Screening for Cytogenetic and Molecular Chromosome Rearrangements in Tunisian Children With Conotruncal Heart Defects

Bochra Gargouri, PhD,1 Nouha Abdelmoula, MD,1 Imen Trabelsi Sahnoun, MD,2 Bouthaina Gargouri, PhD,4 Tarek Rebaï, MD,1 Ahlem Amouri, MD3

(1Department of Histology, Medical University of Sfax, 2Department of Cardiology, Hedi Chaker Hospital of Sfax, 3Department of Histology and Cytogenetics, Pasteur Institute of Tunis, 4Department of Pedopsychatry, Hedi Chaker Hospital of Sfax, Tunisia)

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Abstract

Background: Conotruncal heart defects are cardiovascular malformations that have most been associated with chromosomal 22q11.2 microdeletion.

Methods: To estimate frequency and investigate the clinical features of these microdeletions in unselected patients with conotruncal heart defects, a total of 26 patients originating from southern Tunisia had been prospectively evaluated through cytogenetic and molecular studies. The clinical analysis was performed according to a specific clinical protocol for the diagnosis of congenital cardiovascular malformations. A molecular cytogenetic technique was undertaken by fluorescence in situ hybridization (FISH) using 2 probes: LSI DiGeorge N25 (D22S75) region probe N25/ARSA, and LSI DiGeorge/ VCFs region probe TUPLE1/ARSA. cytogenetic analysis with RHG banding was carried out to detect chromosome rearrangements. All patients have normal karyotype 46,XX or 46,XY.

Results: The frequency of the 22q11.2 microdeletion in the subjects carrying conotruncal heart defects with or without extra-cardiac signs of our series is thus estimated at 3.85% (1/26).

Conclusion: The microdeleted subject is carrying a tetralogy of Fallot.

Materials and Methods

Patients

The study comprised 26 patients selected from the Cardiology Department at the Sfax Hedi Chaker CHU during an 8-month period from June 2004 to January 2005. The 26 sporadic cases included in the study were prospectively selected among patients who were either examined at the external consultation service or hospitalized in the internal service for a congenital cardiopathy during the period of the study and in whom cardiopathy was labelled conotruncal.

The cardiac phenotype was specified by a cardiologist specializing in congenital cardiopathies with a general examination, an echocardiography, and possibly an angiography. Certain patients had already been operated upon whereas others had not. For each patient, a medical card was drawn up on which was recorded all the clinical and para-clinical information, the questioning specifying the antenatal antecedents, and the neonatal and childhood period.

The extracardiac signs, evoking a 22q11.2 microdeletion syndrome, were specified whenever it was possible.

Methods

A 2- to 5-mL sample of venous blood on lithium heparin was taken in a sterile fashion from each patient both for a conventional cytogenetic analysis in the search of chromosomal anomaly and for molecular cytogenetic study by in situ fluorescence hybridization in the search for a 22q11.2 microdeletion.

Concerning the second technique, 2 commercial single-sequence probes specific of the region were used. The targets of the probes were the chromosomes and the extracardiac signs, evoking a 22q11.2 microdeletion syndrome, were specified whenever it was possible.

The perfecting of molecular cytogenetics techniques, and in particular in situ fluorescence hybridization, made it possible to show that most DiGeorge and velo-cardio-facial syndromes were related to the 22q11.2 microdeletion. Later, observations of 22q11.2 microdeletion associated with very partial clinical pictures such as isolated conotruncal cardiopathies or velar were insufficiently reported.

Moreover, the identification of deletion in asymptomatic parents whose children present a typical clinical picture made it possible to further widen the clinical spectrum of the 22q11.2 microdeletion known now as the 22q11.2 microdeletion syndrome. In this paper, we undertake the task of carrying out a longitudinal monocentric exploratory study through which we systematically detect, thanks to the conventional and molecular cytogenetic studies, the presence of chromosomal anomalies and 22q11.2 microdeletion in patients carrying conotruncal cardiopathies recruited during a well-defined period.
**LSI DiGeorge N25 (D22S75) Region Probe**

This probe is a mixture of 2 probes formed by double-fragment DNA, directly marked by 2 fluorochromes of different color.

**LSI DiGeorge/VCFS Region Probe**

This probe consists of a combination of 2 probes: ARSA control probe marked in green, and a probe specific of the distal commonly deleted 22q11.2 area and is marked with rhodamine.

**Results**

All 26 patients had a congenital cardiovascular disease (Table 1).

<table>
<thead>
<tr>
<th>Congenital Cardiovascular Disease</th>
<th>Number</th>
<th>Positive FISH</th>
<th>Negative FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetralogy of Fallot</td>
<td>19 (73.07%)</td>
<td>1/19</td>
<td>18/19</td>
</tr>
<tr>
<td>Double outlet right ventricle</td>
<td>3 (11.53%)</td>
<td>0</td>
<td>3/3</td>
</tr>
<tr>
<td>Transposition of the great arteries with type B interrupted aortic arch</td>
<td>1 (3.84%)</td>
<td>0</td>
<td>1/1</td>
</tr>
<tr>
<td>Agenesis of the pulmonary valves</td>
<td>2 (7.69%)</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Lung atresia with open septum</td>
<td>2 (7.69%)</td>
<td>2/2</td>
<td>2/2</td>
</tr>
</tbody>
</table>

**Table 2. Conotruncal Cardiopathy Cases by Gender**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>15 (57.7%)</td>
<td>46,XY</td>
</tr>
<tr>
<td>Females</td>
<td>11 (42.3%)</td>
<td>46,XX</td>
</tr>
</tbody>
</table>

**Cytogenetic Analysis**

On the cytogenetic level, no chromosomal anomalies in number or structure were noted among our 26 patients. The chromosome chart showed a normal female chromosomal formula 46,XX for the 11 girls and a normal male chromosomal formula 46,XY for the 15 boys (Table 2).

**FISH Analysis**

Of the 26 conotruncal cardiopathy cases, 1 of 26 patients was shown to be deleted at the 22q11.2 locus (Image 2 and 3) and 25 of 26 did not have a 22q11.2 microdeletion (Image 1).

The frequency of the 22q11.2 microdeletion in the subjects carrying conotruncal cardiopathies with or without extracardiac signs in our series is thus estimated at 3.85% (1/26) (Table 1). The microdeleted subject is carrying a regular tetralogy of Fallot. The phenotype of this child of male sex and aged 15 months, is evocative of the velo-cardio-facial syndrome (see clinical observation). The facial dysmorphia was also evocative of a DiGeorge syndrome in 7 other patients in our series not carrying the 22q11.2 microdeletion. For the 26 patients in our series, including the microdeleted child, the state of the thymus, the parathyroids, and the immunological status could not be evaluated due to the death of the microdeleted patient.
**Clinical Observation of the Microdeleted Child**

This is a case of a 15-month-old male child, the offspring of 2 nonconsanguineous parents who have 2 other normal boys. The child was born on time after a normal pregnancy. The neonatal period was marked by several problems: nutrition and sleep apnea. The evolution is marked by a delay in psychomotor acquisitions including head posture, sitting, standing, walking, and speaking.

**Cardiac Echography**

Cardiac echography showed a high and broad interventricular communication and a stenosis on the pulmonary tract. The pulmonary tract is of acceptable quality. It poses the diagnosis of a tetralogy of Fallot.

**The Chromosome Chart**

The patient is a male with a normal karyotype 46,XY.

**Dysmorphia**

Dysmorphia is discrete with a small chin, a small mouth, fine lips, and a characteristic nose with prominent base. The ears are lowly implanted.

**Mental Profile**

The intelligence quotient (IQ) has not been quantified but the child presents a slight mental backwardness with poor language and difficult pronunciation.

**Behavior**

Calm child and cooperative.

**Calcemia**

Normal rate. The immunological checkup mentioned a repeated bronchitis with cognitive disorders, facial dysmorphism, and speech difficulties; it is most probably a case of velo-cardio-facial syndrome rather than DiGeorge syndrome.

Cardiopathies associated with the 22q11.2 microdeletion are congenital and mostly of the conotruncal type.\(^{15}\) These types of cardiac malformations account for approximately 50% of the congenital cardiopathies diagnosed in newborn babies.\(^{1,2,16,17}\) The question that always arises is to know whether the child having an isolated conotruncal cardiopathy or associated other minor extracardiac signs has a 22q11.2 microdeletion, making the assumption that they have a Di-George syndrome or a partially similar one whose expression would be limited to the heart.

Most of the cases (19, 73.07%) were cases of tetralogy of Fallot (73.07%); the other cardiopathies comprised 3 cases of right ventricle with double outlet, 1 case of transposition of the great arteries associated with type B interrupted aortic arch, 2 cases of pulmonary atresy with open septum, and 2 cases of pulmonary valves agenesis.

The FISH technique used in the search of the 22q11.2 microdeletion rests on the targeted application of 2 single-sequence DNA probes that cover the proximal part of the area commonly deleted in DiGeorge and velo-cardio-facial syndromes. These probes are partly complementary of the HIRA gene but cover neither the TBX1 nor the UFD1L genes, which are the 3 major genes that play a prevalent part in the genesis of conotruncal cardiopathies by interaction with neural crest cells. We used as a target the metaphasic chromosomes, which are prepared using the conventional cytogenetic technique. Chromosome charts in RHG bands were thus established for all patients so as not to miss any chromosomal anomaly in number or structure that could be at the origin of the cardiopathies.

No chromosomal anomaly has been found either in number or in structure on the chromosome charts of the 26 patients in our series, in particular structure anomalies involving chromosomes 22. This was an expected result since the conotruncal cardiopathies associated to chromosomal anomalies in number, in general trisomies, are seen within a well-determined syndromic framework, which is not the case in our patients. Moreover, the reciprocal translocations and the structure anomalies are too rare to be evaluated on a rather restricted series like ours. Similarly, the analysis of chromosomes 22 marked in RHG bands (330 bands per haploid genome) did not show anomalies. In fact, only high-resolution techniques (coupled in particular with GTG banding) can make it possible to reveal the microremaniements we expect. These techniques are rather heavy, thus requiring synchronization stages. They were not adopted in our study as the wish was to search for the 22q11.2 microdeletion in particular by adopting the FISH technique, which is more specific, more sensitive, and targeted.

Thus, by in situ fluorescence hybridization, 1 case of 22q11.2 microdeletion was detected in 26 patients. The frequency of the 22q11.2 microdeletion during conotruncal cardiopathies is thus estimated at 3.85% (1/26). This frequency is far lower than the figures reported in the literature.\(^{18,20,21}\)

It should be noted that in most studies in the literature relating to the evaluation of the 22q11.2 microdeletion prevalence, the adopted technique is the one we have used: targeted in situ fluorescence hybridization using the commercial N25 and TUPLE1 probes. It is only in some rare studies that other probes of the type YAC, BAC, or CAP (complementary of particular sequences of the 22q11.2 area) were used.\(^{22}\) Indeed, according to the series of the Goldmunz team in Philadelphia,
which is the reference team as regards congenital cardiopathy genetics, the frequency of the 22q11.2 microdeletion in subjects carrying nonsyndromic conotruncal cardiopathies varies from 18% (9/50) to 29% (5/17). This frequency also varies according to the type of the conotruncal cardiopathy and the age group studied.

In the 2 initial series of Goldmuntz and colleagues, both carried out in 1993, the studies concerned a number of patients comparable with ours with 17 and 50 patients, respectively. The selection criteria of the patients was dominated by the major criterion, which was the presence of conotruncal cardiopathies. Moreover, the subjects carrying conotruncal cardiopathies with evocative signs of a CATCH22 phenotype were excluded from the study.

In other studies, it has been shown that the frequency of the 22q11.2 microdeletion was particularly higher in patients showing conotruncal cardiopathies explored in the neonatal period. Indeed, in the French series of Iserin and colleagues, the frequency of the 22q11.2 microdeletion during conotruncal cardiopathies was approximately 48% (50/104). This study is characterized by the age of the patients who were all newborn babies. Moreover, nearly all the patients showed at least 1 evocative sign of CATCH22 phenotype.

In other studies interested in isolated conotruncal cardiopathies, the frequency of the 22q11.2 microdeletion was low in a way comparable with our figure. For example, in the series of the German team of Voigt and colleagues, the frequency of the microdeletion associated with the isolated conotruncal cardiopathies in a population of different ages (4 days to 58 years) was estimated at 2%. Likewise, the Italian team of Digilio and colleagues reported a very low frequency when it is the case of strictly isolated conotruncal cardiopathies with the exclusion of the cases with even evocative minor signs of a CATCH22 phenotype. The rather low figure found in our series could thus be explained on the one hand by the heterogeneity of the age groups and, on the other hand, by the heterogeneity of the clinical presentation. These 2 factors were inevitable in our study because the selection of the patients was dependent on the scarcity of these pathologies, the prospective and monocentric character of the study, as well as the limited duration of the patient collection (8 months).

Hence, the patient ages varied, ranging from a few months to 30 years. If one considers only the newborn babies (the age group 0 to 2 years; 7 cases), the frequency amounts to 14.3%. The frequency of 22q11.2 microdeletion is particularly higher in patients showing conotruncal cardiopathies explored in the neonatal period and associated with at least one clinical sign (4 cases, 57%, 4/7). Furthermore, the representation of the various clinical signs was heterogeneous in the 7 cases of newborn babies, with an over-representation of the facial dysmorphism.

The isolated and/or syndromic character of the cardiopathy was sometimes difficult to specify, more especially as certain clinical signs such as neonatal hypocalcemia, the immunological checkup centered on the study of T lymphocytes, as well as the radiological aspect of the thymus were not always easy to check. Moreover, if one considers the isolated cardiopathies without signs of facial dysmorphism (18 cases out of 26), the frequency will be null; whereas in the cardiopathies associated with at least 1 clinical sign (8 cases), the frequency will be 12.5% (1/8). The representation of the various conotruncal cardiopathies was heterogeneous in our series, with an over-representation of the tetralogy of Fallot, an absence of the arterial trunk persistence cases, and a very weak representation of the aortic arch interruption.

The prevalence of the 22q11.2 microdeletion, although more important in the neonatal period, also varied according to the type of the conotruncal cardiopathy, but the frequency distribution remained comparable with what was found by Goldmuntz and colleagues. Indeed, during the study of Iserin and colleagues, the 22q11.2 microdeletion was described in 41% (7/17) of the arterial trunks, 89% (16/18) of the interruptions of the aortic arc, 34.5% (19/55) of the tetralogy of Fallot, 40% (2/5) of stenoses of the pulmonary valves, and 66.7% (6/9) of the inter ventricular communications.

In spite of our results, it appears that the 22q11.2 microdeletion constitutes a frequent etiologic factor of the conotruncal cardiopathies (with a frequency that varies from one type to another), which justifies its systematic search or its tracking in any individual carrying a conotruncal cardiopathy.

Moreover, this frequency of the 22q11.2 microdeletion increases when the cardiopathy is associated with other evocative signs of the CATCH22 phenotype.

These clinical signs seem to be subtle in the neonatal period if the clinician is not informed. They make it possible to explain the even higher frequency of the 22q11.2 microdeletion in the cardiopathies detected in the neonatal period. It also appears that the conotruncal cardiopathies of the 22q11.2 microdeletion are subtly different but the distribution of their various types in microdeletion-carrying patients is roughly uniform. IM

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