European Journal of Human Genetics (1999) 7, 903–909

http://www.stockton-press.co.uk/ejhg

ARTICLE

Atypical deletions suggest five 22q11.2 critical regions related to the DiGeorge/velo-cardio-facial syndrome

Francesca Amati[1], Emanuela Conti[1], Antonio Novelli[1], Mario Bengala[1], Maria Cristina Digilio[2], Bruno Marino[2], Aldo Giannotti[2], Orazio Gabrielli[3], Giuseppe Novelli[1] and Bruno Dallapiccola[1]

[1]*Dipartimento di Biopatologia e Diagnostica per Immagini, Università Tor Vergata and CSS-Mendel, Rome*
[2]*Ospedale Pediatrico Bambino Gesù, Rome*
[3]*Clinica Pediatrica, Università di Ancona, Italy*

Deletions of chromosome 22q11.2 have been associated with distinct phenotypes including DiGeorge syndrome (DGS) and velo-cardio-facial (VCFS) syndrome. These diseases result from a failure to form derivatives of the third and fourth branchial arches during development. DGS/VCFS deletions usually encompass about 3 Mb of genomic DNA in more than 90% of patients. However, deletion mapping studies have failed to demonstrate the existence of a single small region of overlap (SRO) and ruled out any obvious correlation between site or size of deletion and severity of clinical phenotype. We describe three patients carrying 'atypical' deletions presenting the DGS/VCFS phenotype. A comparative analysis of deletions in our patients and those previously published has suggested the existence of five distinct critical regions within the 22q11.2 locus. This observation argues that DGS/VCFS results from haploinsufficiency secondary to a complex and as yet unexplained molecular mechanism, probably involving chromatin effects in mediating gene expression throughout the entire region.

Keywords: DiGeorge syndrome; velo-cardio-facial syndrome; deletion map; chromosome 22

Introduction

DiGeorge syndrome (OMIM 188400; DGS) is a severe congenital disorder characterised by immune T cell defect due to thymic hypo-aplasia, parathyroid hypoplasia, and heart outflow tract defects. More than 90% of these patients are hemizygous for a 1.5–3 Mb region within 22q11.2.[1] However, this deletion is associated with a wide spectrum of phenotypes, including velo-cardio-facial syndrome (VCFS; OMIM 192430), conotruncal anomaly face syndrome (contained in OMIM 188400), Cayler syndrome (OMIM 125520), and Opitz-G/BBB syndrome (OMIM 145410; OGS2). This suggests a common origin for all these disorders.[2] The phenotype associated with deletion 22q11.2 may be more complex and include more than 180 symptoms.[3] In addition, subjects with similar molecular defects show inter-individual and intra-familial variability, which does not appear to be obviously related to the size of the deleted region. Smaller deletions are not

Correspondence: Giuseppe Novelli PhD, Department of Biopathology and Diagnostic Imaging, Tor Vergata University of Rome, School of Medicine, Via di Tor Vergata 135-00133 Roma, Italy. Tel: +39+06+72596078; Fax: +39+06+20427313; E-mail: novelli@med.uniroma2.it
Received 17 May 1999; revised 22 July 1999; accepted 5 August 1999

associated with milder or more restricted phenotype. Monozygotic twins have been reported with discordant clinical manifestations.[4] The phenotype resulting from the 22q11.2 deletion has been attributed to a contiguous gene syndrome model.[5] However, a number of patients with non-overlapping deletions and similar phenotype excludes this model as the pathogenic mechanism.[6–9] Molecular studies have defined at least four minimum critical regions, the deletion of which results in the DGS/VCFS phenotype.[8,9] The first small region of overlap (SRO1), about 300 Kb in size, was defined by molecular analysis of patients carrying rare rearrangements, including the translocation between chromosomes 2 and 22 (ADU), associated either with DGS or VCFS phenotypes.[10–12] The second region, SRO2, was suggested by analysis of the DGS patient G, presenting with a deletion telomeric to SRO1.[13] A third critical region, SRO3, was defined by a patient with a deletion distal to SRO1 and SRO2,[8] overlapping with the deletion described by Kurahashi *et al*.[7] A novel deletion, SRO4, adjacent to but not overlapping SRO3, was observed in a mother and her two daughters presenting the full DGS/VCFS phenotype.[9] Yamagishi *et al*[14] have recently reported a DGS/VCFS patient with a 20 Kb *de novo* deletion distal to SRO1, overlapping SRO2 in part. Altogether, these data suggest the existence of several critical regions within the deleted area, and do not support a unique major gene responsible for the primary defect in all patients.[15]

We describe three patients with atypical 22q11.2 deletions and DGS/VCFS phenotype. By comparing the deleted intervals in our patients and those previously reported, the existence of five distinct genomic intervals associated with DGS/VCFS phenotype is inferred.

Materials and Methods

Patients

JK (Figure 1A), a girl, was born by vaginal delivery at term of an uncomplicated pregnancy; birth weight was 2500 g. Neonatal hypocalcaemia was noted in the first week of life. Cardiological evaluation revealed Fallot's tetralogy with absent infundibular septum, right aortic arch, aberrant left subclavian artery. Corrective surgery by homograft conduit was performed. The thymus was absent. At the age of 5 years, weight was 14.4 kg (3rd centile), height 104 cm (25th centile), and OFC 47.5 cm (3rd centile). Clinical examination showed periorbital fullness, hypertelorism, narrow and upwards slanting palpebral fissures, prominent nose with hypoplastic nares, choanal stenosis on the left, small mouth with everted upper lip, micrognathia, high-arched palate with cleft uvula, prominent and dysmorphic ears, long fingers and toes, bilateral inguinal hernias. Motor milestones were largely normal, but language was mildly retarded. Audiological and ophthalmological examinations and renal ultrasound were normal. CD3 cells were reduced (54.4%).

ML (Figure 1B), a girl, was born by Caesarean section at term of an uneventful pregnancy; birth weight was 3100 g. Neonatal hypocalcaemia was recorded. Cardiological evaluation showed Fallot's tetralogy, right aortic arch, restrictive ventricular septal defect caused by accessory tissue of the tricuspid valve, hypertrophic right ventricle with small cavity and suprasystemic pressure. At 8 months corrective surgery by transanular patch was performed. A normal thymus was found at surgery. At 8 years, weight was 22 kg (10th centile), height 122 cm (25th centile), and OFC 52 cm (50th centile). Clinical examination showed narrow and upwards slanting palpebral fissures, large nose, small mouth, high arched palate. Developmental milestones were unremarkable.

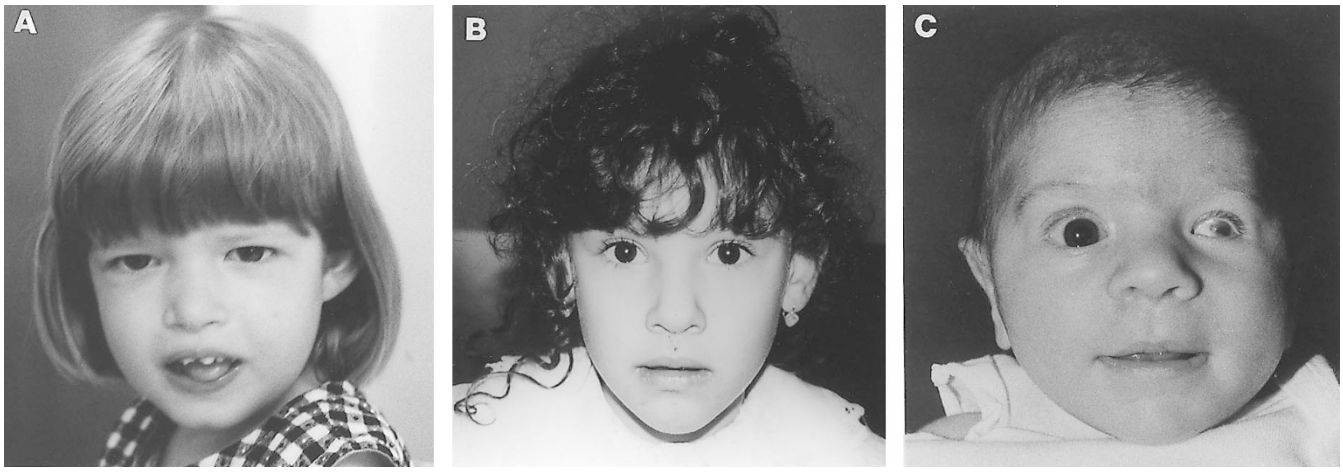

Figure 1 *Facial appearance of patients JK (A), ML (B), and SL (C)*

Audiologic examination showed conductive deafness. Ophthalmologic evaluation and renal ultrasound were normal. CD3 cells were below the normal range (51.9%).

SL, (Figure 1C) a male, was born by Caesarean section for breech presentation. Birth weight was 3053 g, and length 50 cm. Cardiological evaluation disclosed interrupted aortic arch type B and pulmonary valve stenosis. Corrective surgery was performed on the first day of life. At 2 months, weight was 3700 g (< 3rd percentile), and height 52.5 cm (< 3rd centile). Clinical evaluation established a round face with hypertrichosis mainly on the frontal bone, hypertelorism, bilateral coloboma, microphthalamia and corneal clouding on the left, depressed nasal bridge, long philtrum, cleft soft palate, long tapering fingers, proximally implanted thumbs, and cryptoorchidism. Serum calcium was normal. After cardiac surgery the clinical course was unremarkable, and at 21 months psychomotor development was considered to be within normal range.

Marker and FISH Analysis

Chromosome metaphases were prepared from lymphocyte cultures according to standard procedures. DNA was extracted from blood using standard protocols.[16] PCR reactions and FISH analysis were performed according to published protocols.[17,18] A total of 16 probes, including two cosmids (co23, co29), 13 STRP genetic markers spanning 3 Mb of the common deleted region, and an intragenic polymorphism in the *UFD1L* gene[19] were used. Co23 and co29 map within the SRO1. Co29 was isolated by the YAC clone 966a8 as previously described[18] and found to map between D22S75 (probe N25) and HIRA,[20] overlapping with co72F8 and cosmid F39E1 deposited in GenBank (AC 000085 and AC 000094.1). Co23 contains nearly all the *UFD1L* gene and the first two exons of the *CDC45L* gene.[15] Co23 overlaps with co83c5 and co102g9 deposited in GenBank (AC 000087 and AC 000068.1). Both cosmids are currently used in our laboratory for detecting DGS/VCFS patients.[19,21] The STRP markers (cen-D22S427, D22S1638, 22K48-2, 22K48-1, D22S941, D22S944, D22S1623, D22S264, D22S311, D22S1709, D22S306, D22S303, D22S257-tel) have been described by Carlson *et al*[17] and on GDB (http://www.hgmp.mrc.ac.uk). 22k48-1 and 22k48-2 were identified in our lab (GDB 6118474 and GDB 6118487). The following PCR conditions were used to obtain specific amplicons: 1 cycle at 94°C for 5 min, 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s and 1 cycle at 72°C for 7 min. For markers D22S264, D22S1623, D22S944, D222S941, 22K48-1, 22K48-2, and D22S1638 the annealing temperature was 58°C. Genotype analysis was performed by visualising microsatellite alleles on autoradiography. The –277A/G intragenic UFD1L marker was studied by SSCP analysis using the following primers: 5' GCAATAGGTTTTTGGTGTGG 3' (forward primer) and 5' CGAAGACGCTAGGCTCTAAA 3' (reverse primer). These primers were able to amplify two different alleles (A1, A2) with an allelic frequency of 0.65 and 0.35, respectively, in the Italian population. SSCP analysis was performed in a GenePhor electrophoresis unit (Amersham Pharmacia Biotech, Clearbrook Dz. Arlington, IL, USA) using a 12% acrylamide gel at a temperature of 10°C. After electrophoresis acrylamide gels were stained with silver staining (Amersham Pharmacia Biotech).

Results and Discussion

On a total of 90 deletion 22q11.2 DGS/VCFS patients extensively studied with the set of probes described, three had atypical deletions as shown by microsatellite and FISH analyses (Figure 2). The 3.3% is unexpected

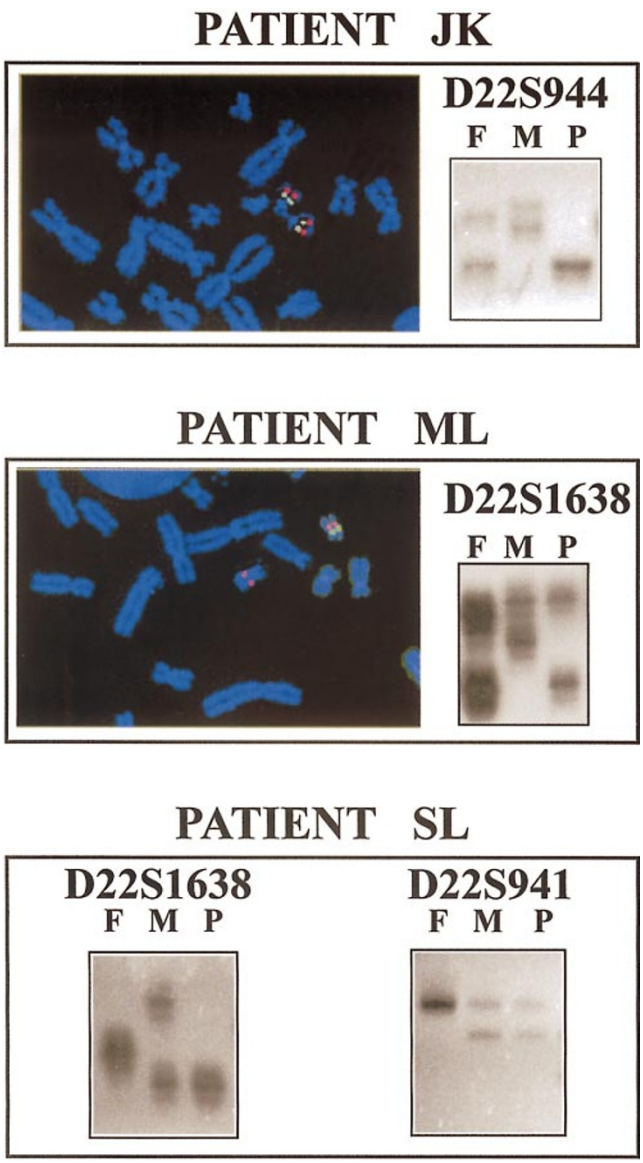

Figure 2 *Microsatellite and FISH analyses of patients carrying 'atypical' deletions. Partial chromosome metaphases of patients JK and ML examined respectively with co23 and co29 (green signals). Red signals are due to a chromosome 22 control probe (co20) mapping outside the commonly deleted region (unpublished). Microsatellite segregation analysis shows the deletion at marker D22S944 in patient JK, and at D22S1638 in patient SL. In contrast, loci D22S1638 and D22S941 result undeleted in patients ML and SL respectively. F: father; M: mother; P: patient*

and is probably due to the fact that in our laboratory a large number of patients with common deletions were not included in this study since molecular analysis with all markers was incomplete or uninformative. Patient JK was hemizygous for D22S944, D22S311, D22S1709 microsatellites markers (Table 1). FISH analysis of metaphases, using co23 and co29, showed two signals. Thus, JK has a deletion of almost 3 Mb which did not include the interval corresponding to SRO1.[20] Patient ML had two alleles at locus D22S1638, whilst co29 and co23 were deleted (Figure 2 Table 1). This deletion narrowed the proximal part of the SRO1 interval and overlaps SRO2. All other distal markers examined revealed heterozygosity or were uninformative (Table 1). Patient SL was hemizygous at the proximal locus D22S1638 (Figure 2), (Table 1). FISH analysis with co29 and co23 showed one and two signals, respectively, on chromosome 22 Table 1). Therefore this patient had a proximal deletion including SRO1. These results are summarised in Figure 3, which includes also other published DGS/VCFS patients with atypical deletions. This deletion map suggests the existence of at least five regions critical for development of the DGS/VCFS phenotype. Interval **1** runs from the proximal breakpoint in patient SL to the proximal breakpoint in patient G,[13] and contains the ADU translocation breakpoint and the loci DGCR6, DGCR5, IDD, TSK1, DGSI/ES2.[22] Interval **2** runs from the proximal breakpoint in patient G to the distal breakpoint in patient JF, and includes the loci GSCL, CTP, CLTCL, HIRA, NLVCF, UFD1L and CDC45L.[22] Interval **3** runs from the distal proximal breakpoint in patient JK to the proximal breakpoint in patient NA and contains the TMVCF locus.[23] Interval **4** runs from the proximal breakpoint in patient NA to the distal breakpoint in patient CTAF, and includes PNUTL1, Gp1Bβ, TBX1, COMT, ARVCF, T10, LZTR1, ZNF74, and HCAT4 loci.[22,24] Interval **5** runs from the proximal breakpoint in patient III:3 to the distal breakpoint in patient JK, and contains the immunoglobulin light chain (IGLC) region.[9] About 90% of DGS/VCFS patients have a similar 3 Mb deletion, which encompasses the five intervals described in this paper. This is not unexpected, since the 22q11.2 region is abundant in low-copy DNA repeats, which induce homologous recombination and mediate deletions.[25] In recent years a number of DGS/VCFS patients with atypical deletions have contributed to defining the SRO critical for this disorder and dissecting the phenotype according to deletion size.[6–9] The identification of an SRO is a crucial step in elucidating the molecular basis of these disorders and identifying gene(s) which contribute to the development of distinct features including cardiac defects, abnormal faces, thymic hypo-aplasia, cleft palate and hypocalcaemia. In this context, a deletion map is a useful aid to fine mapping. Similar attempts have been reported previously.[5] However, complexity of the phenotype delineation has lowered expectations.[26] More recently, Hur *et al*[27] have described two DGS patients carrying an atypical deletion within interal **2**, presumably overlapping with the deletion found in our patient ML. Neither patient exhibited any characteristic facial dysmorphism, suggesting that hemizygosity of this interval predisposes or influences the facial features associated with DGS/VCFS. However, our patient ML strictly overlaps the 'classical' DGS/VCFS phenotype. Phenotypic differences are common in children with a 22q11.2 deletion and probably reflect stochastic events affecting morphogenesis during neural crest cell migration.[28,29] This could explain in part the clinical variability in DGS/VCFS patients. For example, patient SL shows a bilateral coloboma which is a rather unusual malformation in the DGS/VCFS spectrum. However, this finding has been reported in about 3% of VCFS patients.[30] The absence of obvious genotype–phenotype correlation in individuals carrying different deletions excludes this disorder from being a contiguous deletion syndrome.[5,31] The deletion panel described in this paper supports the view that the DGS/VCFS region is not included in a single SRO, further suggesting that this is not a single-gene disorder.[31] These observations argue that DGS/VCFS results from haploinsufficiency secondary to a complex and as yet unexplained molecular mechanism. An attractive model assumes a functional architecture of the 22q11.2 region which is abolished by microdeletions/translocations, irrespective of their location within a genomic interval spanning the commonly deleted 3 Mb.[15,29] Accordingly, it can be envisaged that genes in the region share regulatory element(s) and/or factors which could mediate gene to gene interactions.[31,32] In this respect, the identification from DGS/VCFS intervals **1** and **2** of untranslated transcripts with a putative gene regulation effect is of interest.[33,34] Whether these transcripts affect the chromatin structure, which in turn modifies the transcriptional activity of DGS/VCFS genes, remains to be determined.[35] An alternative possibility is that the DGS/CVFS phenotype results from alteration of the 'chromatin folding code' mediated by the deletion of low-copy DNA repeats within

Table 1 Results of FISH and STRP marker analysis

Patient	*D22S427*	*D22S1638*	*Co29*	*Co23*	*UFD1L*	*22K48-2*	*22K48-1*	*D22S941*	*D22S944*	*D22S1623*	*D22S264*	*D22S311*	*DSS21709*	*D22S306*	*D22S303*	*D22S257*
JK	**100/106**	*120*	**++**	**++**	*A1*	*144*	*71*	*224*	158	*151*	*192*	266	111	*107*	**224/228**	*125*
P	**100**	*120*	**++**	**++**	*A1/A2*	*144*	*71*	*224/248*	158/160	*151/157*	*192/200*	266/272	111/113	*107/111*	**224/228**	*125*
M	**100/106**	*116/120*	**++**	**++**	*A1/A2*	*144*	*71*	*224*	162/170	*151/155*	*192/200*	268	113/115	*105/107*	**228**	*125/133*
ML	*n a*	**114/118**	Del	Del	*A1*	*144*	**73/75**	**226/240**	**164/168**	*153*	**190/210**	**262/274**	**117/129**	**105/111**	*222*	**125/127**
P		**114/120**			*A1*	*144*	**73**	**226**	**164/166**	*153*	**190**	**256/274**	**127/129**	**105/107**	*222*	**125**
M		**118/120**			*A1*	*144*	**75/77**	**240**	**166/168**	*153*	**190/210**	**262/274**	**117**	**111**	*222*	**125/131**
SL	**102/104**	122	Del	**++**	**A1/A2**	**144/146**	*71*	**224/240**	*170*	*157*	**192/194**	**242/248**	**111/113**	**109/111**	*230*	**131/133**
P	**102**	128			**A1**	**144**	*71*	**224**	*170/172*	*157*	**192/196**	**242**	**113/115**	**105/109**	*230*	**131/133**
M	**104/108**	122/128			**A1/A2**	**144/146**	*71*	**224/240**	*170/174*	*157*	**194**	**248**	**111/117**	**107/111**	*228/230*	**133**

P: paternal alleles; M: maternal alleles; n a: not analysed; hemizygosity is indicated by roman text, heterozygosity by bold text, and uninformative allele constellation by italic text. The order of the probes is centromere–telomere.

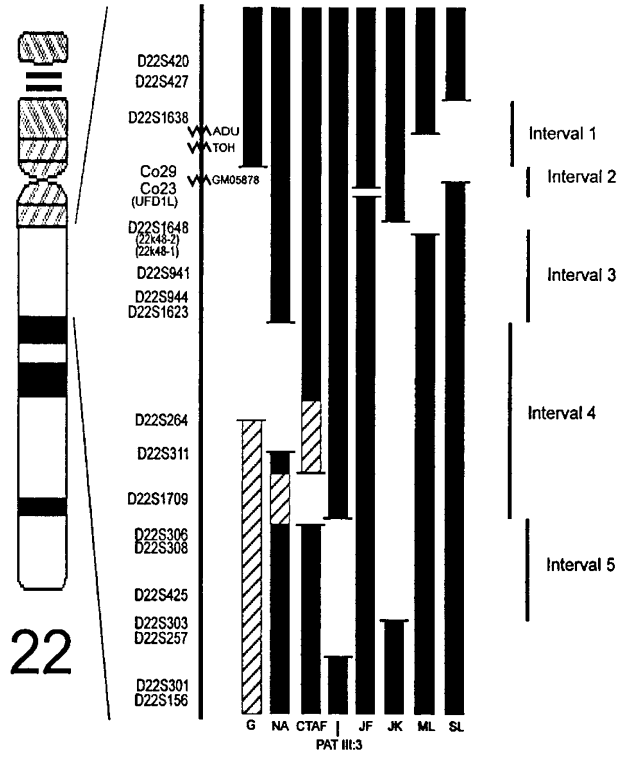

Figure 3 *Deletion map of 22q11.2 critical region. Black represents undeleted regions; hatched segments correspond to regions not analysed. Jagged lines represent breakpoints of patients with translocations involving chromosome 22q11.2. ADU was described by Augusseau* et al,[10] *TOH by Holmes* et al,[11] *GM05878 by Lindsay* et al,[12] *patient G by Levy* et al,[6] *patient NA by O'Donnell* et al,[8] *patient CTAF by Kurahashi* et al,[7] *patient III:3 by Rauch* et al,[9] *patient JF by Yamagishi* et al.[14]

this region. It is well established that low-copy DNA repeats play a role in regulating gene expression.[36] Interestingly, a recent report has shown that chromosome breakpoints in DGS/VCFS patients occur within low-copy DNA repeats (LCR22s),[37] which overlap the five intervals described here. Another contribution to the understanding of DGS/VCFS molecular pathogenesis could be the engineering of mice carrying deletions mimicking the five DGS/VCFS intervals. Finally, we recommend that patients presenting with the DGS/VCFS phenotype in the absence of an obvious deletion 22q11.2 as shown by standard probes (e.g. N25) be analysed by an additional set of probes spanning the five intervals described in this study.

Note added in proof: After submission of this article additional DGS/VCFS patients with atypical 22q11.1 deletions were found (Saitte *et al. Am J Hum Genet* 1999; **65:** 562–566; McQuade *et al. Am J Med Genet* 1999; **86:** 27–33). These patients show a small deletion corresponding to intervals 5 and 4 respectively.

Acknowledgements

We thank Dr B Morrow for critical reading of the manuscript. This work is supported by a grant from Italian Telethon (E.723) and the Italian Ministry of Health

References

1 Driscoll DA, Budarf ML, Emanuel BS: A genetic etiology for DiGeorge syndrome: consistent deletions and microdeletions of 22q11. *Am J Hum Genet* 1992; **50**: 924–933.
2 Scambler PJ: Deletions of human chromosome 22 and associated birth defects. *Curr Opin Genet Dev* 1993; **3**: 432–437.
3 Ryan AK, Goodship JA, Wilson DI *et al*: Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *J Med Genet* 1997; **34**: 798–804.
4 Yamagishi H, Ishii C, Maeda J *et al*: Phenotypic discordance in monozygotic twins with 22q11.2 deletion. *Am J Med Genet* 1998; **78**: 319–321.
5 Budarf ML, Emanuel BS: Progress in the autosomal segmental aneusomy syndromes (SASs): single or multilocus disorders? *Hum Mol Genet* 1997; **6**: 1657–1665.
6 Levy A, Demczuk S, Aurias A, Depetris D, Mattei MG, Philip N: Interstitial 22q11 microdeletion excluding the ADU breakpoint in a patient with DiGeorge syndrome. *Hum Mol Genet* 1995; **4**: 2417–2419.
7 Kurahashi H, Nakayama T, Osugi Y *et al*: Deletion mapping of 22q11 in CATCH22 syndrome: identification of a second critical region. *Am J Hum Genet* 1996; **58**: 1377–1381.
8 O'Donnell H, McKeown C, Gould C, Morrow B, Scambler PJ: Detection of an atypical 22q11 deletion that has no overlap with the DiGeorge syndrome critical region. *Am J Hum Genet* 1997; **60**: 1544–1548.
9 Rauch A, Pfieffer RA, Leipold G, Singer H, Tigges M, Hofbeck M: A novel 22q11.2 microdeletion in DiGeorge syndrome. *Am J Hum Genet* 1999; **64**: 658–666.
10 Augusseau S, Jouk J, Jalbert P, Prieur M: DiGeorge syndrome and 22q11 rearrangements. *Hum Genet* 1986; **74**: 206.
11 Holmes SE, Riazi MA, Gong W *et al*: Disruption of the clathrin heavy chain-like gene (CLTCL) associated with features of DGS/VCFS: a balanced (21;22)(p12;q11) translocation. *Hum Mol Genet* 1997; **6**: 357–367.
12 Lindsay EA, Halford S, Wadey R, Scambler PJ, Baldini A: Molecular cytogenetic characterization of the DiGeorge syndrome region using fluorescence in situ hybridization. *Genomics* 1993; **17**: 403–407.
13 Pragliola A, Jurecic V, Chau CK, Philip N, Baldini A: Goosecoid-like sequences and the smallest region of deletion overlap in the DiGeorge and velocardiofacial syndromes. *Am J Hum Genet* 1997; **61**: 1456–1458.

14 Yamagishi H, Garg V, Matsuoka R, Thomas T, Srivastava D: A molecular pathway revealing a genetic basis for human cardiac and craniofacial defects. *Science* 1999; **283**: 1158–1161.
15 Novelli G, Amati F, Dallapiccola B: UFD1L AND CDC45L: Role of either, neither or both in DiGeorge syndrome and related phenotypes? *Trends Genet* 1999; **15**: 251–253.
16 Sambroock J, Fritsch EF, Maniatis T: *Molecular Cloning, a Laboratory Manual*: 2nd edition. Cold Spring Harbor Laboratory Press: 1989, vol 2, pp 9.16–9.19.
17 Carlson C, Sirotkin H, Pandita R *et al*: Molecular definition of 22q11 deletions in 151 velo-cardio-facial syndrome patients. *Am J Hum Genet* 1997; **61**: 620–629.
18 Pizzuti A, Novelli G, Mari A *et al*: Human homolog sequences to the Drosophila dishevelled polarity gene are deleted in the DiGeorge syndrome. *Am J Hum Genet* 1996; **58**: 722–729.
19 Pizzuti A, Novelli G, Ratti A *et al*: UFD1L, a developmentally expressed ubiquitination gene, is deleted in CATCH 22 syndrome. *Hum Mol Genet* 1997; **6**: 259–265.
20 Halford S, Wadey R, Roberts C *et al*: Isolation of a putative transcriptional regulator from the region of 22q11 deleted in DiGeorge syndrome, Shprintzen syndrome and familial heart disease. *Hum Mol Genet* 1993; **2**: 2099–2107.
21 Novelli A, Sabani M, Caiola A *et al*: Diagnosis of DiGeorge and Williams syndromes using FISH analysis of peripheral blood smears. *Mol Cell Probe* 1999; **13**: 303–307.
22 Lindsay EA, Baldini A: Congenital heart defects and 22q11 deletions: which genes count? *Mol Med Today* 1998; **4**: 350–358.
23 Sirotkin H, Morrow B, Saint-Jore B *et al*: Identification, characterization, and precise mapping of a human gene encoding a novel membrane-spanning protein from the 22q11 region deleted in velo-cardio-facial syndrome. *Genomics* 1997; **42**: 245–251.
24 Sperandeo MP, Borsani G, Incerti B *et al*: The gene encoding a cationic amino acid transporter (SLC7A4) maps to the region deleted in the velocardiofacial syndrome. *Genomics* 1998; **49**: 230–236.
25 Edelmann L, Pandita RK, Morrow BE: Low-copy repeats mediate the common 3-Mb deletion in patients with velo-cardio-facial syndrome. *Am J Hum Genet* 1999; **64**: 1076–1086.
26 Baldini A: Is the genetic basis of DiGeorge syndrome in HAND? *Nat Genet* 1999; **21**: 246–247.
27 Hur H, Kim YJ, Noh CI, Seo JW, Kim MH: Molecular genetic analysis of the DiGeorge syndrome among Korean patients with congenital heart disease. *Mol Cells* 1999; **1**: 72–77.
28 Kurnit DM, Layton WM, Matthysse S: Genetics, chance, and morphogenesis. *Am J Hum Genet* 1987; **41**: 979–995.
29 Bonnet D, Fermont L, Kachaner J *et al*: Tricuspid atresia and conotruncal malformations in five families. *J Med Genet* 1999; **36**: 349–350.
30 Goldberg R, Motzkin B, Marion R, Scambler PJ, Shprintzen RJ: Velo-cardio-facial syndrome: a review of 120 patients. *Am J Med Genet* 1993; **45**: 313–319.
31 Dallapiccola B, Pizzuti A, Novelli G: How many breaks do we need to CATCH on 22q11? *Am J Hum Genet* 1996; **59**: 7–11.
32 Rogan DF, Cousins DJ, Staynov DZ: Intergenic transcription occurs throughout the human IL-4/IL-13 gene cluster. *Biochem Biophys Res Commun* 1999; **255**: 556–561.
33 Sutherland HF, Wadey R, McKie JM *et al*: Identification of a novel transcript disrupted by a balanced translocation associated with DiGeorge syndrome. *Am J Hum Genet* 1996; **59**: 23–31.
34 Pizzuti A, Novelli G, Ratti A *et al*: Isolation and characterization of a novel transcript embedded within HIRA, a gene deleted in DiGeorge syndrome. *Molec Genet Metab* 1999; **67**: 227–235.
35 Huang W-Y, Liew C-C: Chromatin structure and cardiac gene expression. *J Mol Cell Cardiol* 1998; **30**: 1673–1681.
36 Vogt P: Potential genetic functions of tandem repeated DNA sequence blocks in the human genome are based on a highly conserved "chromatin folding code". *Hum Genet* 1990; **84**: 301–336.
37 Edelmann L, Pandita RK, Spiteri E *et al*: A common molecular basis for rearrangement disorders on chromosome 22q11. *Hum Mol Genet* 1999; **8**: 1157–1167.