22q11.2 Deletion Syndrome in Colombian Patients With Syndromic Cleft Lip and/or Palate

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Abstract
The objective of this work was to identify 22q11.2 chromosomal deletion in patients with cleft lip and/or cleft palate and suggestive syndromic phenotype in Colombian patients. We studied 49 patients with cleft lip and/or cleft palate, exhibiting additional clinical findings linked to 22q11.2 deletion syndrome. All patients underwent high-resolution G-banded karyotyping, multiplex ligation-dependent probe amplification, and clinical evaluation by a geneticist. Seven patients presented 22q11.2 deletion and 2 patients had other chromosomal abnormalities. In conclusion, this study contributes with new data for genetic etiology in syndromic conditions of oral fissures.

Keywords
chromosomes, craniofacial morphology, velocardiofacial syndrome, genetics

Introduction
Cleft lip and palate (CLP) is a common congenital malformation in humans affecting orofacial structures. It represents approximately 13% of all reported congenital anomalies (Batra et al., 2003; Prabhu et al., 2012). Oral clefts occur during embryonic development, secondary to alterations during facial processes tissue fusion. Worldwide, they occur approximately in 1 in every 700 live births (Batra et al., 2003; Mossey and Modell, 2012; Prabhu et al., 2012). In Colombia, congenital malformation studies report orofacial cleft frequency at 15.92 per 10 000 births, constituting one of the most frequent malformations in this population (Zarante et al., 2010). Orofacial clefts include isolated cleft lip (CL), isolated cleft palate (CP), or CLP.

Generally, these anomalies produce facial, dental, speech, and hearing development impairment, as well as problems in psychosocial behavior in affected individuals (Mossey et al., 2009). In clinical terms, orofacial clefts can be classified as nonsyndromic, or syndromic clefts if they occur along with other congenital malformations (Kohli and Kohli, 2012; Prabhu et al., 2012). Orofacial clefts are caused by a complex interaction among environmental and genetic factors, such as mutations or chromosomal alterations (Brito et al., 2012; Kohli and Kohli, 2012; Prabhu et al., 2012).
The prevalence of the 22q11.2 deletion is estimated at 1 in 4000 to 1 in 6000 live births. It affects more than 30 genes and has been considered one of the most common chromosomal alterations in humans (Goodship et al., 1998; Lindsay, 2001; Óskarsdóttir et al., 2004; Shprintzen, 2008). Over 90% of 22q11.2 probands have de novo deletion; the remnants inherit from one progenitor (Shprintzen, 2008; Bassett et al., 2011).

Palatine anomaly is one of the most frequent clinical characteristics in patients with this deletion (Monteiro et al., 2013). It has been reported that in the presence of 22q11.2 deletion, up to 71% of patients may have palatine anomalies. Moreover, up to 11% have an apparently isolated CP, including submucous CP, thus becoming the most common genetic syndrome associated with palatine clefts (Friedman et al., 2011; McDonald-McGinn et al., 2013). The elevated palatine anomaly frequency in 22q11.2 deletion syndrome, added to the scant information on this association in the Colombian population, underlined the need to evaluate its presence in patients with syndromic orofacial clefts. Therefore, the purpose of this study was to determine 22q11.2 deletion presence in patients with cleft lip and/or cleft palate (CL/P) accompanied by other phenotypic characteristics associated with 22q11.2 deletion syndrome.

Materials and Methods

Patient Selection

Forty-nine patients with CL/P exhibiting additional clinical characteristics associated with 22q11.2 deletion syndrome (syndromic CL/P) were analyzed. Patients were selected from Malformation Surveillance Database in Bogotá, Human Genetics Institute (HGI), Javeriana University; genetic consultations at the HGI, Javeriana University; Cleft Lip and Palate Program, School of Dentistry, Javeriana University; Healing the Children Mission 2013, Neiva; “Grupo Nace una Sonrisa” Comfamiliar Risaralda, Pereira; and medical institutions in other Colombian cities. Patients, parents, or guardians were provided with information on the study, and subsequently informed consent was obtained from all participants. This study was approved by the ethics committee of Pontificia Javeriana University’s School of Medicine.

Clinical Analysis

A clinical geneticist evaluated all patients in search for additional congenital anomalies or clinical signs associated with CL/P. Patients with isolated oral cleft (nonsyndromic) or with specific known syndromes were excluded from the study.

Cytogenetic Analysis

High-resolution karyotyping cell cultures were prepared using RPMI 1640 culture medium for human peripheral blood cells (Sigma-Aldrich, St Louis, Missouri), 10% fetal bovine serum (Gibco, Life Technologies, Waltham, Massachusetts), and phytohemagglutinin (Gibco, Life Technologies). To obtain prometaphase chromosomes, cells were synchronized with amethopterin (methotrexate; 10⁻⁵ M; Sigma-Aldrich). Block was released by 10⁻³ thymidine (Sigma-Aldrich). For each patient, 25 G-banded metaphase cells at 550 to 700 band stage were analyzed for the presence of critical deletion region or other chromosomal alterations.

Multiplex Ligation-Dependent Probe Amplification

Molecular Analysis of the 22q11.2 Region

DNA was obtained from peripheral blood samples (QIAamp DNA Blood Mini Kit; Qiagen, Hilden, Germany). 22q11.2 chromosomal region copy number variations (CNVs) were analyzed with SALSA multiplex ligation-dependent probe amplification (MLPA) probemix P250-B1 DiGeorge (MRC-Holland Foundation, Amsterdam, the Netherlands). In addition, copy number status on 4q, 6p, 9q, 10p, 17p, and 22q13 regions, responsible for different disorders with 22q11.2 deletion syndrome phenotypic features, was also analyzed by the same kit. Multiplex ligation-dependent probe amplification reaction was carried out following the manufacturer’s instructions (MRC-Holland Foundation). PCR products were evaluated on ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, California). Data analysis was performed with Gene Scan version 2013 Coffalyser.Net software (MRC-Holland Foundation).

Statistical Analysis

Clinical characteristics frequency comparison between 22q11.2 deletion-carrier patient group and noncarrier group was determined by χ² test. Statistical significance level was set at 5% (P < .05).

Results

A total of 49 patients who met the study inclusion criteria were analyzed: 30 (61.2%) males and 19 (38.7%) females, ranging from 0 to 49 years of age. Twenty-five (51%) patients presented CP, 15 (30.6%) had CLP, 4 (8.2%) of all studied cases were CL. Likewise, 4 (8.2%) of 49 had palatine velum insufficiency and 1 (2%) individual presented bifid uvula. A large percentage of studied patients had other characteristics associated with 22q11.2 deletion syndrome, such as facial dysmorphism (33 patients, 61.2%), cognitive impairment and/or developmental alterations (22 patients, 52.3%), recurrent infections (12 patients, 28.5%), and congenital cardiac defects caused by conotruncal structural defects (13 patients, 31%; tetralogy of Fallot, interrupted aortic arch, atrial septal or ventricular septal defects, vascular rings).

Even though 22q11.2 deletion was not observed with cytogenetic banding, other chromosomal abnormalities were detected in 2 (4%) patients by this method. The 2 cases presented palatine anomaly, facial dysmorphism, cardiac abnormalities, and developmental delay, among others. The first case showed a 46, XY, der(4)t(4;8)(p16;p23) Html karyotype.
Additionally, MLPA analysis revealed a partial trisomy in the 8p23.1 region (rsa 8p23.3p23.1 (SALSA MLPA P250-B1 DiGeorge)x3). It is noteworthy to point out, as mentioned in the methodology, DiGeorge MLPA SALSA P250-B1 kit also included control probes binding to the 8p23.1 region. Last, array CGH confirmed MLPA findings and detected a deletion in the 4p16 region. The second case exhibited a 46, XX, der(21)inv(21)(q21.1q22.2)del(21)(q22.2) karyotype. Subsequently, array Comparative Genomic Hybridization (CGH) for this patient disclosed complex chromosome 21 long-arm intrachromosomal rearrangements represented by deletions and duplications.

Multiplex ligation-dependent probe amplification analysis identified 22q11.2 deletion in 7 (14.3%) patients: 6 (85.7%) exhibited typical ~ 3 Mb deletion located between low copy repeats (LCRs), LCR-A and LCR-D (cases 1, 2, 3, 4, 6, and 7; Table 1). Moreover, 1 (14.3%) patient presented an ~ 1.8 Mb deletion, distal from the typical deletion region located between LCR-D and LCR-F (case 5; Table 1). In this group, 4 (57%) patients had CP, 2 (29%) patients presented palatine velum insufficiency, and 1 (14%) patient had bifid uvula. These 7 patients exhibited facial dimorphism and had recurrent infections. Cognitive impairment and/or developmental alterations were observed in 6 (85.7%) patients. Furthermore, 5 (71.4%) exhibited congenital cardiac defects and 6 (85.7%) displayed other clinical alterations (Table 1). A significant difference in immunological phenotype was observed when comparing clinical characteristics between 22q11.2 deletion carrier group and noncarrier group (Table 2).

**Discussion**

Cleft lip and/or palate is a multifactorial clinical condition that can appear either in isolated or syndromic form. Its etiology is related to monogenic mutations and chromosomal anomalies (Brito et al., 2012; Kohli and Kohli, 2012; Prabhu et al., 2012). In affected individuals, this congenital alteration can have consequences on speech, hearing, physical appearance, and psychological development, which may lead to adverse results in health and social integration (Mossey et al., 2009; Prabhu et al., 2012). Pathogenetic factor identification for oral anomalies may contribute to recognize its cause, therefore assist in family counseling. In addition, it can aid in guiding treatment and clinical monitoring.

In this study, 49 patients with CL/P and other congenital alterations associated with 22q11.2 deletion syndrome were evaluated. Deletion was detected in 7 (14.3%) patients. Vieira et al. (2015) reported a higher percentage (35%) in a similar study carried out in 100 patients with palatine anomalies and suspected deletion. Conversely, Prabodha et al. (2012) did not identify 22q11.2 deletion in 162 evaluated patients with CP, even though 56.79% of patients exhibited other accompanying congenital anomalies related to deletion phenotype. Additional studies have reported an association between CL/P and 22q11.2 deletion; however, patient clinical condition was not specified (Sivertsen et al., 2007; Bashir et al., 2008).

There is considerable variation depending on the population studied. Sivertsen et al. (2007) described in Norwegian children with isolated CP a 22q11.2 deletion frequency in 1 (1.8%) of 57 patients, whereas in English children with CL/P, Bashir et al. (2008) reported deletion in 9 (6.7%) of 134 patients. However, these studies do not clarify whether the analyzed group presented syndromic or nonsyndromic cleft condition. Nevertheless, Bashir et al. (2008) recommended deletion confirmation because of its implications on affected patient treatment. Likewise, Reish et al. (2003) emphasized on 22q11.2 deletion syndrome identification importance for pathology recognition during clinical suspicion. In general, studies carried out in groups including all patients with nonsyndromic orofacial clefts record lower 22q11.2 deletion frequency than our study (14.3%), which included only patients with syndromic CL/P (Reish et al., 2003; Sivertsen et al., 2007; Prabodha et al., 2012). Our data are in agreement with Vieira et al. (2015), suggesting microdeletion routine testing importance for patients with CP and additional congenital anomalies.

22q11.2 deletion syndrome has a wide phenotype spectrum (Tobias et al., 1999; McDonald-McGinn and Sullivan, 2011). In our study, 7 (100%) patients affected by 22q11.2 deletion exhibited facial dysmorphism, with frequent infections suggestive of an immunological phenotype. Six (85%) exhibited cognitive development impairment and 5 (71.4%) presented congenital cardiac malformations (Ventricular Septal Defect [VSD], tetralogy of fallot). These are the most commonly described characteristics in patients with 22q11.2 deletion (Tobias et al., 1999; McDonald-McGinn et al., 2013). Despite differences in population number, compared to the study by Vieira et al. (2015) with a larger sample, our results are similar. Our data are also in agreement with Lay-Son et al.’s (2012) study in Chilean patients with 22q11.2 deletion and palatal abnormalities. Furthermore, Wu et al. (2013) reported on 43 Chinese patients with velocardiofacial syndrome, all with facial abnormalities, highlighting the importance of this phenotype as a key indicator for 22q11.2 deletion syndrome diagnosis.

All our 22q11.2 deletion carrier patients showed some degree of immunological compromise, specifically recurrent respiratory infections. It has been reported that 77% of all 22q11.1 deletion patients may have partial combined immunodeficiency due to the presence of thymus hypoplasia. Additionally, they can have recurrent respiratory infections as a cause of other complications, such as aspiration pneumonia, palatine velum incompetence, and gastroesophageal reflux, mainly in individuals with congenital cardiac disease (Sullivan, 2004; McDonald-McGinn et al., 2013).

In our study, 30 patients exhibited palatine anomaly and deletion was confirmed in 7 patients. Of these, 4 (57%) exhibited CP, 2 (29%) velopharyngeal insufficiency, and 1 (14%) bifid uvula. Deletion was not detected in patients with CL with or without cleft palate (CL ± P). Although the analyzed group was small, our results agree with data reported by McDonald-McGinn and Sullivan (2011) and McDonald-McGinn et al. (2013), but differ slightly from those of Vieira et al. (2015)
<table>
<thead>
<tr>
<th>N</th>
<th>Age</th>
<th>Sex</th>
<th>Karyotype</th>
<th>MLPA Result</th>
<th>Fissure Type</th>
<th>Cardiac Anomaly</th>
<th>Facial Dysmorphism</th>
<th>Immunological Phenotype</th>
<th>Development Alteration/Cognitive Alterations</th>
<th>Other Clinical Findings</th>
<th>Analysis of Parents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>M</td>
<td>46, XY</td>
<td>3 Mb TDR</td>
<td>Clef palate</td>
<td>Absent</td>
<td>Midfacial hypoplasia, triangular facies, overfolded helix</td>
<td>Recurrent respiratory tract infection</td>
<td>Learning impairment and speech disorders</td>
<td>Microcephaly, cutaneous syndactyly on hands,</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>F</td>
<td>46, XX</td>
<td>3 Mb TDR</td>
<td>Clef palate</td>
<td>VSD</td>
<td>Epicantic folds, midfacial hypoplasia, prognathism</td>
<td>Frequent respiratory and dermatological infections, immunological analysis revealed partially combined immunodeficiency</td>
<td>Speech disorder</td>
<td>von Willebrand disease type II, symptomatic focal epilepsy, hypothyroidism, keratitis, chronic sinusitis, bilateral conductive hearing loss, bipolar affective disorder</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>F</td>
<td>46, XX</td>
<td>3 Mb TDR</td>
<td>Clef palate</td>
<td>VSD</td>
<td>Microcephaly, brachycephaly, hirsutism, oblique palpebral fissures, telecanthus, micrognathia</td>
<td>Recurrent respiratory tract infection and otitis media</td>
<td>Psychomotor development retardation</td>
<td>Generalized hypotonia</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>F</td>
<td>46, XX</td>
<td>3 Mb TDR</td>
<td>Clef palate velum</td>
<td>VSD</td>
<td>Posteriorly rotated ears, upturned nares, upturned palpebral fissures</td>
<td>Recurrent respiratory tract infection</td>
<td>Psychomotor development retardation</td>
<td>Absent</td>
<td>ND</td>
</tr>
<tr>
<td>5a</td>
<td>49</td>
<td>M</td>
<td>46, XY</td>
<td>1.8 Mb LCR-D to -F deletion</td>
<td>Palate velum insufficiency</td>
<td>Absent</td>
<td>Upturned palpebral fissures, midfacial hypoplasia, high nasal root</td>
<td>Recurrent herpes zoster infections, immunological analysis revealed partially combined immunodeficiency</td>
<td>Psychomotor development retardation and mild cognitive impairment</td>
<td>Sensorineural hearing loss, hypothyroidism, bilateral inguinal hernia</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>M</td>
<td>46, XY</td>
<td>3 Mb TDR</td>
<td>Bifid uvula</td>
<td>Tetralogy of Fallot interventricular communication</td>
<td>High nasal bridge, midfacial hypoplasia</td>
<td>Repetitive respiratory tract infection</td>
<td>Speech impairment</td>
<td>Absent</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>M</td>
<td>46, XY</td>
<td>3 Mb TDR</td>
<td>Palate velum insufficiency</td>
<td>VSD</td>
<td>High nasal bridge, elongated facies, almond-shaped eyes, external ear malformation</td>
<td>Recurrent respiratory tract infection</td>
<td>Absent</td>
<td>Short stature, bladder wall thickening</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: F, female; LCR, low copy repeats; M, male; MLPA, multiplex ligation-dependent probe amplification; NA, not analyzed; ND, not deletion; TDR, typical deleted region; VSD, ventricular septal defect.

*Patient with 22q11.2 distal deletion.
Table 2. Clinical Findings of Patients With and Without 22q11.2 Deletion.

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Patients With 22q11.2 Deletion</th>
<th>Patients Without 22q11.2 Deletion</th>
<th>P Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facial dysmorphology</td>
<td>7 (100%)</td>
<td>28 (66.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>Immunological phenotype</td>
<td>7 (100%)</td>
<td>7 (16.6%)</td>
<td>0.019b</td>
</tr>
<tr>
<td>Development alteration/ cognitive alterations</td>
<td>6 (85.7%)</td>
<td>21 (50%)</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiac anomalies</td>
<td>5 (71.4%)</td>
<td>13 (31%)</td>
<td>NS</td>
</tr>
<tr>
<td>Total Patients</td>
<td>7 (100%)</td>
<td>42 (100%)</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations: NS, nonsignificant.

aStatistical significance level was 5% (P < .05), χ² test.
bP < .05, significant.

and Lay-Son et al. (2012), most likely due to differences in sample selection and clinical analysis.

Oral cleft frequency varied among patients with 22q11.2 deletion syndrome, mainly explained by already known great phenotypic spectrum variation among individuals (Robin and Shprintzen, 2005; Shprintzen, 2008). As suggested by Driscoll et al. (2006), possible factors contributing to this variability are gender, ethnicity, and environmental factors during embryonic development. Additionally, the presence of oral cleft could be influenced by modifier genes effect located outside the deletion region. Collectively, our data support CP presence is a characteristic condition of 22q11.2 deletion syndrome. Therefore, it is important to evaluate it in these patients to achieve specific diagnosis, treatment, and identification of genetic origin, which in turn are requisite to evaluate genetic risks and provide family counseling.

De novo 22q11.2 deletion origin was established in 3 of the 7 patients; no parents were available for the remainder. Reports describe approximately 90% of cases are de novo (Oskarsdottir et al., 2004; Shprintzen, 2008).

On the other hand, cytogenetic abnormalities unrelated to 22q11.2 deletion have been reported in patients with suspected 22q11.2 deletion syndrome (Fernández et al., 2008). Two of our cases with palatal anomaly and clinical signs suggestive of 22q11.2 deletion syndrome had an abnormal karyotype. Duplication of the 8p21.2 region has been associated with generation of a phenotype overlapping with 22q11.2 deletion syndrome, mainly cardiac malformations (Barber et al., 2010). However, patients with clinical characteristics, such as heart disease, CP, and delayed neurodevelopment, have also been described in 4p deletion (South et al., 2008). Thus, both alterations would be contributing to the described patient phenotype. Regarding the patient with chromosome 21 abnormalities, the phenotypic overlap with 22q11.2 deletion syndrome could be related to 21q region deletion (Lyle et al., 2009), which is characterized by cardiac malformations, CP, psychomotor retardation, and facial dimorphism.

Conclusions

Results showed an important number of patients with palatine abnormalities may be 22q11.2 deletion carriers, suggesting the importance of considering 22q11.2 deletion in differential diagnosis in individuals with any palatine anomaly, accompanied by congenital facial or cardiac malformations. These analyses should be directed to clarify diagnosis and benefit patients with 22q11.2 deletion syndrome with adequate follow-up and therapeutic management. Multiplex ligation-dependent probe amplification analysis proved to be a simple and efficient tool in detecting not only classic 22q11.2 deletion but also some CNVs in different chromosomes regions possibly associated with the phenotype. In addition, cytogenetic banding analysis was a useful tool in the visualization of different chromosomal alterations that may cause a deletion-like phenotype, undetected by MLPA. Combination of cytogenetic banding and molecular diagnostic tools can provide greater possibilities for achieving etiological diagnosis for this type of clinical entities.

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Declaration of Conflicting Interests

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