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ORIGINAL ARTICLE

TGFB3 and *BMP4* polymorphism are associated with isolated tooth agenesis

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Abstract

Objective. To evaluate the association of the polymorphisms in the *TGFB3* gene (rs2268626) and the *BMP4* gene (rs17563) with isolated human tooth agenesis. ***Materials and methods:*** One hundred and seventy-five unrelated individuals (125 control individuals without tooth agenesis and 50 cases with tooth agenesis) were evaluated using a case-control design. The participants of the study were recruited through the Dental School of the Federal University of Rio de Janeiro, Brazil. Genotyping of the selected polymorphisms for *TGFB3* (48 individuals with tooth agenesis and 125 control cases) and *BMP4* (46 individuals with tooth agenesis and 88 control cases) were carried out by real-time PCR using the Taqman assay method from a genomic DNA isolated from buccal epithelial cells of all individuals. ***Results.*** Significant statistical differences were found for genotype frequencies between tooth agenesis and *TGFB3* control samples ($p = 0.026$). In addition, significant differences were also observed for allele and genotype frequencies between unilateral tooth agenesis and *TGFB3* control samples ($p = 0.014$ and 0.004 for allele and genotype frequencies, respectively). For *BMP4,* genotype distribution had a statistically significant difference between groups ($p = 0.047$). The GG genotype of *BMP4* was more frequent in individuals with three or more missing teeth than in the control group ($p < 0.0001$). ***Conclusions.*** These results indicate that polymorphisms in the *TGFB3* gene and in *BMP4* genes contribute to tooth agenesis. Nonetheless, the extents to which this polymorphism may actually contribute to the tooth agenesis status should be clarified.

Key Words: *bone morphogenetic protein, craniofacial abnormalities, tooth abnormalities, transforming growth factor beta*

Introduction

Tooth agenesis is the most common congenital dental anomaly in humans and it is characterized by developmental absence of teeth. It can occur in association with other genetic diseases or as an independent trait. Non-syndromic tooth agenesis shows wide phenotypic heterogeneity [1–4]. The prevalence of tooth agenesis varies substantially from 2.6–11.3% (excluding third molars) [5–7]. Tooth agenesis may lead to several dysfunctions with a variety of orthodontic and prosthetic problems [8].

Tooth development is a complex process that involves many interactions. The interaction in the odontogenic epithelia mesenchyme repeatedly involves basic organogenic cascades, which include the signaling of Fgf, Bmp, Shh and Wnt. Any disturbances in the tightly balanced signaling cascades may result in dental anomalies [9,10]. Although the exact mechanism that leads to tooth agenesis remains largely unclear, there is evidence supporting a genetic etiology for this dental anomaly. Genes involved in the ectodermal organogenesis serve as potential candidates.

Transforming growth factor β (*TGF*β) superfamily signaling is essential for tooth development [11]. This superfamily consists of more than 35 members that include *TGF*β and bone morphogenetic proteins (*BMPs*) [12–14]. Previous studies have indicated that

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*TGF*β superfamily signaling and its cognate receptors are important regulators of early tooth development [15–17].

Activin is a member of the TGFβ superfamily. Animal models demonstrated that incisors and mandibular molar teeth fail to develop beyond a rudimentary bud in activin-β mutant mice [18]. The gene *BMP4* has also been related to be expressed in the presumptive dental epithelium during early tooth morphogenesis [19,20]. Therefore, the aim of this work was to investigate if polymorphisms in *TGFB3* and *BMP4* are associated with isolated human tooth agenesis.

Materials and methods

Subjects

The tooth agenesis group and the control group consisted of unrelated individuals recruited through the Dental School of the Federal University of Rio de Janeiro, RJ, Brazil. Individuals with oral clefts and syndromes were excluded based on the *International Classification of Diseases, Ninth Revision* (ICD-9). A total of 175 individuals were included in this study: 125 controls and 50 with tooth agenesis. A group of 48 individuals with tooth agenesis and 125 controls were genotyped for *TGFB3*; and a group with 46 individuals with tooth agenesis and 88 controls were genotyped for *BMP4*. The local Research and Ethics Committee approved the study (approval #150A/2009). All participating individuals or parents/legal guardians allowed participation in the study by signing a free informed consent.

Diagnostic criteria for tooth agenesis

Tooth agenesis was considered when at least one permanent tooth was missing during development (excluding third molars). All cases were clearly evident from the radiographic examination. Tooth agenesis was defined based on the age of the individuals and when initial tooth formation could be visible in the radiographs [7].

DNA samples and genotyping

Genomic DNA for molecular analysis was extracted from buccal cells by the previously reported method [21]. 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 [22] by Agilent Technologies (Stratagene Mx3005P). Applied Biosystems supplied the assays and reagents (Foster City, CA).

Statistical analysis

The data were subsequently processed and analyzed using the Epi Info3.3.2 statistical software package (http://www.cdc.gov/epiinfo). Chi-square or Fisher's exact tests determined if tooth agenesis or its subgroup (type, side, arch) was preferentially associated with *TGFB3* or *BMP4* genotypes and alleles. Differences were considered significant when $p \leq 0.05$.

Results

Table I presents the basic demographics of the studied population and shows that there are no significant differences in ethnicity and gender for *TGFB3* and *BMP4* samples.

The genotype distributions for both polymorphisms were in Hardy–Weinberg equilibrium.

In the tooth agenesis group, 24 individuals presented one missing tooth; 24 individuals presented two, three or four missing teeth; and the two remaining individuals presented oligodontia (six missing teeth).

Significant statistical differences were found for genotype frequencies between tooth agenesis and controls for the *TGFB3* sample ($p = 0.026$). In addition, significant differences were also observed for allele and genotype frequencies between unilateral tooth agenesis and controls for the *TGFB3* sample ($p = 0.014$ and 0.004 for allele and genotype frequencies, respectively) (Table II). The genotype CC was not observed in this population.

Table I. Characteristics of the population studied for *TGFB3* and *BMP4*.

	TGF*B3*			BMP4		
Characteristics	Tooth agenesis ($n = 48$)	Control ($n = 125$)	$p^\star$	Tooth agenesis ($n = 46$)	Control ($n = 88$)	$p^\star$
Gender						
Male	18	56	0.384	17	38	0.486
Female	30	69		29	50	
Ethnic group						
Caucasian	32	80	0.742	33	60	0.671
Afro-descendent	16	45		13	28	

*Chi-Square test; statistically significant if $p \leq 0.05$.

Table II. Frequency of *TGFB3* allele and genotype in tooth agenesis and control subjects.

		Alleles, *n* (%)			Genotypes, *n* (%)		
Subjects	*n*	C	T	*p*⋆	CT	TT	*p*⋆
Controls	125	47 (18.8)	203 (81.2)	—	47 (37.6)	78 (62.4)	—
Tooth agenesis							
All tooth agenesis	48	27 (28.1)	69 (71.9)	0.058	27 (56.3)	21 (43.8)	*0.026*
Upper lateral incisor	22	12 (27.3)	32 (72.7)	0.195	12 (54.5)	10 (45.5)	0.134
Premolar	21	10 (23.8)	32 (76.2)	0.448	10 (47.6)	11 (52.4)	0.383
Others	8	6 (37.5)	10 (62.5)	0.069	6 (75.0)	2 (25.0)	0.058
Affected side							
Unilateral	23	16 (34.8)	30 (65.2)	*0.014*	16 (69.6)	7 (30.4)	*0.004*
Bilateral	25	11 (22)	39 (78)	0.600	11 (44.0)	14 (56.0)	0.548
Dental arch							
Maxilla	23	14 (30.4)	32 (69.6)	0.072	14 (60.9)	9 (39.1)	*0.037*
Mandible	19	10 (26.3)	28 (73.7)	0.278	10 (52.6)	9 (47.4)	0.211
Both	6	3 (25.0)	9 (75.0)	0.704	3 (50.0)	3 (50.0)	0.673

*Chi-Square test and Fisher's exact tests; control group was used as a reference; italics indicates statistical significance ($p \leq 0.05$).

For *BMP4* (Table III), the AA genotype was identified in eight individuals (one from the tooth agenesis group and seven from the control group), whereas the GG genotype was found in 13 individuals (eight from the tooth agenesis group and five from the control group). The AG genotype was identified in 113 individuals (37 from the tooth agenesis group and 76 from the control group). Genotype distribution was statistically significantly different between groups ($p = 0.047$). The GG genotype was more frequent in individuals with three or more missing teeth than in the individuals belonging to the control group ($p < 0.0001$).

Discussion

Human tooth agenesis interprets complex traits, in which many genetic factors are involved in distinct phenotypes [4]. Many fundamental questions related to the etiology of tooth agenesis remain to be clarified [9]. Studies of genes involving tooth agenesis offer an insight into the genetic pathways that control the

Table III. Frequency of *BMP4* allele and genotype in tooth agenesis and control subjects.

		Alleles, *n* (%)			Genotypes, *n* (%)		
Subjects	*n*	A	G	*p*⋆	AA+AG	GG	*p*⋆
Controls	88	90 (51.1)	86 (48.9)	—	83 (94.3)	5 (5.7)	—
Tooth agenesis							
All tooth agenesis	46	39 (42.4)	53 (57.6)	0.173	38 (82.6)	8 (17.4)	*0.029*
Upper lateral incisor	22	17 (38.6)	27 (61.4)	0.137	17 (77.3)	5 (22.7)	*0.012*
Premolar	21	17 (40.5)	25 (59.5)	0.214	17 (81)	4 (19)	*0.045*
Others	6	6 (50.0)	6 (50)	0.939	5 (83.3)	1 (16.7)	0.334
Affected side							
Unilateral	20	17 (42.5)	23 (57.5)	0.324	16 (80)	4 (20)	*0.036*
Bilateral	26	22 (42.3)	30 (57.7)	0.263	22 (84.6)	4 (15.4)	0.106
Dental arch							
Maxilla	23	19 (41.3)	27 (58.7)	0.234	19 (82.6)	4 (17.4)	0.066
Mandible	17	17 (50.0)	17 (50.0)	0.903	16 (94.1)	1 (5.9)	0.973
Both	6	3 (25.0)	9 (75.0)	0.079	3 (50.0)	3 (50.0)	*0.000*

*Chi-Square test and Fisher's exact tests; control group was used as a reference; italics indicates statistical significance ($p \leq 0.05$).

development of human dentition. To our knowledge, this is the first report that investigates *TGFB3* and *BMP4* polymorphisms in human tooth agenesis.

During tooth development, the epithelium and mesenchyme interact through different groups of signaling molecules and their receptors that comprise the (TGFβ) superfamily and (BMP). The perturbation of any of these odontogenic signaling pathways can affect tooth development and may contribute to dental alteration [3,23,24].

Bmp4 has been extensively studied in the developing mouse tooth and it is thought to play an important role in the dental development [25]. Moreover, *Pax9* and *Msx1* encode transcription factors that are known to be essential for changing in odontogenic potential from the epithelium to the mesenchyme. It was suggested that these molecules play an important role in the maintenance of *Bmp4* expression in the mesenchyma, which ultimately drives morphogenesis of the dental organ [26]. In the developing mouse tooth, these transcription factors and growth factors are closely linked together and regulate each other, forming a molecular network that controls tooth formation [27]. Ogawa et al. [26] demonstrate that Pax9 is able to directly regulate *Msx1* expression and interact with MSX1 at the protein level to enhance its ability to transactivate *Bmp4* expression during tooth development. Interestingly, mutations in the *MSX1* [28–31] and *PAX9* [32–34] also cause tooth agenesis in humans.

In our study, we found that polymorphisms in *TGFB3* and *BMP4* were associated with tooth agenesis in humans and preferential associations were observed.

In the *TGFB3* analysis, we noted that differences were observed for maxillary tooth agenesis but not for mandible tooth agenesis. Interestingly, this gene was associated with another common development alteration in the maxilla, as oral cleft [35,36] and in some instances oral clefts and tooth agenesis may share the same genetic background [36,37].

Notwithstanding, the analysis of *BMP4* revealed an association when both arches were simultaneously affected. Following a similar pattern, individuals with three or more missing teeth were strongly associated with *BMP4*. We may hypothesize that *BMP4* may be preferentially involved in the more severe cases of tooth agenesis.

One additional worthwhile observation was observed. Subtle random deviations from perfect bilateral symmetry is called 'fluctuating asymmetry' and was previously observed in tooth agenesis. In a Brazilian population, unilateral cases of tooth agenesis were more common than bilateral cases [6]. We have recently proposed that distinctive genes could influence tooth agenesis laterality [6] and, by the light of the results presented here, *TGFB3* and *BMP4* were associated only with unilateral forms of tooth agenesis.

TGFB3 was preferentially associated with other types of tooth agenesis (such as lower incisors, canines and molars), whereas *BMP4* was preferentially associated with upper lateral incisors and premolars. These reflect the fact that different types of teeth have had different genetic factors, as previously suggested by Vieira [4].

In summary, our findings demonstrated that *TGFB3* (rs2268626) and *BMP4* (rs17563) are involved in tooth agenesis and will assist in understanding the development and prevention of tooth agenesis. The extent to which this polymorphism may actually contribute to the tooth agenesis status is still to be clarified. Thus, 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 tooth agenesis in different populations. Also, functional studies would help to better understand the effect of this polymorphism during odontogenesis.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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