Prevalence of 22q11.2 microdeletion in 121 patients with cardiac malformation

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Abstract

BACKGROUND: It is known that 22q11.2 microdeletion is a submicroscopic chromosomal anomaly with cardiac and extra-cardiac manifestations. The prevalence and manifestations in north India have not been well characterized.

OBJECTIVES: This study was designed to determine the prevalence of 22q11.2 microdeletion in congenital cardiac malformation cases referred for surgery from north India and to assess ability of clinical criteria to predict the presence or absence of 22q11.2 microdeletion.

METHODS: A total of 121 cardiac malformation cases requiring surgery (conotruncal as well as non conotruncal heart defect) were prospectively screened for 22q11.2 microdeletion using fluorescence in situ hybridization test. Detailed clinical information was obtained from all cases.

RESULTS: Four out of 121 patients (prevalence 3.3%) had 22q11.2 microdeletion (all with tetralogy of Fallot). In all of these four patients, typical dysmorphic features of 22q11.2 microdeletion were present. None of the cases with isolated cardiac defect (n=52) was positive for the deletion.

CONCLUSIONS: Our work showed that 22q11.2 microdeletion is over-recognized with isolated congenital cardiac malformation. The absence of typical phenotypic features in cases of congenital cardiac malformation makes it difficult to justify investigation for deletion. Screening for 22q11.2 microdeletion should be considered in those cardiac malformation cases with extra-cardiac manifestations in particular facial dysmorphism.
Background

The 22q11.2 microdeletion syndrome is characterized by a hemizygous deletion of chromosome 22q11.2 locus. The 22q11.2 microdeletion syndrome is the most common microdeletion syndrome [1]. Mutation of the TBX1 gene located in 22q11.2 has been suggested as a major determinant of the syndrome [2]. The deletion usually arises spontaneously and is relatively common (1 in 4,000 to 6,000 live births) [1,3]. Wilson, et al. (1993) [4] acronymed the common defects associated with this deletion as CATCH 22 syndrome (Cardiac abnormality, Abnormal facies, T cell deficit due to Thymic hypoplasia, Cleft palate and Hypocalcemia). The 22q11.2 microdeletion is found in patients with DiGeorge syndrome, velocardiofacial syndrome and conotruncal anomaly face syndromes [5]. Typically, the associated cardiac anomalies involve the conotruncus and include lesions such as tetralogy of Fallot (TOF), pulmonary atresia/ventricular septal defect (PA/VSD), and truncus arteriosus (TA) [5].

Studies have tried to ascertain the incidence of this condition in population [1,6,7], heart disease [8-15], psychiatric disease [16-18], neonatal hypocalcimia [19] and velopharyngeal insufficiency [20]. However, there are very few studies reported from India and mostly as case reports [21-23] and one as prospective study [24]. The prevalence and manifestations in north India have not been well characterized. This study was aimed to investigate the incidence of 22q11.2 microdeletion in cases (originated from north India) with congenital cardiac malformation (conotruncal as well as non conotruncal) and its association with other congenital anomalies (extracardiac manifestation), with emphasis on facial dysmorphism.
**Material & Methods**

From August 2006 to July 2009, 121 cases of structural heart defects (conotruncal or non-conotruncal) that required surgery and associated with or without extra-cardiac anomalies or dysmorphic features were enrolled prospectively into the study. They were all referred to our institution from various states of north India for surgical management. All of the study patients was identified prospectively from the elective cardiac catheterization or operative procedure schedules, or as diagnosed patients admitted for additional evaluation under cardiology or cardiac surgery or pediatric genetics. Recruitment at the time of a hospitalization or invasive procedure allowed for detailed evaluation. A few older patients were recruited from the outpatient setting.

All patients underwent cardiac as well as clinical genetics evaluation including echocardiography as well as CT/catheter angiography. Clinical genetics evaluation was made as per guidelines (Table 1) of Tobias, *et al* (1999) [25]. The relevant morphological features were recorded.

Molecular cytogenetics study was carried out both in interphase as well as metaphase cells. EDTA as well as heparinized blood sample was collected from affected individuals (0.5–1 ml each). Interphase cell suspension was prepared by standard methods [22]. Blood nucleated cells washed in phosphate buffer saline solution three times before 30 minutes hypotonic treatment (50 mMol KCl) and fixation in methanol:acetic acid solution (3:1 ratio). Cells re-suspended in 100 ul fresh fixative. Approximately 20 ul cell suspension was used to prepare a slide. Metaphase nuclei were prepared from phytohaemagglutinin stimulated human peripheral blood lymphocytes using standard cytogenetic technique.

Deletion status was determined by FISH using non-commercial DNA probes that included the chromosome region 22q11.2. Bacterial artificial chromosome clones (BAC)/Phage artificial chromosome clones (PAC) named RP5-882I5 (22q11.2), RP11-22M5 (22q11.22) & CTA-154H4 (22q11.21) and spanning approximately 2 mega base (Mb) in length from 22q11.21 through 22q11.23 (micro deletion detection limit of >90% in patients with DiGeorge anomaly) were obtained from European Resource Centre for Molecular Cytogenetics, University of Bari, Italy (www.biologia.uniba.it; curtesy Professor Mariano Rocchi). The clones were received as bacterial (E.coli; resistant to chloramphenicol) LB agar stab culture. Sub culture in LB agar plate before growing in large amount in LB medium. Probe DNA was extracted using a commercial BAC extraction kit (Sigma, India). All probes were labelled by nick translation method with FITC-12-dUTP (Roche) or TRITC-
12-dUTP (Roche) or Cy3 (Amersham, UK). Working concentrations of probe DNA was maintained for the study were between 100-200 ng/μl.

Slide washed in acetic acid for 2 min and dehydrated in 70, 90, 100%, 3 min each in ethanol series. Nuclei on the slide treated with pepsin (100mg/ml) in 0.01N HCL for 20 min at 37°C, rinsed in bi-distilled water and once in PBS and post-fixed in 1 % par formaldehyde in PBS for 10 min at 4°C. Slides rinsed in PBS, twice in bi-distilled water and then dehydrated through ethanol series as before. The hybridization buffer (60% formamide, 2x SSC, 10% dextran sulfate Sigma Aldrich USA) containing labeled probe was applied to the slides under a circular cover slip (11mm in diameter). The probes and nuclear DNA denatured at 76°C for 6-7 min. Hybridization performed in a dark moist chamber at 37°C for overnight FISH. After hybridization cover slips were removed and slides were washed initially with NP40 0.03% solution at 72°C for 2-3 min and thereafter with NP40 0.01% solution for 2 min at room temperature. Then slides dehydrated in ethanol series, as before and mounted in antifade (Vector, USA) with 1μg/ml 4,6 diaminidino-2- phenylindol (DAPI; Sigma USA). The slides were screened by Olympus BX 51 fluorescent microscope with a 100 watt mercury bulb using 100X plan-appochromatic objective and single band pass filter for DAPI, FITC and TRITC and a Triple band pass filter for DAPI, TRITC and FITC (Olympus Japan). FISH image was captured through spectral imaging system. A total of more than 1000 interphase nuclei and at least 5 metaphase nuclei were scored from each case. A presence of two signals in 100% metaphase and 90% interphase cells were considered as normal whereas demonstration of one signal in 100% metaphase or 85-90% interphase cells considered as deletion positive. When the presence of both one and two signal in metaphase and interphase (>15% deletion) cells were observed then cases was considered as mosaicism.
Results

A total of 121 consecutive cases with congenital structural heart defects requiring surgical treatment were offered screening and studied by FISH. The mean age was 11.65 years, range 5 days to 29 years. There were 84 males and 37 females. Among 121 cases sixty three patients had conotruncal heart defects (tetralogy of Fallot in sixty one) and 58 had non-conotruncal (ventricular septal defect in 13, atrial septal defect in 6 and multiple cardiac anomalies in 39 cases) heart defect (Table 2). Typical or some clinical features of 22q11.2 microdeletion was seen in 69 cases (32 cases typical; 21 cases facial dysmorphology and 16 cases hypocalcemia besides cardiac defects). Isolated heart defect was seen in 52 cases. Out of 121 patients four patients had hemizygous 22q11.2 microdeletion (3.3%); 2 as complete and 2 as mosaic forms (Tables 2 and 3; Fig.1&2). No patient with isolated cardiac malformation was found to have a 22q11.2 microdeletion. No patient also had karyotypic abnormality.
Discussion

The association of conotruncal cardiac defects with hemizygosity of locus on chromosome 22q11.2 is one of the evidence for genetic aetiologies of congenital heart defects [26,27]. As molecular probes for this locus are readily available, the diagnosis of a chromosome 22q11.2 microdeletion is now routinely performed [28,29]. However, it remains unknown why this haploinsufficiency shows such a wide range of penetrance and expressivity.

In this prospective study we have presented FISH result of 22q11.2 microdeletion study on 121 cases of structural cardiac malformation requiring surgery and found four cases of 22q11.2 microdeletion (3.3%). Among 121 cases sole cardiac defect was noted in 52 (i.e., isolated cardiac defects) and remaining 69 had extra-cardiac features in addition to cardiac defect. All four microdeletion cases also had extra-cardiac manifestations (4 out of 69 i.e., 5.8%) besides cardiac defect (tetralogy of Fallot) or 6.4% (in TOF) or 8.7% (in TOF with extra-cardiac malformations; Table 2). This frequency is towards lower side than what has been reported in published reports (TOF 6% to 21%, PA/VSD 32% to 48%) [4,30]. This discrepancy may possibly be explained by a higher attrition rate in western countries. Indeed, another report from western India [24] have found a frequency of 5.7% which is much higher than our overall prevalence of 3.3%. In contrast to this Indian report we did not find any 22q11.2 microdeletion in isolated ASD (nil vs 6.6%) or isolated VSD cases (nil vs 12%).

Chromosome 22 microdeletion occur de novo in most cases, however in 8% it may be inherited (50% risk for transmission). Early diagnosis is important because of its diverse medical complications and hereditary implications (recurrence in next pregnancy). Retrospective examination of clinical data by Tobias et al. (1999) [25] had revealed useful guidelines for clinical diagnosis of the condition (Table 1). Authors suggested FISH analysis should be performed on patients who meet one of the criteria in column A. Any patient with a conotruncal cardiac anomaly, even in isolation, should be investigated for the presence of the deletion, because this problem occurs frequently (~50%). Alternatively, the possession or history of two features in column B or one feature in column B in addition to one in column C are regarded as sufficient to merit FISH investigation. These guidelines were devised with the aim of achieving high sensitivity in the initial detection of patients for whom the FISH analysis should be considered. Our experience through this study does not support FISH analysis on isolated cardiac malformation even if it is of conotruncal anomalies. The ability of the clinical criteria laid down by Tobias et al (1999) [25] seems inadequate and needs to
incorporate more criteria through meta analysis for better pick up of microdeletion positive cases. We have also found poor correlation between the presence of typical dysmorphic features and 22q11.2 microdeletion (typical features without 22q11.2 microdeletion).

We have encountered with a patient of 22q11.2 microdeletion (mosaic) with trigonocephaly derived from craniosynostosis of the metopic suture. This case is a rare case of 22q11.2 microdeletion and trigonocephaly.

We observed prevalence of 22q11.2 microdeletion of 3.3% in patient with major cardiac malformation. Chromosome 22q11.2 micro deletion is common underlying cause in cases with major cardiac malformation with extra cardiac manifestation (5.8%). However, its role in cardiac malformation cases without extra cardiac manifestations is doubtful. We suggest routine FISH should be offered in cases with structural malformation of heart requiring surgery associated with extra cardiac anomalies (e.g., dysmorphic features). This may facilitate carrier detection and prevention of another baby with this malformation through prenatal diagnosis.
Consent

Written informed consent was obtained from the parent of patients for publication with any accompanying images.

Competing interests

No financial & non financial competing interests

Authors’ contributions

AH formulated activity plan, checked results & interpreted results besides principal investigator of the project funded by ICMR, India. He also had reviewed clinical findings, prepared manuscript and will respond to the quarries of reviewers. He will be the guarantor of the manuscript. MJ carried out all FISH related activity under guidance of AH besides worked as research fellow for the project under AH. IC also carried out FISH related activity under guidance of AH. MK was involved in clinical management of the cases. All authors read and approved final manuscript.

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References


Table 1 shows clinical features that should lead to consideration of FISH analysis for a possible 22q11.2 deletion (derived from Tobias, et al 1999) [25]

<table>
<thead>
<tr>
<th>Column A</th>
<th>Column B</th>
<th>Column C</th>
</tr>
</thead>
<tbody>
<tr>
<td>The presence of one of the following</td>
<td>Two or more of the following core features</td>
<td>One core feature plus one of these associated features</td>
</tr>
<tr>
<td>Conotruncal cardiac anomaly such as Fallot’s tetralogy, interrupted aortic arch, truncus arteriosus or major aorto-pulmonary collateral arteries</td>
<td>Characteristic facial abnormalities viz. broad bulbous nose, square shaped tip of nose, short filtrum, telecanthus, slanting eyes, low set ears, etc</td>
<td>Long slender fingers and hands</td>
</tr>
<tr>
<td>Parent of an affected child</td>
<td>Non-conotruncal congenital cardiac defect</td>
<td>Short stature</td>
</tr>
<tr>
<td></td>
<td>Learning difficulties/developmental delay</td>
<td>Hypotonia</td>
</tr>
<tr>
<td></td>
<td>Cleft palate, velopharyngeal insufficiency or swallowing difficulty</td>
<td>Renal abnormalities or Potter sequence</td>
</tr>
<tr>
<td></td>
<td>Hypocalcaemia</td>
<td>Psychiatric (especially bipolar) disorders</td>
</tr>
<tr>
<td></td>
<td>Immunodeficiency or thymic hypoplasia</td>
<td>Family history of congenital cardiac defects</td>
</tr>
</tbody>
</table>
Table 2 shows details of structural cardiac malformation in relation to extra-cardiac malformations.

<table>
<thead>
<tr>
<th>Defects</th>
<th>With extra-cardiac malformation</th>
<th>Without extra-cardiac malformation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conotruncal Anomalies</strong></td>
<td>47</td>
<td>16</td>
<td>63</td>
</tr>
<tr>
<td>TOF</td>
<td>46 (all 4 +ve cases i.e., 8.7% of 22q11.2 microdeletion belong to this group)</td>
<td>16</td>
<td>62 (6.4% in all TOF)</td>
</tr>
<tr>
<td>Truncus Arteries (TA)</td>
<td>01</td>
<td>00</td>
<td>01</td>
</tr>
<tr>
<td>Non-Conotruncal Anomalies</td>
<td>22</td>
<td>36</td>
<td>58</td>
</tr>
<tr>
<td>Isolated VSD</td>
<td>00</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Isolated ASD</td>
<td>00</td>
<td>06</td>
<td>6</td>
</tr>
<tr>
<td>Multiple anomalies</td>
<td>22</td>
<td>17</td>
<td>39</td>
</tr>
<tr>
<td>(2 or more combinations of pulmonary stenosis, VSD, ASD, AVSD, coarctation of aorta, PDA, etc)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>69</td>
<td>52</td>
<td>121</td>
</tr>
</tbody>
</table>

(3.3%)
Table 3 shows clinical manifestations of patients with 22q11.2 microdeletion (n=4)

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Gender</th>
<th>Religion</th>
<th>Cardiac Abnormality</th>
<th>Extra cardiac Abnormality</th>
<th>FISH Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>96 months</td>
<td>Female</td>
<td>Muslim</td>
<td>TOF</td>
<td>FD, LD, BD</td>
<td>93% interphase cells with hemizygous deletion</td>
</tr>
<tr>
<td>2</td>
<td>14 months</td>
<td>Male</td>
<td>Hindu</td>
<td>TOF</td>
<td>FD, LD, HT</td>
<td>98.5% interphase cells with hemizygous deletion</td>
</tr>
<tr>
<td>3</td>
<td>33 months</td>
<td>Male</td>
<td>Hindu</td>
<td>TOF</td>
<td>FD, LD, HT, SS</td>
<td>Mosaic; 8% normal cells</td>
</tr>
<tr>
<td>4</td>
<td>18 months</td>
<td>Male</td>
<td>Hindu</td>
<td>TOF</td>
<td>FD, LD, CS</td>
<td>Mosaic; 15% interphase cells with hemizygous deletion</td>
</tr>
</tbody>
</table>

TOF = tetralogy of Fallot; FD= facial dysmorphology; LD=learning difficulty; BD= behavioral disorder; HT=hypotonia; SS=short stature; CS=craniostenosis (metopic sutures)
Legends to Figures

Figure 1 is showing front view (A) of face indicating broad nose, square shaped tip of nose, small filtrum, hypertelorism, telecanthus, squint and low set ears and FISH results (B) with (one signal) hemizygous deletion of a patient with pure 22q11.2 microdeletion syndrome.

Figure 2 is showing front view (A) of face indicating broad nose, square shaped tip of nose, small philtrum, hypertelorism, telecanthus, squint and low set ears and FISH results (B) with (1 signal; arrows) as well as without (two signal) hemizygous deletion of a patient with mosaic 22q11.2 microdeletion syndrome.
Figures

Fig. 1 A

Fig. 1 B
Additional files provided with this submission:

Additional file 1: copy right.jpg, 242K
http://www.biomedcentral.com/imedia/1551252902304542/supp1.jpeg