The use of fluorescence in situ hybridization in the diagnosis of DiGeorge anomaly

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DiGeorge anomaly comprises hypoparathyroidism, thymic aplasia, conotruncal cardiac anomaly and dysmorphic features. Most cases result from a microdeletion within chromosome 22q11. We report a case of documented DiGeorge anomaly and the use of high-resolution cytogenetic analysis and the fluorescence in situ hybridization technique are discussed.

HKMJ 1995;1:263-265

Key words: DiGeorge syndrome; In situ hybridization, fluorescence

Introduction

The DiGeorge anomaly (DGA) is a developmental field defect involving the third and fourth branchial arches and pouches and is characterised by hypoparathyroidism, recurrent infection secondary to thymic aplasia, conotruncal cardiac defect, and dysmorphic features.[1,2] Various chromosomal abnormalities are responsible, including Mendelian disorders (e.g. velocardiofacial syndrome, Zellweger syndrome), teratogenic exposure (alcohol, retinoids, insulin) and other associations (e.g. CHARGE association).

In 1981, a Finnish family with DGA was found to have an autosomal translocation involving chromosomes 20 and 22,[3] and the putative gene was suggested to be located at sites on these two chromosomes. More recently, with the assistance of high-resolution cytogenetic techniques, visible interstitial deletion of 22q11.2 has been identified in approximately 25% of DGA patients.[4] Using probes and cosmids from the DiGeorge chromosome region (DGCR), deletion of 22q11 was detected in 83% of DGA patients by DNA dosage analysis and fluorescence in situ hybridization (FISH).[5] In this paper, a case of documented DGA is described and the use of high-resolution cytogenetic analysis and FISH are discussed.

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Case report

The patient, the baby boy of a non-consanguinous healthy couple, was delivered at 41 weeks gestation by Caeserian section, with a birth weight of 3.1 kg. The antenatal history was uneventful. No history of maternal diabetes, alcohol or other drug exposure was noted. The patient was noted to have cyanosis on day 2 and his echocardiogram showed ventricular septal defect, pulmonary atresia, absence of right pulmonary artery with collaterals, overriding aorta and anonymous left pulmonary artery from ascending aorta.

On day 20 he developed twitching of all limbs and his serum calcium level was low. Total serum calcium was 1.09 mmol/L, phosphate 3.49 mmol/L, and albumin 25 g/L. The parathyroid hormone PH level was 0.7 pmol/L (normal range, 1.16-5.67 pmol/L). The seizures stopped after calcium replacement and phenobarbital therapy.

Physical examination revealed a round face, micrognathia, and mild anteverted nostrils. No other dysmorphic feature was noted. Lymphocyte subset by flow cytometry revealed: white cell count of 13.77×10^9/L, lymphocyte count of 1.46×10^9/L, B cells (CD19) 0.798×10^9/L, and T cells (CD3) 0.298×10^9/L. Axillary lymph node biopsy showed lymphoid depletion. A chest X-ray showed absent thymic shadow, and ultrasound scan of the mediastinum found a small thymus.

Cytogenetic analysis

Metaphase chromosome spreads from three-day lymphocyte cultures were prepared according to stand-

ard methods.[6] Routine cytogenetic studies such as G banding and high-resolution prometaphase analysis were conducted using standard procedures.[6]

Fluorescence in situ hybridisation

The specific probe D22S75 DGCR and chromosome 22 control probe D22S39 were purchased as digoxigenin-labelled DNA (Oncor Inc., Gaithersburg, Md, US). The slides were ribonuclease-treated (Merck & Co, Darmstadt, Germany)(100 ug/ml at 37°C for one hour), washed in 2 x SSC (standard saline citrate), pH 7.0, twice for two minutes each time, and dehydrated through an alcohol series. The air-dried slides were denatured in 70% formamide (Oncor Inc., Gaithersburg, Md, US)/2 x SSC, pH 7.0 at 70°C for three minutes before the application of the prepared probes. Hybridization was performed at 37°C overnight. The slides were washed twice in 50% formamide/2 x SSC at 42°C for five minutes each and again (twice) in 2 x SSC for five minutes. Probe detection and signal amplification were carried out as described elsewhere.[7] The slides were then counterstained with propidium iodide (Oncor Inc., Gaithersburg, Md, US) and DAPI (Oncor Inc., Gaithersburg, Md, US) before being analysed under a fluorescence microscope (Leica Mikroskopie und Systeme GmbH, Heerbrugg, Switzerland) with a 100-watt fluorescent lamp. Photographs were taken using the PSI MacProbe system (Perceptive Scientific Instrument Inc., League City, Tx, US).

Results

Cytogenetics

Interstitial deletion of 22q11 was suspected in high-resolution G-banded prometaphase (800 bands level, Fig 1).

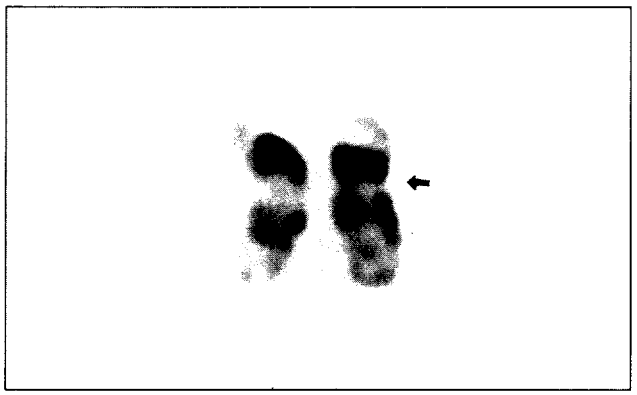

Fig 1. Prometaphase chromosome 22 from the DiGeorge anomaly patient. The arrow indicates the suspected microdeleted region

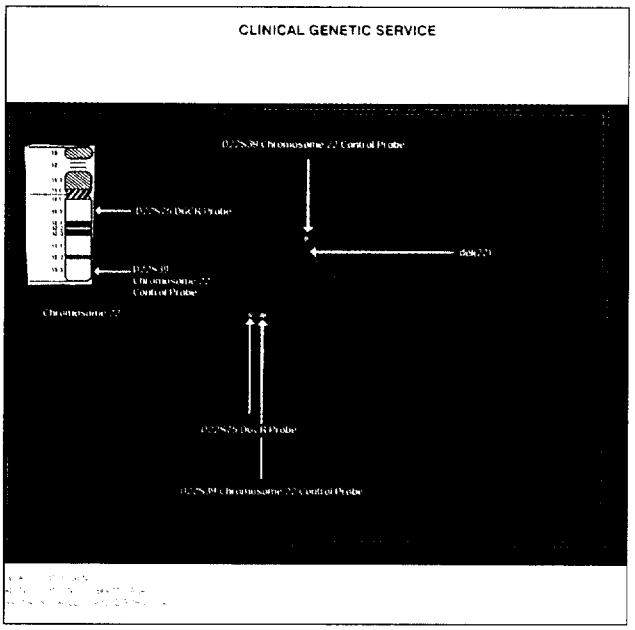

Fig 2. Fluorescence in situ hybridization of DiGeorge chromosome region (DGCR) probe on metaphase chromosome from the DiGeorge anomaly patient. The chromosomes 22 were identified with D22S39 control probe marking the distal long arm of both homologues. The band 22q11 was identified by the D22S75 DGCR probe which was nearer to the centromere. The relative position of the two probes is shown in the idiogram. A hybridization signal for DGCR probe was visualised on only one copy of the chromosome 22, consistent with a deletion

Fluorescence in situ hybridization

Both chromosomes 22 were identified using the D22S39 control probe which labelled the distal long arm of both homologues. The band 22q11 was identified with the D22S75 DGCR probe which was nearer to the centromere. The relative position of the two probes is shown in the idiogram (Fig 2). The false negative rate for the probe D22S75 in our experiments was zero. Of the 100 control metaphases, both chromosome 22 homologues were labelled by the control probe. In our patient, 11 metaphases were analysed. All showed two D22S39 signals on the chromosome 22 homologues. However, only one hybridization signal for D22S75 DGCR probe was visualised, which was consistent with a deletion (Fig 2). The apparent difference in colour of the signals was due to the difference in intensity of the fluorescein isothiocyanate signals.

Discussion

The DiGeorge anomaly is a rare congenital condition which has a wide range of clinical manifesta-

tions. The aetiology is heterogeneous. Most patients are associated with a deletion within 22q11. The present study demonstrated the use of FISH in the diagnosis of a patient with this anomaly.

Although deletion 22q11 is very common in patients with DGA, high-resolution cytogenetic analysis only detects this microdeletion in 25% of all patients. In our patient, high-resolution cytogenetic analysis was suggestive of interstitial deletion of 22q11, but not conclusive. The demonstration of deletion was highly dependent on the experience of the cytogeneticist, the quality of banding, and the spreading of the chromosomes. By comparison, FISH offers a relatively simple and rapid screening method for detecting the microdeletion. It also carries a high sensitivity.[8]

For the present, FISH cannot replace traditional high-resolution banding techniques in the diagnosis of DGA. Apart from the microdeletion of 22q11, other chromosomal abnormalities have also been reported in association with DGA. Several cases of monosomy10p13[9] and one case of 17p13 deletion[10] have been reported and they appear to contain another possible DGA locus. Hence, both high-resolution cytogenetic analysis and FISH should be performed in all cases of DGA.

The demonstration of microdeletion in DGA patients is important for genetic counselling. If microdeletion is detected in the proband, parental blood should also be screened. If no deletion is found in the parents, they can be reassured that the recurrence risk in the next pregnancy will be very low.[11] However, if the same deletion is identified in one of the parents, the recurrence risk will be one in two. In the latter situation, prenatal diagnosis should be offered as FISH is able to detect the deletion in cultured amniocytes and chorionic villi.

Apart from DGA, interstitial deletion of 22q11 has also been observed in velocardiofacial syndrome, conotruncal anomaly face syndrome, and cases of isolated congenital heart disease. It is thought that these conditions are all part of one clinical spectrum and the term CATCH 22 has been proposed to include the group as a whole.[11]

Acknowledgement

We would like to thank Dr Margaret Chan, the Director of Health, for permission to publish this paper.

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