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Frequency of a 22q11 deletion in patients with conotruncal cardiac malformations: a prospective study

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Abstract Recent molecular studies have revealed that a 22q11 deletion is frequently detected in DiGeorge syndrome (DGS), velo-cardio-facial syndrome (VCFS), and conotruncal anomaly face syndrome (CTAFS). As one of the major clinical manifestations in these three syndromes is conotruncal cardiac malformation, we prospectively studied the frequency of a 22q11 deletion in a group of patients with conotruncal cardiac malformation. Fluorescence in situ hybridization (FISH) analyses using N25 (D22S75) DiGeorge Chromosome Region probe were performed on 64 patients with conotruncal cardiac malformation, who visited our clinic from October 1993 to January 1994. Of the 64 patients studied, a 22q11 deletion was detected in 5 patients (7.8%): 3 out of 30 patients with tetralogy of Fallot, one of three with interruption of the aortic arch, and one hemitruncus patient. No deletion was found in 16 patients with complete transposition of the great arteries, 8 with double outlet right ventricle and 2 with aortopulmonary window. In these five patients with 22q11 deletion, patient 1 was clinically diagnosed as having DGS, patients 2 and 3 had CTAFS, and patient 4 had VCFS. Patient 5 could not be dysmorphologically evaluated. It was noteworthy that all patients with a 22q11 deletion, except a non-evaluated patient, had some symptoms of syndromes DGS, CTAFS or VCFS, and that we failed to identify a non-syndromic 22q11 deletion positive patients in the present series of 64 patients.

Conclusion This study suggests that it is advisable to bear 22q11 deletion in mind when a patient with conotruncal cardiac anomalies has some other features of DGS, VCFS or CTAFS.

Key words 22q11 deletion · DiGeorge syndrome · Velo-cardio-facial syndrome · Conotruncal anomaly face syndrome

Abbreviations *CTAFS* conotruncal anomaly face syndrome · *DGS* DiGeorge syndrome · *DORV* double outlet right ventricle · *FISH* fluorescence in situ hybridization · *IAA* interrupted aortic arch · *PA* pulmonary atresia · *TA* truncus arteriosus communis · *TGA* complete transposition of the great arteries · *TOF* tetralogy of Fallot · *VCFS* velo-cardio-facial syndrome

Introduction

Conotruncal cardiac malformation, a cardiovascular anomaly involving the aortic arch system or the arterial pole of the heart, is a cardinal feature of the following three syndromes: DiGeorge syndrome (DGS), velo-cardio-facial syndrome (VCFS) and conotruncal anomaly face syndrome (CTAFS). DGS (MIM 188400) is a developmental field defect of the third and fourth pharyngeal pouches characterised by aplasia/hypoplasia of the thymus/parathyroid glands and by conotruncal cardiac malformation [6]. VCFS (MIM 192430) [6] is an autosomal dominant disorder in which patients manifest cleft palate, heart malformation, speech and learning disabilities, and typical facial characteristics. CTAFS (Takao syndrome) comprises a dysmorphic facial appearance and outflow tract defects of the heart [7].

These three syndromes have similarities in facial appearance, including a prominent nose with a square nasal root and a narrow alar base, retrognathia, narrow palpebral fissures, and minor ear anomalies [9]. Moreover, recent molecular analyses have revealed that many patients with these syndromes have a deletion within the long arm of chromosome 22 (22q11). The frequency of patients with a 22q11 deletion was reported as 83% in DGS, 68% in VCFS [4], and 100% in CTAFS [1]. As one of the major clinical manifestations in these three syndromes is conotruncal cardiac malformation, we prospectively studied the frequency of a 22q11 deletion in a group of patients with conotruncal cardiac malformations.

Patients and methods

Sixty-four patients, 40 males and 24 females ranging from 1 month to 16 years of age, visited our hospital as outpatients for periodic check-ups or as new patients from October 1st 1993 to January 15th 1994 and participated in this study. These patients were diagnosed as having conotruncal cardiac malformation by echocardiography, cardiac catheterisation or both. The major conotruncal cardiac malformations of these patients were tetralogy of Fallot (TOF; $n = 30$), complete transposition of the great arteries (TGA; $n = 16$), double outlet right ventricle (DORV; $n = 8$), interrupted aortic arch (IAA; $n = 3$), congenital corrected transposition ($n = 3$), truncus arteriosus communis (TA; $n = 2$), aortopulmonary window ($n = 1$), and one hemitruncus (origin of the right pulmonary artery from the ascending aorta).

Standard chromosome preparations were obtained from peripheral blood lymphocytes of each patient. The Oncor N25 (D22S75) DGS Chromosome Region (DGCR) probe combined with the pH17 (D22S39) chromosome 22 control probe both labelled with digoxigenin were used for fluorescence in situ hybridization (FISH) analyses. FISH was essentially done according to the manufacture's protocol with a slight modification. Briefly, slides were denatured in 70% formamide/2 × SSC at 70°C for 2 min, and immediately dehydrated by passing them for 5 min each into 70% and 100% ethanol, and air dried. Ten microlitres of the combined probe was mixed with 1.5 µl of 50% dextran sulphate, 20 µg bovine serum albumin and 2.5 µl formamide, and then hybridized in situ to denatured chromosome preparations at 37°C for 12–18 h. After hybridization the slides were washed in 50% formamide/2 × SSC at 42°C for 15 min, in 2 × SSC and 1 × SSC at room temperature each for 15 min. The hybridizing probe was visualized using fluorescein-labeled antidigoxigenin antibody. The chromosomes were counter-stained with propidium iodide and then observed using a Nikon fluorescent microscope. Chromosome constitution was also studied using the standard G-banding method in each patient.

Results

FISH analyses revealed that the DGCR probe signal was seen on only one of the chromosomes 22, which is consistent with a deletion, in 5 out of 64 patients examined (Fig. 1). The clinical features of these five patients are shown in Table 1. Cardiac malformations in these patients with a deletion were diverse; three were TOF (containing TOF and PA which has been classified as TA type IV), one IAA and one hemitruncus. Patient 1 was diagnosed as having DGS (Fig. 2). Patients 2 and 3 were diagnosed as having CTAFS. Although the facial appearance of patient 3 was somewhat different from that of CTAFS, his photograph at age 6 years showed typical facial features consistent with CTAFS. Patient 4 was diagnosed as having VCFS. Patient 5 could not be dysmorphologically evaluated, because he had died. G-banding analysis at around the 550-band level of resolution revealed that no abnormalities in other chromosomes were detected in all of the patients.

Discussion

We prospectively studied the frequency of a 22q11 deletion in a series of 64 patients with conotruncal cardiac malformations. Out of the 64 patients studied, a 22q11 deletion was detected in 5 patients (7.8%): 3 out of 30

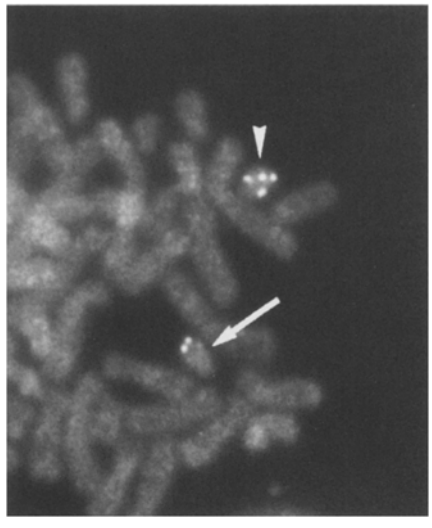

Fig. 1 A metaphase spread from patient 3 hybridized with probes N25 and pH17. No signal for N25 is seen on one chromosome 22 homologue *(arrow)*. An *arrowhead* indicates normal chromosome 22

Table 1 Main clinical features of the five patients with 22q11 deletion

Patient	Sex	Age (years)	Congenital heart defect	Thymic hypoplasia	Cleft palate	Hypo-calcaemia	Facial dysmorphism	Clinical diagnosis
1	M	1/12	IAA	+	-	+	Hypertelorism, blunted nose, small mouth, micrognathia, ear anomalies	DGS
2	M	5/12	Hemitruncus	+	-	-	Hypertelorism, short palpebral fissures, small mouth, low-set ears	CTAFS
3	M	15	TOF, PA	-	-	-	Bloated eyelids, low nasal root	CTAFS
4	F	16	TOF, PA	-	+	-	Long face, long nose with squared nasal root, hypoplastic alae nasi	VCFS
5	M	2/12	TOF	+	-	-	Not evaluated	Not evaluated

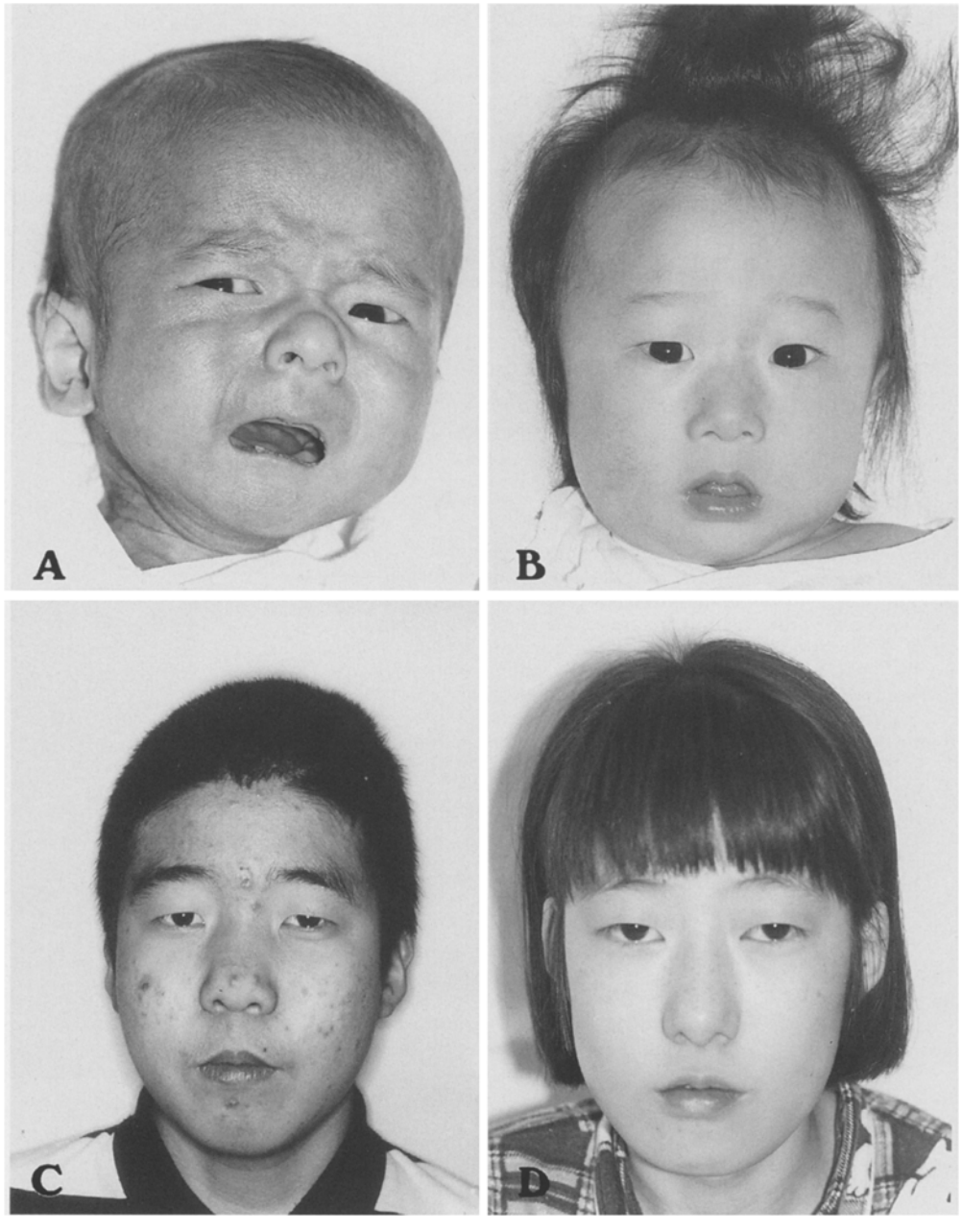

Fig. 2 A–D Facial appearances of four patients with a 22q11 deletion. Patient 1 with DGS (**A**), patient 2 (**B**) and 3 (**C**) with CTAFS, and patient 4 with VCFS (**D**)

TOF patients, one of three IAA, and one hemitruncus patient. No deletion was found in one AP window, 16 TGA, eight DORV, and two TA patients. It was noteworthy that retrospective evaluation of the clinical features of these 22q11 deletion positive patients except patient 5 revealed that every patient had either DGS (patient 1), CTAFS (patients 2, 3), or VCFS (patient 4). Patient 5 could not be dysmorphologically evaluated because he had died. This means that there were no non-syndromic 22q11 deletion positive patients with a conotruncal defect in the present 64 patients. Although we could not fully evaluate whether the 22q11 deletion negative patients were syndromic or not, this might suggest that non-syndromic patients with an isolated conotruncal cardiac anomaly are not likely to have a 22q11 deletion.

To our knowledge, there have been two reports evaluating the frequency of a 22q11 deletion in a group of conotruncal cardiac malformations. Wilson et al. [8] investigated 40 patients with TOF and found two patients (5%) with a 22q11 deletion. Goldmuntz et al. [5] studied 17 non-syndromic patients with one of the three most common conotruncal defects: TA (4 patients), IAA (3) and TOF (10). Patients with other cardinal features of DGS were excluded in their study. Out of these 17 patients, a 22q11 deletion was found in 5 (29%): 3 with TA and 2 with TOF. They described that the 22q11 deletion positive patients were mildly dysmorphic but did not have characteristics of a specific syndrome. However, these two previous studies were performed on patients with selective types of conotruncal cardiac anomalies (TOF, TA or IAA) most commonly seen in DGS and VCFS. Therefore the present study seems to be the first report of investigating the frequency of a 22q11 deletion in patients with non-selective conotruncal cardiac anomalies.

In this study we used only one cosmid probe (N25) that was located in the DGCR for FISH to identify a 22q11 deletion. Previous studies showed that a consistent deletion (> 750 kb in size) was found in 22q11, encompassing the probes from N25 to R32, in patients with DGS, VCFS and isolated conotruncal cardiac anomalies [2, 3, 5]. Therefore, we assumed that the majority, if not all, of the patients with 22q11 deletions seemed to be identifiable by FISH with the N25 probe alone. It is obvious, however, that the identification of the actual gene(s) in DGCR that is critical to conotruncal development would be needed for full evaluation of the 22q11 gene(s) associated conotruncal cardiac anomalies.

In conclusion, the present study suggests that the frequency of a 22q11 deletion in patients with non-selective types of conotruncal cardiac anomaly may be quite high at nearly 10%. It is noteworthy, as mentioned earlier, that all but one 22q11 deletion positive patients had either DGS, VCFS or CTAFS, and that we failed to identify a non-syndromic 22q11 deletion positive patient in the present series of 64 patients. This might suggest that non-syndromic conotruncal cardiac anomaly patients are not likely to have a 22q11 deletion. It would be advisable that children with conotruncal cardiac anomalies should be carefully examined to detect other morphological abnormalities of DGS, CTAFS or VCFS. Once these are found, a molecular cytogenetic analysis is then indicated.

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References

1. Burn J, Takao A, Wilson D, Cross I, Momma K, Wadey R, Scambler P, Goodship J (1993) Conotruncal anomaly face syndrome is associated with a deletion within chromosome 22q11. J Med Genet 30: 822–824
2. Driscoll DA, Budarf ML, Emanuel BS (1992) A genetic etiology for DiGeorge syndrome: Consistent deletions and microdeletions of 22q11. Am J Hum Genet 50: 924–933
3. Driscoll DA, Spinner NB, Budarf ML, et al (1992) Deletions and microdeletions of 22q11.2 in velo-cardio-facial syndrome. Am J Med Genet 44: 261–268
4. Driscoll DA, Salvin J, Sellinger B, Budarf ML, McDonald-McGinn DM, Zackai EH, Emanuel BS (1993) Prevalence of 22q11 microdeletions in DiGeorge and velocardiofacial syndromes: implications for genetic counseling and prenatal diagnosis. J Med Genet 30: 813–817
5. Goldmuntz E, Driscoll D, Budarf ML, Zackai EH, McDonald-McGinn DM, Biegel JA, Emanuel BS (1993) Microdeletions of chromosomal 22q11 in patients with congenital conotruncal cardiac defects. J Med Genet 30: 807–812
6. McKusick VA (1992) Entries 188400 and 192430 In: Mendelian inheritance in man. Catalogs of autosomal dominant, autosomal recessive, and X-linked phenotypes, 10th edn. Johns Hopkins Univ Press, Baltimore, p1078–1079, 1137
7. Takao A, Terai M, Ando M, Momma K (1985) Cardiovascular malformations in the conotruncal anomaly face syndrome. In: Doyle EF, Engle MA, Gersony WM, et al (eds) Pediatric cardiology. Proceedings of the second World Congress. Springer Verlag, Berlin Heidelberg New York, pp 926–928
8. Wilson DI, Scambler PJ, Goodship JA, Burn J (1992) Deletion within chromosome 22q11 is a major cause of isolated heart defects and most causes of DiGeorge and velocardiofacial syndromes (abstr P16). Proceedings of the British Paediatric Association Meeting April 7–10, Warwick, UK
9. Wilson DI, Burn J, Scambler P, Goodship J (1993) DiGeorge syndrome: part of CATCH 22. J Med Genet 30: 852–856