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Use of Fluorescence In Situ Hybridization (FISH) in the Diagnosis of DiGeorge Sequence and Related Diseases

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Abstract

The proximal portion of human chromosome 22q has been implicated in the pathogenesis of a clinically diverse group of conditions including DiGeorge sequence (DGS), velocardiofacial syndrome, and CHARGE association as well as isolated conotruncal heart anomalies. Frequently, overlap in the clinical presentation of these syndromes occurs and, recently, the presence of microdeletions on chromosome 22q11.2 with varying frequencies has been demonstrated in these syndromes. Using fluorescence in situ hybridization (FISH), we assessed 20 consecutive patients who were cytogenetically and clinically evaluated for a suspected syndrome that could be due to a microdeletion of chromosome 22q11.2. After cytogenetic testing and full clinical evaluation, we compared the results by FISH with the final clinical diagnosis and karyotype results. We found that microdeletions of 22q11.2 were detected in three of the five patients who were evaluated for DGS. The three cases with microdeletions appeared clinically to have DGS while the two negative cases were more atypical. High-resolution banding techniques did not detect a microdeletion in any of the cases; however, one of the 20 patients had a translocation between chromosomes 13 and 22. This patient also had a microdeletion of 22q11.2 detected by FISH and clinical features of DGS. None of the patients who were evaluated for disorders related to DGS showed microdeletions. We conclude that FISH is a useful, easily applied technique for the diagnosis of 22q11.2 microdeletion syndromes, particularly DGS. This test may also be useful in genetic counseling and in both prenatal and postnatal diagnoses.

Keywords

DiGeorge sequence; Chromosome 22; Fluorescence in situ hybridization; Microdeletion syndromes.

A number of screening and diagnostic tests have been developed for the evaluation of congenital disorders. The proximal portion of human chromosome 22q has been implicated in the pathogenesis of various developmental disorders, including DiGeorge sequence (DGS) (4–7,14,16), velocardiofacial syndrome (VCFS) (2,7,8,12,13), and isolated congenital conotruncal heart defects (10,17). Overlap in the clinical presentation of these syndromes occurs and, accordingly, microdeletions on chromosome 22q11.2 have been

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demonstrated in each of these disorders. The acronym CATCH 22 association (*C*ardiac defects, *A*bnormal facies, *T*hymic hypoplasia, *C*left palate, and *H*ypocalcemia) has been proposed as an encompassing term for this group of disorders (11,14,15). In addition, CHARGE association, (*C*oloboma, *H*eart defects, *A*tresia of choanus, *R*etardation (growth and/or mental), *G*enital defects, and *E*ar abnormalities) has also been shown to be associated with a microdeletion of chromosome 22q11 in rare cases (9).

Microdeletions of chromosome 22q11.2 can be detected occasionally with cytogenetic high-resolution banding techniques (8,16), restriction fragment length polymorphisms (4,6,14), or DNA dosage analysis (6). Recently, the use of cosmid probes has allowed for the detection of microdeletions using fluorescence in situ hybridization (FISH) (5,7,9,13). Although FISH is relatively new, it is simple, rapid, and less work intensive than other techniques. Hence, we report our experience of screening for chromosome 22q11.2 microdeletions in 20 consecutive patients with suspected DGS, CHARGE association, or related disorders by using FISH.

MATERIALS AND METHODS

Patients

Twenty consecutive patients with clinical features suggestive of a 22q11.2 microdeletion syndrome were referred to the cytogenetics laboratory for chromosomal analysis (Table 1). Of these 20 patients, five, seven, and eight patients had suspected DGS, CHARGE association, or a related disorder, respectively.

High-Resolution Chromosomal Analysis and Fluorescence in situ Hybridization (FISH)

Metaphase chromosome spreads were prepared from peripheral blood lymphocytes harvested routinely. Chromosomal karyotyping and high-resolution banding were performed following pretreatment with actinomycin C as previously described (3). FISH was performed using a commercially available digoxigenin-labeled DNA kit (Oncor, Gaithersburg, MD, U.S.A.), according to manufacturer's protocols. Briefly, chromosome spreads were cohybridized with two digoxigenin-labeled cosmid probes. One cosmid probe was directed at the site of the deletion (chromosome 22q11.2, locus D22S75) (6), and the other probe was directed to another region of chromosome 22 (22q13.3 locus D22S39) (1) and served as an internal control. Fluorescein-isothiocyanate-labeled anti-digoxigenin monoclonal antibodies were used for detection, and samples were counterstained with propidium iodide. At least 20 chromosome spreads were scored in each case by using an Olympus BH-2 fluorescent photomicroscope (Olympus Optical, Tokyo, Japan). Ektachromatic 400HC color-slide film (Kodak, Rochester, NY, U.S.A.) was used throughout this study.

RESULTS

DiGeorge Sequence

In the present study, five patients were evaluated for possible DGS. Four of the five had a normal karyotype by standard or high-resolution banding techniques. However, FISH

detected a 22q11.2 microdeletion in three of the five (Fig. 1 and Table 1). Two of these three patients with a microdeletion of chromosome 22q11.2 had a normal karyotype. The third patient with a microdeletion had a chromosome translocation involving chromosomes 13 and 22 [45,XY, −13, −22, + der13 t(13:22)(q33;q11).

Since the karyotype and FISH studies were carried out on patients in which a diagnosis had not been firmly established, we compared the results of the FISH analysis with the clinical diagnosis after a complete clinical evaluation (Table 1). The three patients with microdeletions of chromosome 22 of the 20 patients studied had features of DGS, including hypocalcemia, thymic hypoplasia, heart defects, and facial anomalies. Two patients (numbers 4 and 5, Table 1) who did not have a microdeletion of chromosome 22q11.2 did not have a clinical picture entirely compatible with DGS. Patient 4 was identified with only isolated tetralogy of Fallot, and patient 5 had congenital complex heart abnormalities and multiple endocrine defects associated with panhypopituitarism.

CHARGE Association and Other 22q11.2 Microdeletion Syndromes

Microdeletions of chromosome 22q11.2 were not detected in any of the seven patients referred for CHARGE association. Six of the seven patients had normal chromosome karyotypes. The remaining patient was referred with features of CHARGE association (patient 8, Table 1) but had a trisomy 21. After full clinical evaluation, only two of these seven patients were given a diagnosis of CHARGE association. Subsequently, the remaining five were given a variety of diagnoses (Table 1).

Of the remaining eight cases, two were referred for possible VATER association (*V*ertebral defects, *A*nal atresia, *T*racheo-esophageal fistula with *E*sophageal atresia, and *R*enal dysplasia) or a chromosome 22 microdeletion syndrome, two for VCFS, and five to rule out a chromosome 22 abnormality. All of these patients had normal karyotypes, including the two cases evaluated by high-resolution banding. None of these eight patients had microdeletions of chromosome 22 detected by using FISH. The “rule out” VCFS case had only isolated facial anomalies. The two possible VATER subjects did not fit into any other particular syndrome category, each having complex congenital heart defects with duodenal atresia and tracheo-esophageal fistulas. Three of the five samples sent to rule out a chromosomal 22 abnormality were from patients with complex heart disease. Of the remaining two patients, one was not identified with a specific syndrome after clinical genetics evaluation, and the other patient was thought to have a VATER variant.

DISCUSSION

We have shown that DGS is associated with a microdeletion in chromosome 22 that can be readily detected by FISH. A microdeletion could not be detected by karyotyping using high-resolution chromosome techniques. Early studies in the genetics literature have shown an association among DGS, VCFS, CHARGE, isolated conotruncal defects, and deletions of chromosome 22q11.2. Previous studies have demonstrated the presence of these microdeletions in well-characterized cases. However, there is a paucity of studies of consecutive cases referred to a cytogenetic laboratory because of the clinical suspicion of a syndrome associated with a deletion of chromosome 22q11.2 and reported in the pathology

literature. Our study shows that FISH is useful in identifying microdeletions in patients with suspected syndromes associated with a specific chromosome abnormality.

The frequency of microdeletions detected is related to the patient population that is studied. We reported microdeletions in all three of the cases of DGS and, as importantly, microdeletions were not detected in the remaining two subjects suspected to have DGS before detailed clinical evaluation. Our study agrees with previous reports that cases of DGS have a high frequency of microdeletions (7,16).

Microdeletions were not detected in any of the remaining 15 cases that were not referred to rule out DGS. Microdeletions of chromosome 22 apparently occurs much less frequently in syndromes with clinical features similar to those seen in DGS. Hence, none of the remaining cases had a CATCH 22 or CHARGE association after full clinical evaluation and, accordingly, microdeletions were not detected.

FISH has several advantages over classic cytogenetic techniques. As shown in this study, FISH is more sensitive than conventional karyotyping or using high-resolution banding techniques. FISH is also less labor intensive than karyotyping, DNA dosage analysis, or restriction fragment length analysis. In addition, FISH has been useful in prenatal diagnosis using amniotic fluid cells (7).

The observation that a broad spectrum of clinical presentations including DGS (immunologic), VCFS (dysmorphic), and conotruncal anomalies (cardiologic) can have microdeletions of chromosome 22 implies a multifactorial etiology to these syndromes. The precise mechanism by which deletions in chromosome 22q11.2 contribute to these syndromes is not well defined. However, the cosmid probe used for FISH extends over 35 kb of contiguous DNA sequence (6), and such a large span of DNA may encompass several genes. The specific gene(s) within this region is not well characterized. However, a variation in the size and position of the deletion may explain the broad clinical presentation and overlap with several syndromes or congenital defects. The correlation between genotype and phenotype will require continued investigation.

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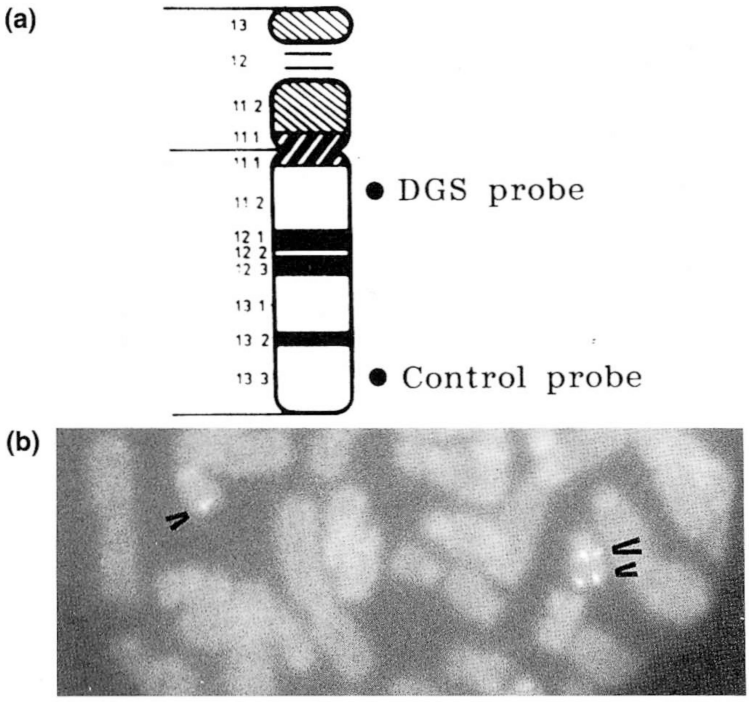

FIG. 1.

a: Chromosome 22 idiogram with the location of the hybridization signals for the DiGeorge sequence (DGS) and control probes. **b:** Fluorescence in situ hybridization using a cosmid probe (D22S75) from chromosome 22q11.2 for the detection of microdeletions in DGS. The DGS probe and an internal control probe (D22S39) are shown cohybridized to chromosome 22 from a partial metaphase of patient 3. The control probe (D22S39) is shown on the long arm in both chromosome 22s (small arrows) while the DGS probe (D22S75) is hybridized to only the normal chromosome 22 (large arrow), indicating a microdeletion of chromosome 22q11.2 in one of the chromosomes.

Table 1.

Clinical features and laboratory findings in twenty consecutive patients presenting to the cytogenetics laboratory with features of syndromes associated with microdeletions of chromosome 22

Patient (age, gender)	Reason for referral	Abnormal clinical features	High-resolution chromosome results	22q11 FISH results	Final clinical diagnosis
1 M 2 d	r/o DGS	Leukocytopenia, CHD	46, XY	Deletion	DGS
2 M 2 d	r/o DGS	Hypocalcemia, CHD, hypertelorism	45, XY, −13, −22, +der (13)t(13;22) (q33;q11)	Deletion	DGS
3 M 5 y	r/o DGS	VSD, hypocalcemia, ear anomalies, lymphocytopenia, umbilical hernia	46, XY	Deletion	DGS
4 F 14 m	r/o DGS	CHD	46, XX	Normal	No recognizable syndrome
5 F 1 m	r/o DGS	CHD, multiple endocrine defects	46, XX	Normal	Septo-optic dysplasia with panhypopituitarism and CHD
6 F 3 d	r/o CHARGE	Bilateral colobomata, left cataract, ASD, choanal atresia, external ear deformities, hearing deficit	46, XX	Normal	CHARGE
7 F 2 y 9 m	r/o CHARGE	Choanal atresia, ASD, bilateral optic coloboma, hearing deficit, microphthalmia, esotropia	46, XX	Normal	CHARGE
8 M 1 d	r/o CHARGE	Duodenal atresia, ASD, PDA, choanal atresia, low-set ears, syndactyly	47, XY, +21	Normal	Down syndrome and acrocephalosyndactyly type I
9 F 7 d	r/o CHARGE	ASD, growth retardation, external ear abnormalities	46, XX	Normal	CHARGE
10 F9 m	r/o CHARGE	Bilateral ocular coloboma	46, XX	Normal	No recognizable syndrome
11 M 20 d	r/o CHARGE	Hypospadias, micrognathia, inguinal hernia, cardiomegaly	46, XY	Normal	No recognizable syndrome
12 M 19 d	r/o CHARGE	Cleft lip, bilateral coloboma, PDA, unusual ear shape	46, XY	Normal	No recognizable syndrome
13 F 21 m	r/o 22q deletion	TOF, PDA	46, XX	Normal	Multifactorial CHD
14 M 3 d	r/o 22q deletion	TOF, abnormal facies	46, XY	Normal	VATER
15 M 1 d	r/o 22q deletion	Pulmonary atresia, TGV, VSD with tricuspid valve override, PDA	46, XY	Normal	Multifactorial CHD
16 M 1 d	r/o 22q deletion	Micrognathia, cleft palate	46, XY	Normal	No recognizable syndrome
17 F 3 d	r/o 22q deletion	VSD, TGV, PDA, pulmonary atresia with VSD	46, XX	Normal	Multifactorial CHD
18 M 19 m	r/o VCFS	Cleft palate and lip, microcephaly, abnormal facies	46, XY	Normal	No recognizable syndrome
19 F 1 d	r/o VATER	VSD, ASD, PDA, coarctation of aorta, esophageal atresia, duodenal atresia, tracheo-esophageal fistula	46, XX	Normal	No recognizable syndrome
20 F 1 d	r/o VATER	Esophageal atresia, TOF, duodenal atresia, tracheo-esophageal fistula	46, XX	Normal	No recognizable syndrome

ASD, atrial septal defect; CHARGE, coloboma, heart defects, atresia of choanus, retardation (growth and/or mental), genital defects, and ear abnormalities; CHD, congenital heart disease; DGS, DiGeorge sequence; PDA, patent ductus arteriosus; TGV, transposition of great vessels; TOF, tetralogy of Fallot; VATER, vertebral defects, anal atresia, tracheo-esophageal fistula with esophageal atresia, and renal dysplasia; VCFS, velo-cardiofacial syndrome; and VSD, ventricular septal defect.