YAC contains two 10p regions corresponding to WI-9659 and D10S570. WI-9659 maps proximally to D10S1705, and is dizygous in GM3470, D10S570 maps distally to D10S1705 (according to the Généthon linkage map, March 1996), and is hemizygous in GM3470. Another, rather unlikely, cause for the contrasting results would be a complex rearrangement in GM3470. The proximal breakpoint maps within the YAC 805e6, which shows a weaker signal on the del(10p) chromosome compared with the normal chromosome. Therefore, the deletion of GM3470 encompasses about 15 cM.

FISH analysis was also used to define the 10p breakpoint interval of the translocation (10p;14q) in patient GM10207. We mapped the breakpoint within the 4 cM interval between D10S552 and D10S189.

Parental origin

The parental origin of *de novo* deletions in patients AMS, LEM, HOV, ROB, and KAN were determined. Four terminal deletions were found to be of paternal (LEM, HOV, ROB, KAN) and one of maternal (AMS) origin. The parental origin of the *de novo* deletion could not be determined in patients GM3470, MEG, MAR, GM6936, BIH, and TAT because of a lack of parental DNA. The deletion in patient MAJ is due to a maternal pericentric inversion.[15]

Discussion

Critical DGS2 Region

In order to define a haploinsufficiency region for DGS on chromosome 10p, we performed molecular deletion analysis in 12 patients with partial monosomy 10p. Genotype–phenotype correlation studies are potentially complicated by cases derived from parental translocations or inversions in which both monosomy and trisomy of different chromosomal segments are present. To exclude interferences from such double segment aneuploidies, we concentrated on cases with monosomy 10p alone. The only exception is patient MAJ, who carries a cytogenetically invisible trisomy of distal 10q, minimising the amount of the trisomic material.[15] It is known from several studies that there is a wide variability in the phenotypic features of patients with deletions or duplications of the same chromosomal region, such as partial monosomy 4p in patients with Wolf syndrome, and partial monosomy 5p in *cri du chat* syndrome.[45] For this reason, the presence of a particular trait in a patient carries more weight than its absence. Nine patients are informative for the determination of an haploinsufficiency region for DGS/VCFS, since they show features of DGS (LEM, MAJ, HOV, ROB, TAT, KAN) or VCFS (MEG, MAR).

The smallest region of overlap (SRO) is defined by the terminal deletion in patient LEM and the interstitial deletion of patient MEG (Figure 2). Patient LEM shows clinical signs of immunodeficiency and has persistent hypoparathyroidism, but no heart defect. Hypoparathyroidism alone is known from 22q11 deletions,[12] and therefore we conclude that this patient can

be used for the determination of the critical region for DGS2. Patient MEG shows typical features of the VCFS including ventricular septal defect, cleft palate and T-cell deficiency.[31]

The only YAC which was found to be dizygous in both patients LEM and MEG (747h7) carries a deletion. For the distal YAC 918h11 (D10S547) patient LEM is hemizygous and patient MEG is dizygous. For the proximal YAC 773c3 (D10S585), the situation is reversed. Thus the boundaries of the SRO are defined by these two YACs, which both overlap with YAC 747h7. For further definition of the SRO, FISH was performed with PACs corresponding to loci adjacent and between these YACs. Both LEM and MEG were hemizygous for the PACs 323N1 and 204F19 corresponding to D10S585 and WI-2389, respectively. All patients of the present study with features of DGS or VCFS are hemizygous for these two PACs, which define the minimal SRO in the DGS/VCFS patients of this study. Our results refine the DGS2 deletion interval defined and estimated to encompass about 2 Mb by Daw *et al*,[36] although the exact breakpoints have yet to be determined. The breakpoint in patient LEM narrows the interval by approximately 1 Mb. LEM is dizygous for the YAC 773c3, while the patients analysed by Daw *et al* are hemizygous. According to the genetic map the critical DGS2 region is approximately 1 cM, which is the distance given between loci D10S585 and D10S547. So far no genes have been mapped to the DGS2 region. However, due to the possibility of position effects, which are well known for several haploinsufficiency genes in humans,[44] a candidate region of at least 500 kb around the SRO has to be considered. Genes in the vicinity of the 10p translocation breakpoint associated with cleft lip and ventricular septum defect (VSD) are unlikely to be involved in DGS2, because the breakpoint interval maps about 12 cM distal to the DGS2 region.

In general, there is a chance that the pathogenesis of DGS2/VCFS2 might be more complex and several loci separated by larger intervals might contribute to the DGS phenotype. The possibility of a haploinsufficiency locus causing congenital hypoparathyroidism alone without T-cell deficiency and heart defect has been stressed by molecular analysis of two terminal deletion patients with a breakpoint just distal to the critical DGS2 region.[45] Interestingly, patient MEG, who is dizygous for the region deleted in these two patients did not show hypoparathyroidism, while all DGS patients with a terminal deletion of our study did.

Phenotypic Spectrum of DGS2 versus DGS1

Regarding the phenotypic expression of the DGS spectrum in patients with partial monosomy 10p, there are both similarities and differences to DGS1. Both partial monosomy 10p and partial monosomy 22q11 are associated with a variable expression of the DGS, with a significant proportion of patients presenting only a subset of the DGS features. The VCFS belongs to the phenotypic spectrum of both partial monosomy 10p and partial monosomy 22q11. Patients AMS and BIH of this study have no features of the DGS/VCFS spectrum, but they are hemizygous for the critical DGS2/VCFS2 region. This provides evidence for a reduced penetrance of DGS2/VCFS2, which is also well known for DGS1/VCFS1.

Renal abnormalities have been found in 36% of patients with partial monosomy 22q11[46] and in six of the nine DGS2/VCFS2 patients of this study. Thus, abnormalities of the urinary tract might be more common in DGS2/VCFS2 than in DGS1/VCFS1. Heart defects in monosomy 10p involve tetralogy of Fallot, atrial septal defect, ventricular septal defect, aortic valve stenosis and pulmonary stenosis, and thus appear to be more heterogeneous than in patients with partial monosomy 22q11. While the common deletion region in DGS1 and VCFS1 encompasses about 2 Mbp, with identical breakpoints in about 95% of patients, the size of the deletions in the patients of this study varies from about 20 to 40 Mbp. Fine mapping gave no evidence for a clustering of breakpoints. Thus, regarding renal abnormalities and heart defects, the differences observed between DGS1/VCFS1 and DGS2/VCFS2 are compatible with a variable set of deleted genes in partial monosomy 10p.

The parental origin of the deletion is known in 6/12 deletion patients investigated. There is no evidence for imprinting effects. Both patients MAJ and AMS have deletions of maternal origin, and present with all typical DGS features or no DGS feature, respectively. The four deletions of paternal origin (in patients LEM, HOV, ROB, KAN) were associated with a variable spectrum of DGS features.

Tentative Mapping of other 10p Disease Loci

The deletions described in this study range from at least 10 cM in patient AMS to almost 50 cM in patients TAT and KAN. The deletion patients present with several phenotypic features, which are not part of the DGS/VCFS spectrum. This enables the tentative mapping of

disease loci other than DGS to the short arm of chromosome 10.

Ptosis is not very common in chromosomal aneuploidy syndromes and was observed in six patients of this study (MAR, MAJ, HOV, ROB, TAT, KAN). The molecular data of these patients suggest that gene(s) on 10p responsible for this phenotype map between D10S552, which flanks the interstitial deletion of MAR distally, and D10S223, which flanks the terminal deletion of MAJ.

Hearing loss was found in three of eight patients in this study (BIH, MAJ, HOV) and has been described in at least four other patients with partial monosomy 10p.[30,34,47,48] Because several patients have been reported at an age before hearing loss might be obvious, and because in other reports no information on this feature is given, we argue that hearing loss might be more common in partial monosomy 10p than reported to date. The deletion breakpoints of patients BIH, MAJ and HOV suggest that gene(s) involved in hearing loss map distal to D10S1705, for which BIH with a terminal deletion is dizygous. More investigations are necessary to determine the type of hearing loss in the affected patients.

Probes from the refined deletion interval can now be used to screen for 10p microdeletions in patients where monosomy 22q11 has been excluded. Criteria for the characterisation of candidate genes from the DGS2 deletion region include spatial and temporal expression patterns, sequence homologies to known developmental domains including DGS1 candidates and, last but not least, knock-out mice experiments.

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