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22q11 Microdeletion Studies in the Heart Tissue of an Abortus Involving a Familial Form of Congenital Heart Disease

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Microdeletion of chromosome 22 is responsible for DiGeorge syndrome, Velo Cardio Facial syndrome, and conotruncal defects. Here, we report on a case of microdeletion 22q11.2 in the heart tissue of a miscarried fetus in a family whose two children had died due to complex congenital heart disease. Fluorescence in situ hybridization (FISH) analysis in the couple revealed that the mother was mosaic for microdeletion of chromosome 22q11.2 in 10% of her peripheral lymphocytes. Prenatal diagnosis was offered to her in her third pregnancy. On routine ultrasonography at 10 weeks, the overall view of the heart was normal. However, before any further tests could be performed, she miscarried at 16 weeks. FISH studies on the heart tissue of the abortus revealed 22q11.2 microdeletion with two different cell lines. This suggests the importance of performing FISH studies when there is a history of congenital heart disease, even though ultrasonography shows a normal view of the heart. J. Clin. Lab. Anal. 20:160–163, 2006. © 2006 Wiley-Liss, Inc.

Key words: CHD; FISH; familial; chromosome 22q11.2

INTRODUCTION

Although chromosome 22q11.2 deletion has been documented postnatally (1–3), prenatal diagnosis of this abnormality, particularly with a mosaic form, has not been reported. We describe here what we believe to be the first study of a miscarried fetus where mosaicism for a deletion 22q11.2 was detected. Surprisingly, routine sonography was apparently normal.

CASE REPORT

A 29-year-old woman was referred to our clinic for prenatal diagnosis because her two previous children had died due to cyanotic congenital heart disease. A two-dimensional echo of the first child had revealed a complex congenital heart defect (complete atrio ventricular (AV) canal defect). No other clinical records of the first child were available. The photograph of the second child showed clinical features of Rubinstein-Tyabi syndrome. Available clinical records for the second child indicated: microcephaly, downward and lateral slanting of eyes, bilateral cleft palate, hypoplastic mandible, low set ears, broad thumbs, and multiple congenital heart defects that included tetralogy of fallot with pulmonary atresia, single large nonrestrictive ventricular septal defect (VSD), secundum type of ASD, and patent ductus with multiple arteriopulmonary collaterals. Karyotypic studies of this child indicated a normal chromosomal complement. No further studies could be conducted on both the children because they had died. Their mother was 16 weeks pregnant when she was referred to our clinic. She was carefully examined for minor findings of velo cardio facial syndrome (VCFS) such as: short stature; characteristic findings of facies, heart, limbs, toes; and subnormal intelligence. We found her to be clinically normal. Ultrasonography done at 10 weeks of gestation revealed a biparietal diameter and femur length equivalent to that level of gestation. The heart was apparently normal and similar to the normal development of the heart during that particular time of pregnancy. Before 3D ultrasono-

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graphy could be undertaken, the mother miscarried at 16 weeks. Autopsy findings of the miscarried fetus revealed dysmorphic face, no cleft lip or palate, closed eyes, normal limbs and fingers, normal toes, and normal anal opening. Internally, various organs were in a normal position and the heart appeared normal externally. Microscopically, sections of the heart and lungs revealed no signs of inflammation. Further studies on the other tissues were not possible due to marked softening and autolysis, making them unsuitable for processing.

METHODS

Cytogenetic and Fluorescence In Situ Hybridization Investigation

Cytogenetic investigation was carried out by standard GTG banding (500–750 band level) on metaphases obtained by a standard leukocyte culture technique. Fluorescence in situ hybridization (FISH) was performed using a DiGeorge syndrome probe (Vysis, Illinois) at locus 22q11.2 (red) with a distal control probe at locus 22q13 (green). FISH studies on leukocytes were performed according to the instructions of the manufacturer with slight modifications. In short, slides were dehydrated in 70%, 85%, and 100% ethanol for 5 min each. Slides were then codenatured along with the probe mixture at 72–74°C on a hot plate for 5 min, coverslipped, and sealed with rubber cement. Hybridization took place at 37°C overnight in a humidified box. Stringent washes in 0.4 × saline sodium citrate (SSC)/0.3% (Octylphenoxy)Polyethoxyethanol;Octylphenyl - Polyethylene Glycol (IGEPAL) (Sigma, St. Louis, MO) for 2 min and 1 × SSC/0.1% IGEPAL for 1 min followed. Slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI)/antifade and were screened under an epifluorescent Axioskop 2 plus microscope (Carl Zeiss, Germany) using a triple band pass filter, equipped with a cooled-charged coupled device. Image analysis was done using software from Metasystems (Metasystems, Germany).

FISH on Heart Tissue

Whole nuclei from paraffin embedded sections of heart tissue were extracted and were used for FISH studies (4,5). A total of 10–20 μL of whole nuclei extracted from paraffin sections was dropped on precleaned, warm slides. Slides were cooled to room temperature and then treated with proteinase K (Sigma) 2 mg/mL final concentration at 37°C for 30 min to 1 hr. The slides were then washed in 1 × Phosphate Buffered Saline (PBS) for 5 min. Slides were then dehydrated in an ethanol series of 70%, 85%, and 100% for 5 min each. A 5–10 μL probe mixture was applied to target DNA affixed to a glass slide, mounted with a cover slip, and sealed with rubber cement. Denaturation was carried out by codenaturing the slides along with the probe mixture at 75–80°C on a hot plate for 5–10 min. Slides were incubated in a humidified box at 37°C overnight.

Posthybridization washing was done in 0.4 × SSC/IGEPAL-0.3% at 45°C for 30 sec to 1 min. Cells were counterstained with DAPI/antifade and were observed under an epifluorescent Axioskop 2 plus microscope using a triple band pass filter, equipped with a cooled-charged coupled device. Image analysis was done using software from Metasystems. A minimum of 100 cells were screened.

RESULTS

An analysis of 20 metaphases each, from both the parents, revealed a normal Karyotype. FISH studies on the couple were performed; 600 interphase cells and 50 metaphases were analyzed for each patient. FISH studies on the father were normal. The studies indicated mosaicism for a deletion at 22q11.2 region in the mother (Fig. 1A and B). Out of 600 cells screened, 540 showed normal chromosome 22 while the remaining 60 cells (10%) showed microdeletion on chromosome 22q11.2. Out of the 50 metaphases analyzed in the mother, five (10%) showed a microdeletion on 22q11.2. Studies on the lymphocytes of five normal controls revealed a deletion on 22q11.2 in 0.5% of the 600 interphase cells studied, which was comparable to the results obtained on the patients and their relatives (0.7%). FISH studies on the metaphase plates of five normal controls (50 metaphases/control) revealed hybridization signals on all the metaphases, confirming 100% efficiency of the probe. Lymphocyte and metaphase analysis by FISH was performed by conducting two different sets of experiments for each normal control as well as for the patients. Thus, the mother was confirmed to be a carrier of 22q11.2 microdeletion. FISH analysis was also carried out on her parents and sisters, which showed a normal status of chromosome 22. A total of 600 interphase cells and 50 metaphases were counted for each of the family members.

FISH analysis of the fetal heart tissue showed two cell lines, i.e., normal (13%) and 22q11.2 deletion (85%) (Fig. 1C and D). The percentage of 22q11.2 deletion in the heart tissue was high (85% abnormal), which might have resulted in the miscarriage of the fetus. FISH studies were also carried out on normal heart tissue (five samples) to check the probe efficiency and to rule out false, positive results in the heart tissue. The percentage for monosomy of chromosome 22 seen in the control

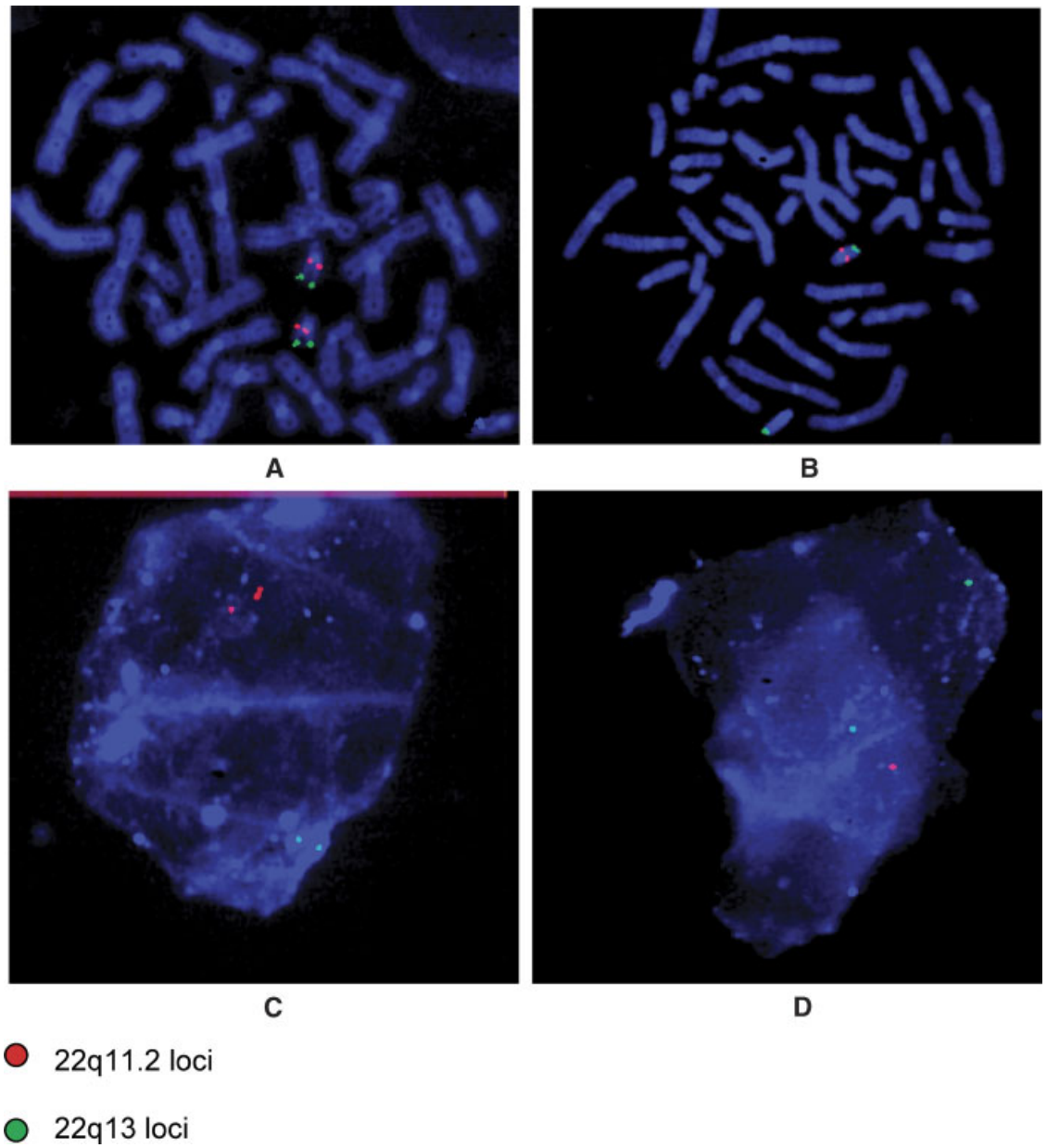

Fig. 1. **A:** FISH studies showing normal chromosome 22. **B:** Metaphase showing microdeletion on Ch22q11. **C:** Heart tissue showing normal chromosome 22. **D:** Heart tissue showing microdeletion 22q11.2.

heart tissue was almost similar, i.e., 1.8% when compared to the abortus fetus, which was 2%. The percentage of 22q11.2 deletion in the normal heart tissue was 3.2%, and 95% of the cells showed a normal hybridization pattern, i.e., cells showing two green and two red signals.

DISCUSSION

The association of conotruncal cardiac defects with hemizygosity of a locus on chromosome 22 is one of the most dramatic and clinically important pieces of evidence to show a genetic etiology in congenital heart defects (6). Because molecular probes for this locus are readily available, the diagnosis of chromosome 22 microdeletion can now be performed in fetuses and neonates with conotruncal defects (7–9). However, it remains unknown as to why this haploinsufficiency shows such a wide range of penetrance and expressivity.

Chromosome 22 microdeletion occurs de novo, in most cases, with only 8% being inherited (10). When one parent carries the deletion, either symptomatic or not, the fetus has a 50% risk of inheriting it. In our study, the mother was clinically asymptomatic and was

a carrier of mosaicism for a submicroscopic del 22q11.2. Her previous two children had died of complex cyanotic heart disease and the third (the miscarried fetus) revealed deletion in 85% of the cells. Thus, intrafamilial genotypic variability with the progeny being more severely affected than the parent could be explained by anticipation due to a change in the extent of microdeletion during fetal development. It is highly unlikely that the fetus received a normal chromosome 22 from the mother and in the course of cell divisions, the chromosome somehow reconstituted into two clones, i.e., a normal chromosome 22 and a clone with del 22q11.2.

We hypothesize that the chromosome 22 that the fetus inherited from the mother must have been more susceptible for this aberration (deletion) and in the early stage of fetal development must have received some external stimuli that led to the microdeletion resulting in two clones. An explanation for the event might be the influence of some environmental factors, socioeconomic background, or a de novo event that might have caused the microdeletion in the fetus. However, the limitations in the present study are that the microdeletion investigations were limited to only heart tissue, which prevents us from drawing a complete conclusion on the extent of 22q11.2 microdeletion in the miscarried fetus.

Thus, we stress that in familial cases with severe heart defects, chromosome 22 microdeletion studies should be performed and prenatal testing with DiGeorge probe be carried out in spite of normal ultrasound findings for the heart. However, more studies on familial cases are necessary to validate our findings on mosaicism.

We conclude that efforts should be made to increase the prenatal detection of severe cardiac defects. Routine fetal sonography used in developing countries may not be able to identify those defects. In high-risk patients, color Doppler, as well as detailed cardiovascular scans, should be mandatory in addition to chromosome 22 microdeletion studies. This will be important in order to establish a cardiac prognosis and to provide adequate counseling.

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