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Studies of genes in the *FGF* signaling pathway and oral clefts with or without dental anomalies

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Keywords

FGF signaling pathway; polymorphisms; cleft lip/palate; tooth agenesis

To the Editor:

Orofacial clefts comprise a large fraction of all human birth defects, affecting approximately one in every 500 to 1000 births worldwide, and are notable for their significant lifelong morbidity and complex etiology [Murray, 2002; Cox, 2004]. The clinical manifestations of these defects are diverse, ranging from isolated clefts of the lip to complete bilateral clefts of the lip, alveolus and palate [Fogh-Andersen, 1942].

It has been proposed that clefting is part of a complex malformation that can be associated with dental anomalies resulting from the disturbed development of the dentition [Stahl et al., 2006]. In that context, we have shown that dental anomalies outside the cleft area could be used as additional features for the generation of more sophisticated cleft subphenotypes [Letra et al., 2007].

The fibroblast growth factor (*FGF)* signaling pathway regulates multiple developmental processes, including craniofacial development [Nie et al., 2006]. Mutations in *FGFR1* cause autosomal dominant Kallmann syndrome, which includes clefts in 30% of the cases and tooth agenesis in 7% [Dodé et al., 2003]. Sequencing of the coding region of genes in the *FGF* signaling pathway in nonsyndromic cleft cases revealed that missense and nonsense mutations in *FGF* genes might contribute to approximately 3% of nonsyndromic cleft lip and palate [Riley et al., 2007].

Considering that oral clefts and tooth agenesis may be part of the same phenotypic spectrum, we investigated if polymorphisms in *FGF* genes were associated with cleft subphenotypes that included dental anomalies. To confirm the proposed hypothesis, we used a case-control design and 966 individuals (484 cases with oral clefts and 482 control individuals without clefts or family history of clefting). Study subjects were ascertained through the Hospital of

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Rehabilitation and Craniofacial Anomalies (HRAC) and Bauru Dental School, of the University of São Paulo, Bauru, SP, Brazil.

The study was conducted with the consent of the participants and approved by the Research and Ethics Committee of the University of São Paulo and by the University of Pittsburgh Institutional Review Board. In the case of children under 15 years of age, authorization was also requested from their parents or from the individual legally in charge of the child.

Genomic DNA of all individuals was isolated from buccal epithelial cells by the proteinase K digestion followed by ammonium acetate extraction method [Aidar and Line, 2007]. The SNPs used in this study were chosen from previous studies where associations with oral clefts [Riley et al., 2007] and breast cancer [Easton et al., 2007; Hunter et al., 2007] were described. Details of the studied polymorphisms are shown in Table I.

Genotyping of the selected polymorphisms was carried out by real-time PCR using the Taqman method [Ranade et al., 2001] in an ABI 7900 automatic instrument (Applied Biosystems, Foster City, CA). Assays and reagents were supplied by Applied Biosystems (Applied Biosystems, Foster City, CA). Differences in the frequencies of the alleles of each polymorphism between cases and controls by each cleft subphenotype were assessed by usingthe odds ratio and 95% confidence intervals.

To avoid the effects of population stratification, we excluded all individuals with reported African or Japanese ancestry. Individuals in both case and control groups were thus of European origin. Control individuals consisted of 282 healthy, non-related people, aged 4–94 years (average age, 36.8 yrs), the great majority who were patients and students at Bauru Dental School.

Individuals with clefts were examined clinically and through their medical records so that we could determine the cleft type and side to describe each individual's cleft status. Cleft status was based on cleft completeness (comprised of primary and secondary palates entirely clefted) or incompleteness, and on laterality (left, right, bilateral). The authors (AL and RM — each with vast experience in dentistry) examined all patients. They had access to all previous dental and radiographic records, and performed additional oral and radiographic examination. In this manner, all extracted or avulsionated teeth were not mistakenly included in the analysis as congenitally missing teeth. Central and lateral incisors and canines when absent in the same side of the cleft were not counted as congenitally missing teeth. Dental anomalies such as tooth agenesis (including hypodontia and oligodontia), microdontia, supernumerary teeth, tooth malposition (rotation or inclination), impaction, shape anomalies, and transposition were assessed clinically and through radiographs and were recorded for each individual. For every anomaly, the inclusion criterion was that at least one permanent tooth was affected (children 8 years old or younger were excluded, mainly because sometimes premolar tooth buds are not visible at younger ages). Instances of anomalies adjacent to the cleft area (affecting maxillary central incisors, lateral incisors, or canines) were not included, because the absence of such teeth was likely the consequence of developmental anomalies at the cleft side. Genotype and allele distributions were within Hardy-Weinberg equilibrium (data not shown).

All analyses and observed results are presented in Table II. An increased risk for complete unilateral cleft lip and palate (CL/P) was seen for individuals carrying variant alleles in *FGF10* (OR=1.52; 95% C.I.: 1.13-2.04). Increased risk was also found for individuals with unilateral right CL/P carrying variant alleles of *FGF3* (OR=1.83; 95% C.I.: 1.21-2.77). When tooth agenesis data was considered in the analysis, an increased risk for individuals with bilateral CL/P with associated tooth agenesis carrying variant alleles of *FGF10* (OR=1.95; 95% C.I.: 1.08-3.52) and *FGFR2* (OR=2.02; 95% C.I.: 1.14-3.59) could be seen.

We did not observe any increase in risk when considering other dental anomalies like supernumerary teeth, impacted teeth or mal positioned teeth (data not shown).

Our results partially corroborate the association data presented by Riley et al. [2007] in which several genes (*FGF3*, *FGF7*, *FGF10*, *FGF18*, and *FGFR1*) demonstrated a trend for association with nonsyndromic cleft lip and palate. In addition, the diversity in the binding specificity of *FGF* receptors for *FGFs* clearly can lead to a large combinatorial set of possible interactions. It has been demonstrated that *FGF3* activates the b splice forms of *FGFRs* 1 and 2 [Ornitz et al., 1996] and that *FGFR2*b binds to *FGF10* [Yeh et al., 2003]. It is interesting to observe that increased risks of CL/P in our study were found for alleles in *FGF3*, *FGF10*, and *FGFR2*.

Our observations that some *FGF* genes may be associated with cleft subphenotypes (namely bilateral cleft lip and cleft palate with tooth agenesis) reinforce the theory that dental anomalies could be an extension of the cleft phenotype and should be carefully considered upon examination of the cleft patient.

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Table I

Summary of the SNPs studied

SNP	*gene*	*locus*	*reference*
rs1448037	*FGF10*	5p13-p12	Riley et al., 2007
rs4073716	*FGF18*	5q34	Riley et al., 2007
rs13317	*FGFR1*	8p11.2-p11.1	Riley et al., 2007
rs2981582	*FGFR2*	10q26	Easton et al., 2007
rs1219648	*FGFR2*	10q26	Hunter et al., 2007
rs4631909	*FGF3*	11q13	Riley et al., 2007
rs4980700	*FGF3*	11q13	Riley et al., 2007
rs2413958	*FGF7*	11q13	Riley et al., 2007

	FGF10 rs1448037	*FGF18* rs4073716	*FGFR1* rs13317	*FGFR2* rs2981582	*FGFR2* rs1219648	*FGF3* rs4631909	*FGF3* rs4980700	*FGF7* rs2413958
Cleft phenotype (N) vs. Controls (N)	**ODDS ratio (95% confidence interval)**							
All Clefts (379) vs. Controls (281)	1.27 (1.0 - 1.16)	1.00 (0.8 - 1.26)	0.80 (0.62 - 1.05)	0.98 (0.79 - 1.23)	0.87 (0.69 - 1.08)	1.26 (1.01 - 1.58)	1.17 (0.93 - 1.47)	1.17 (0.90 - 1.52)
CL/P (326) vs. Controls (281)	1.30 (1.02 - 1.64)	0.97 (0.77 - 1.22)	0.79 (0.60 - 1.04)	1.03 (0.82 - 1.30)	0.86 (0.69 - 1.09)	1.25 (0.99 - 1.58)	1.19 (0.94 - 1.51)	1.17 (0.89 - 1.54)
Bilateral CL/P (125) vs. Controls (281)	1.17 (0.85 - 1.61)	0.89 (0.65 - 1.21)	0.65 (0.44 - 0.96)	0.83 (0.61 - 1.14)	0.76 (0.56 - 1.04)	1.16 (0.85 - 1.58)	1.08 (0.79 - 1.48)	1.23 (0.85 - 1.78)
Complete Bilateral CL/P (103) vs. Controls (281)	1.13 (0.80 - 1.58)	0.87 (0.62 - 1.22)	0.67 (0.45 - 1.02)	0.82 (0.59 - 1.43)	0.78 (0.56 - 1.09)	1.30 (0.94 - 1.81)	1.20 (0.86 - 1.67)	1.35 (0.91 - 2.00)
Incomplete Bilateral CL/P (22) vs. Controls (281)	1.40 (0.73 - 2.69)	0.98 (0.51 - 1.87)	0.59 (0.26 - 1.37)	0.92 (0.48 - 1.76)	0.66 (0.34 - 1.26)	0.63 (0.31 - 1.27)	0.64 (0.32 - 1.27)	0.82 (0.37 - 1.80)
Unilateral CL/P (201) vs. Controls (281)	1.38 (1.05 - 1.80)	1.02 (0.78 - 1.33)	0.87 (0.64 - 1.19)	1.17 (0.90 - 1.52)	0.93 (0.72 - 1.21)	1.31 (1.01 - 1.71)	1.26 (0.97 - 1.65)	1.14 (0.84 - 1.55)
Complete Unilateral CL/P (137) vs. Controls (281)	**1.52 (1.13 - 2.04)**	1.01 (0.75 - 1.36)	1.02 (0.73 - 1.42)	1.07 (0.80 - 1.44)	0.89 (0.66 - 1.19)	1.47 (1.09 - 1.98)	1.28 (0.95 - 1.73)	1.08 (0.76 - 1.52)
Incomplete Unilateral CL/P (64) vs. Controls (281)	1.10 (0.73 - 1.66)	0.56 (0.39 - 0.80)	0.76 (0.46 - 1.23)	1.42 (0.96 - 2.09)	1.04 (0.70 - 1.53)	1.00 (0.67 - 1.51)	1.22 (0.82 - 1.83)	1.28 (0.81 - 2.02)
Unilateral Left CL/P (140) vs. Controls (281)	1.32 (0.98 - 1.78)	1.06 (0.79 - 1.42)	0.83 (0.59 - 1.17)	1.21 (0.90 - 1.62)	0.94 (0.70 - 1.27)	1.14 (0.85 - 1.53)	1.08 (0.80 - 1.46)	1.11 (0.79 - 1.56)
Unilateral Right CL/P (61) vs. Controls (281)	1.50 (1.0 - 2.24)	0.93 (0.62 - 1.40)	0.98 (0.61 - 1.56)	1.09 (0.73 - 1.62)	0.90 (0.61 - 1.34)	**1.83 (1.21 - 2.77)**	1.81 (1.20 - 2.73)	1.22 (0.76 - 1.95)
Cleft Palate only (53) vs. Controls (281)	1.09 (0.71 - 1.69)	1.25 (0.81 - 1.92)	0.89 (0.54 - 1.48)	0.72 (0.46 - 1.10)	0.90 (0.58 - 1.37)	1.37 (0.90 - 2.07)	1.05 (0.68 - 1.60)	1.14 (0.69 - 1.87)
Cleft Phenotype with tooth agenesis vs. Cleft Phenotype without tooth agenesis								
All clefts with tooth agenesis (106) vs. All clefts without tooth agenesis (273)	1.17 (0.83 - 1.61)	0.99 (0.71 - 1.36)	0.87 (0.76 - 1.72)	1.08 (0.78 - 1.50)	1.01 (0.73 - 1.41)	1.05 (0.76 - 1.46)	0.95 (0.68 - 1.32)	0.92 (0.62 - 1.35)
CL/P with tooth agenesis (94) vs. CL/P without tooth agenesis (232)	1.21 (0.85 - 1.72)	1.10 (0.77 - 1.57)	0.95 (0.61 - 1.46)	1.08 (0.76 - 1.52)	1.10 (0.78 - 1.56)	0.98 (0.69 - 1.38)	0.94 (0.66 - 1.34)	0.84 (0.56 - 1.28)
Bilateral CL/P with tooth agenesis (34) vs. Bilateral CL/P without tooth agenesis (91)	**1.95 (1.08 - 3.52)**	1.04 (0.57 - 1.89)	0.89 (0.41 - 1.93)	1.89 (1.05 - 3.40)	**2.02 (1.14 - 3.59)**	0.69 (0.38 - 1.24)	0.90 (0.49 - 1.66)	0.79 (0.39 - 1.61)
Unilateral CL/P with tooth agenesis (60) vs. Unilateral	0.92 (0.59 - 1.42)	1.05 (0.67 - 1.64)	0.96 (0.57 - 1.62)	0.87 (0.37 - 0.10)	0.76 (0.49 - 1.19)	1.18 (0.76 - 1.83)	1.12 (0.71 - 1.73)	0.87 (0.52 - 1.46)

	FGF10 **rs1448037**	***FGF18*** **rs4073716**	***FGFR1*** **rs13317**	***FGFR2*** **rs2981582**	***FGFR2*** **rs1219648**	***FGF3*** **rs4631909**	***FGF3*** **rs4980700**	***FGF7*** **rs2413958**
Cleft phenotype (N) vs. Controls (N)	**ODDS ratio (95% confidence interval)**							
CL/P without tooth agenesis (141)								
Right Unilateral CL/P with tooth agenesis (24) vs. Right Unilateral CL/P without tooth agenesis (37)	1.27 (0.60 - 2.67)	2.06 (0.95 - 4.46)	0.68 (0.28 - 1.66)	1.24 (0.59 - 2.60)	1.34 (0.64 - 2.82)	1.49 (0.69 - 3.25)	1.48 (0.68 - 3.21)	0.90 (0.37 - 2.17)
Left Unilateral CL/P with tooth agenesis (36) vs. Left Unilateral CL/P without tooth agenesis (104)	0.74 (0.42 - 1.30)	0.85 (0.49 - 1.49)	1.11 (0.58 - 2.14)	0.61 (0.35 - 1.05)	0.55 (0.31 - 0.98)	0.96 (0.55 - 1.66)	0.86 (0.49 - 1.50)	0.84 (0.44 - 1.59)