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ORIGINAL RESEARCH

Genetic Association for Caries Susceptibility among Cleft Lip and/or Palate Individuals

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ABSTRACT

Aim: To evaluate the association of the polymorphisms in the TGFB3 gene (rs2268626), and the BMP4 gene (rs17563) with dental caries in two different groups (noncleft group and oral cleft group) from a cleft center located at Rio de Janeiro, Brazil.

Materials and methods: A total of 486 unrelated children and adolescents with or without caries were evaluated using a cohort design. Data on oral health habits was obtained through a questionnaire and caries data was collected by clinical examination. Genotyping of the selected polymorphisms for TGFB3 and BMP4 were carried out by real-time PCR using the TaqMan assay method from a genomic DNA isolated from buccal epithelial cells of all children and adolescents.

Results: No association was found between BMP4 polymorphism and caries among individuals from both groups. For TGFB3 polymorphism, significant differences were observed for allele and genotype frequencies between caries free and caries affected individuals in oral cleft group (p = 0.013 and 0.006 for allele and genotype frequencies respectively).

Conclusion: Our findings provide evidence suggesting that TGFB3 may be involved in caries susceptibility in oral cleft group.

Clinical significance: In the future, the possibility of identifying genes related to caries susceptibility can lead to counseling of the individual that carries gene alterations, with the aim of working on preventive measures for caries as well as bioengineering treatments.

Keywords: Dental caries, Transforming growth factor beta, Genetic predisposition to disease, Genetic susceptibility, Genetic polymorphism.

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INTRODUCTION

Dental caries is the most common chronic disease in children and adolescents. This condition is thought to be multifactorial with an interaction between genetic and environmental factors but also with strong evidence for a genetic component in the etiology of this disease.[1-6]

Researches using animals and human epidemiological studies have contributed to a greater understanding of genetic factors in caries susceptibility. Studies in mice have demonstrated evidence of a genetic component in caries susceptibility in the H2 region on chromosome 17,[7] and regions of chromosome 1, 2, 7 and 8.[8] Studies on twin pairs estimated the heritability for caries.[9,10]

Recent studies have sought to compare individuals with distinct caries experiences, similarly influenced by confounding factors, such as diet, oral hygiene habits, fluoride exposure and access to dental care. Genes that may be related to saliva flow and diet preferences were proposed. A protective locus for caries in the X chromosome may explain the gender differences seen in this disease frequency.[11] Deeley et al[1] suggest that variation in amelogenin may contribute to caries susceptibility in a Guatemalan-Mayan. Other study[2] with Turkish children suggest that variation in amelogenin, ameloblastin and tuftelin contribute to caries susceptibility.

In addition, variation in enamelin appeared to interact with levels of *S. mutans* infection.[2] In a Brazilian population, genetic variations in MMP^{13} $gene^{6}$ and MMP^{20} $gene^{5}$ may contribute to individual differences in caries susceptibility. Possible association of $DEFB^{1}$ gene to increased or with decreased caries experience was proposed.[3]

Despite improved trends in levels of dental caries due to prevention measures to improve oral health and prevent disease, caries remains prevalent in certain population groups,[12,13] such as in oral cleft children.[14-17] The possible association between these two conditions highlighted the possibility that genes involved in oral cleft etiology may also be involved in caries susceptibility.

Transforming growth factor β (TGFB) superfamily consists of more than 35 members that include TGFB and bone morphogenetic proteins (BMPs).[18-20] Tgfb signaling pathways might induces the expression of many diverse genes necessary for the coordination of biomineralization,[21] including enamel maturation[22] and palatal growth.[23]

Therefore, the aim of the present work was to examine the relationship between dental caries and TGFB3 (rs2268626) and BMP4 (rs17563) polymorphisms in two different groups (noncleft group and oral cleft group) from a cleft center located at Rio de Janeiro, Brazil.

MATERIALS AND METHODS

Subjects

Eligible unrelated children and adolescents from 3 to 19 years were enrolled in two different groups (noncleft group and oral cleft group) using a cohort design from February 2009 to July 2011. Nonsyndromic oral cleft group was composed of nonsyndromic subjects ascertained through a public reference hospital of oral cleft rehabilitation in Rio de Janeiro, Brazil; none of the subjects were diagnosed with median cleft. To further reduce possible etiological heterogeneity, we excluded those clefts with additional unspecified multiple malformations. Noncleft group was recruited at Pediatric Dental Clinic in Federal University of Rio de Janeiro, Brazil. Both institutions are located in the northern part of the city of Rio de Janeiro. The institution, where the subjects were recruited, is located in the Southeast of Brazil, the most densely populated and industrialized region of the country. The ethnicity definition was ascertained based on self-reported information. The Southeast region of Brazil comprises an ethnic admixture of Caucasian and African descents. The remaining 0.5% of the population is Amerindian or Asian descents.[24]

All children and adolescents or parents/caregivers answered a questionnaire about fluoride exposure history and oral hygiene habits. Information was also sought on the child's frequency of ingesting cakes, cookies and sweets between meals on the day prior to completing the questionnaire.

In the current study, we compared children and adolescents living in the same area and with similar cultural backgrounds and access to dental care in an attempt to reduce the influence of environmental confounders.

The Research and Human Ethics Committee of the Health Department of the city of Rio de Janeiro, Brazil (113/09) approved the study. All participating individuals or parents/legal guardians allowed participation in the study by signing a free informed consent.

Determination of Caries Experience

All individuals received an oral and dental examination performed by two pediatric dentists.[14] Cohen's kappa values for agreement between examiners were 0.91. Caries was diagnosed in primary and permanent teeth by visual examination and was registered if there was definite visual evidence with a breach in the enamel and extension into dentin. Subjects were seated in a dental chair, and examinations were done with the use of a flashlight and mouth mirror. Caries were assessed using the DMFT (decayed, missing teeth due to caries, filled teeth) and/or dmft indexes according to the criteria recommended by the World Health Organization guidelines. Individuals were classified according to the caries experience level. Individuals free from caries experience (dmft/DMFT = 0) were compared with individuals with caries experience (dmft/DMFT $\geq$ 1).

DNA Samples and Genotyping

Genomic DNA for molecular analysis was extracted from buccal cells by the previously reported method.[25] Genetic polymorphisms in the TGFB3 gene (rs2268626) and in the BMP4 gene (rs17563) were genotyped by real-time polymerase chain reactions using the TaqMan method[26] by Agilent Technologies (Stratagene Mx3005P). Assays and reagents were supplied by Applied Biosystems (Foster City, CA). Marker information is included in Table 1.

STATISTICAL ANALYSIS

The data were subsequently processed and analyzed using the Statistical Package for the Social Sciences (SPSS 16.0). The student's test and Chi square test were used to analysis the mean age, type of dentition, gender, ethnicity, tooth brushing habit, use of dental floss daily and dietary factors between caries experience and caries free groups.

Caries experience was analyzed together (noncleft group and oral cleft group) and stratified (noncleft group and oral

Table 1: Details on the genetic markers studied in individuals

Gene	*Average heterozygosity*[a]	*SNP*	*Flanking sequence* [b]	*Locus*	*MAF*[b]
TGFB3	0.377 ± 0.216	rs2268626	GGGTGGGCTCAGCACC[C/T]GACCAGCTGCAGGGCG	14q24.3	0.225
BMP4[c]	0.461 ± 0.135	rs17563	CATCCCTGAGAACGAGG[C/T]GATCTCCTCTGCAGA	14q22.2	0.347

[a]Obtained from the UCSC genome; [b]Browser on Human 2012 assembly (Available at: http://genome.ucsc.edu); [c]Obtained from ENTREZ SNP database (Available at: http://www.ncbi.nlm.nih.gov/sites/entrez); Missense — Val152Ala

Table 2: Demographic data and risk factors for caries in the study subjects (n = 486)

	Noncleft group			*Oral cleft group*		
	Caries experience (n = 222)	*Caries free (n = 145)*	*p-value**	*Caries experience (n = 77)*	*Caries free (n = 42)*	*p-value**
Mean age (SD)	8.84 (±3.02)	9.65 (±3.64)		11.57 (±3.64)	11.83 (±4.06)	
Type of dentition (%)						
Permanent dentition	37 (16.7)	49 (33.8)		37 (48.1)	20 (47.6)	
Mixed dentition	156 (70.3)	72 (49.6)	**<0.001**	34 (44.1)	21 (50)	0.460
Primary dentition	29 (13)	24 (16.6)		6 (7.8)	1 (2.4)	
Gender (%)						
Female	104 (46.8)	66 (45.5)	0.803	33 (42.9)	21 (50)	0.455
Male	118 (53.2)	79 (54.5)		44 (57.1)	21 (50)	
Ethnicity (%)						
Caucasian	131 (59)	90 (62.1)	0.558	32 (41.6)	20 (47.6)	0.524
Afro-descendants	91 (41)	55 (37.9)		45 (58.4)	22 (52.4)	
Tooth brushing (%)						
1X	17 (7.7)	11 (7.6)		3 (3.9)	0 (0)	
2X	81 (36.5)	46 (31.7)	0.532	20 (26)	13 (31)	0.397
3X or more	111 (50)	82 (56.6)		48 (62.3)	28 (66.6)	
No answer	13 (5.8)	6 (4.1)		6 (7.8)	1 (2.4)	
Use of dental floss daily (%)						
Yes	64 (28.8)	58 (40)		22 (28.6)	15 (35.7)	
No	147 (66.2)	81 (55.9)	**0.028**	49 (63.6)	26 (61.9)	0.543
No answer	11 (5)	6 (4.1)		6 (7.8)	1 (2.4)	
Dietary factors (ingest sweets between meals) (%)						
Yes	137 (61.7)	58 (40)		42 (54.5)	24 (57.1)	
No	74 (33.3)	79 (54.5)	**<0.001**	29 (37.7)	17 (40.5)	0.948
No answer	11 (5)	8 (5.5)		6 (7.8)	1 (2.4)	

Note: *Chi-square and Fisher's exact tests

cleft group only). Chi-square test was also performed to evaluate if subgroups were preferentially associated with the variations in TGFB3 and BMP4 genotypes and alleles. Differences were considered significant when $p \leq 0.05$. Moreover, the standard Chi-square test was used to test for deviation from Hardy-Weinberg equilibrium. Marker information is included in Table 1.

RESULTS

A total of 486 individuals were included in this study: 187 (38.5%) were caries-free individuals, while 299 (61.5%) presented caries experience. The demographic characteristics and risk factors for caries noncleft and oral cleft groups were summarized in Table 2. In oral cleft group, the univariate analysis showed that there were no significant differences in demographics and adherence to preventive oral health habits, such as tooth brushing, use of dental floss daily and dietary factors (ingest sweets between meals) between caries-free and caries-affected individuals. In noncleft group, regarding the risk factors for caries, statistical differences were observed between caries-free and caries-affected individuals in dietary factors ($p < 0.001$) (*see* Table 2).

No significant association between caries presence and genotype or allele distributions for BMP4 polymorphism was found in oral cleft and noncleft groups, and the results were shown in Table 3.

For TGFB3 polymorphism, significant differences were observed for allele and genotype frequencies between caries-free and caries-affected individuals in oral cleft group ($p = 0.013$ and 0.006 for allele and genotype frequencies

respectively) (Table 4). The frequencies of the CC, CT and TT genotypes of TGFB3 in oral cleft group were 29.3 and 70.7% in caries-free individuals and were 55.8 and 44.2% in individuals with caries experience respectively. The genotype CC was observed in only two individuals in oral cleft group and 13 individuals in noncleft group.

DISCUSSION

A significant decline in dental caries in many countries became evident and has been accompanied by the phenomenon of polarization. It is defined as a small percentage of people with high dental caries rate and a large percentage of caries-free people.[27] The polarization may reflect measures of prevention and control of dental caries, based in the population strategy, which seeks to control the determinants of incidence in the population as a whole. This preventive strategy leaves to identify high-risk susceptible individuals and do not offer them some individual protection.[28] Considering the multifactorial nature of the caries disease, many fundamental questions related to the etiology of dental caries remain to be clarified. Genetic factors that underlie individual differences in caries susceptibility may help to elucidate this situation.

Results of previous studies about caries experience in individuals born with oral clefts have remained controversial. Several reports suggested that individuals born with oral cleft are at an increased risk for dental caries:[15,16] however, others did not confirm this association.[14,17] Although, in this study, the caries experience in individuals born with oral clefts is not higher in comparison to control group, some demographic data and risk factors for caries differ in these two groups. It is important to emphasize that both groups analyzed had a similar lifestyle and were dependent on the public health service. As a result, we decided to stratify the sample into two groups — noncleft and oral cleft groups and perform the analysis of the two groups together and stratified. The purpose of this stratification is to avoid possible confounding factors, since, genes involved in oral cleft etiology may also be involved in caries susceptibility.

One of the challenges of studying multifactorial disease, such as dental caries, is the fact that many genetic and environmental factors contribute to the disease. Studies of genes involved in caries susceptibility offer an insight into the genetic pathways that control the development of human enamel and dentition.

Lip, palate and tooth bud development have a close embryological relationship. Genes that play an important role in lip and palate formation and, later in tooth development,[29] may be involved in the occurrence of oral cleft and later in caries susceptibility. Thus, we chose to study BMP4

Table 3: Frequency of BMP4 (rs17563) allele and genotype in oral cleft and noncleft groups

Subjects	*Alleles (%)*		*p**	*Genotypes*			*p**
	C	*T*		*CC*	*CT*	*TT*	
Noncleft group							
Caries experience (n = 217)	177 (40.8)	257 (59.2)	0.791	40 (18.4)	97 (44.7)	80 (36.9)	0.225
Caries free (n = 137)	109 (39.8)	165 (60.2)		18 (13.1)	73 (53.3)	46 (33.6)	
Oral cleft group							
Caries experience (n = 77)	65 (42.2)	89 (57.8)	0.328	18 (23.4)	29 (37.6)	30 (39)	0.651
Caries free (n = 42)	30 (35.7)	54 (64.3)		8 (19.1)	14 (33.3)	20 (47.6)	
All subjects							
Caries experience (n = 294)	242 (41.2)	346 (58.8)	0.479	58 (19.7)	126 (42.9)	110 (37.4)	0.284
Caries free (n = 179)	139 (38.8)	219 (61.2)		26 (14.5)	87 (48.6)	66 (36.9)	

Note: *Chi-square test, bold emphasis indicates statistical significance ($p \leq 0.05$)

Table 4: Frequency of TGFB3 (rs2268626) allele and genotype in oral cleft and noncleft groups

Subjects	*Alleles (%)*		*p**	*Genotypes*		*p**
	C	*T*		*CC + CT*	*TT*	
Noncleft group						
Caries experience (n = 220)	95 (21.6)	345 (78.4)	0.199	90 (40.9)	130 (59.1)	0.353
Caries free (n = 144)	74 (25.7)	214 (74.3)		66 (45.8)	78 (54.2)	
Oral cleft group						
Caries experience (n = 77)	45 (29.2)	109 (70.8)	**0.013**	43 (55.8)	34 (44.2)	**0.006**
Caries free (n = 41)	12 (14.6)	70 (85.4)		12 (29.3)	29 (70.7)	
All subjects						
Caries experience (n = 297)	140 (23.6)	454 (76.4)	0.908	133 (44.8)	164 (55.2)	0.573
Caries free (n = 185)	86 (23.2)	284 (76.8)		78 (42.2)	107 (57.8)	

Note: *Chi-square and Fisher's exact tests, bold emphasis indicates statistical significance ($p \leq 0.05$)

(rs17563) and TGB3 (rs2268626) genes, since previous studies demonstrated that polymorphic variant in these genes are involved with tooth agenesis[30] and oral cleft.[31-34]

To the best of our knowledge, this is the first report that investigates BMP4 and TGFB3 polymorphisms and their associations in caries experience. BMP4, a member of the TGFB superfamily, located to 14q22-23, is expressed in the presumptive dental epithelium during early tooth morphogenesis.[35,36] In mice, BMP4 expression was also detected in the odontoblasts and ameloblasts, indicating a persistent role for BMP4 in the development and differentiation of both odontoblasts and ameloblasts.[37] BMP4 acts in both, a paracrine and autocrine fashion, to coordinate both the processes of dentin and enamel formation.[38] Despite this participation, our present results do not show a statistically significant association between caries presence and genotype or allele distributions in oral cleft and noncleft groups. Indeed, the several genetic and environmental factors involved probably can account differently to the development of the malformation in population with distinct ethnic background. Moreover, experimental studies with distinct models and population may also contribute to confirm or refute the findings of this study.

Results of the present study indicate that TGFB3 polymorphism shows an association with dental caries susceptibility in oral cleft group. However, the exact role of TGFB3 in enamel and/or dentin cannot be explained by the current knowledge of tooth development. The extent to which this polymorphism may actually contribute to dental caries status is still to be clarified. It is reasonable to hypothesize that TGFB3 and its cognate receptor may be involved in regulating early tooth formation and tooth mineralization may also be affected. Thus, functional studies are necessary to confirm our findings.

Following the tendency, further investigations with other polymorphisms in these genes as well as with larger number of samples are necessary to confirm the involvement of TGFB3 and BMP4 with dental caries in different population.

CONCLUSION

1. TGFB3 (rs2268626) may be involved in caries susceptibility in oral cleft group.
2. Since little is still known about the host genetic factors influencing susceptibility, further studies will assist in understanding the development and prevention of dental caries.

CLINICAL SIGNIFICANCE

The identification of genes related to caries will improve our understanding about the etiology of oral conditions. In the future, the possibility of identifying genes related to caries susceptibility can lead to counseling of an individual that carries gene alterations, with the aim of working on preventive measures for caries as well as bioengineering treatments.

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