European Review for Medical and Pharmacological Sciences 2015; 19: 2188-2194

Association of *BMP4* polymorphisms with isolated tooth agenesis in a Chinese Han population: a case-control study

M. GONG, Y.-J. QIAN, N. GU, W. WANG, H. WANG, L. MA, J.-Q. MA, W.-B. ZHANG, Y.-C. PAN, L. WANG

Jiangsu Key Laboratory of Oral Diseases, Nanjing Medical University; Department of Orthodontics, Affiliated Hospital of Stomatology, Nanjing Medical University, Nanjing, China

Miao Gong, Yajing Qian and *Ning Gu* contributed equally to the work

Abstract. – OBJECTIVE: Tooth agenesis is a common craniofacial anomaly in human beings. Mounting evidence has demonstrated that the bone morphogenetic protein 4 gene (*BMP4*) plays an important role in tooth development. This case-control study was designed to evaluate the association of the polymorphism rs17563 in *BMP4* gene with susceptibility of isolated human tooth agenesis in a Chinese Han population.

PATIENTS AND METHODS: 335 tooth agenesis cases and 444 healthy controls were included in this study.

RESULTS: Although no significant association was observed either in the overall or stratified analysis between the types and the severity of missing teeth. However, significant difference was observed between the anterior and posterior tooth agenesis (APTA) cases and the controls (p = 0.018 for allele distribution and OR = 0.39, 95% CI = 0.15-0.99). Furthermore, the heterozygote (TC) and dominant model (CC+TC) were associated with decreased risk of APTA compared with the control (p_{het} = 0.018, OR_{het} = 0.39, 95% CI_{het} = 0.15-0.99 and p_{dom} = 0.042, OR_{dom} = 0.34, 95% CI_{dom} = 0.13-0.87, respectively).

CONCLUSIONS: These results indicated that rs17563 in *BMP4* gene was potentially associated with APTA in Chinese Han population and further independent studies are required to verify these findings.

Key Words:
Tooth agenesis, *BMP4*, Single nucleotide polymorphisms, Case-control study.

Introduction

Tooth agenesis is one of the most common craniofacial anomalies with congenital lack of more than one tooth, which may contribute to the orofacial malocclusion and masticatory function disorder. Excluding the third molar, the incidence of missing teeth in permanent dentition varies from 2.6% to 11.3% depending on demographic and geographic profiles[1]. Tooth agenesis can be divided into three types according to the number of missing teeth: hypodontia (< 6 permanent teeth missing); oligodontia (> 6 permanent teeth missing); and anodontia (all permanent teeth missing). They may occur as sole anomaly (isolated or non-syndromic) or parts of multiple congenital anomalies (such as Down syndrome, ectodermal dysplasia and orofacial clefts)[2].

Development of human dentition depends on the epithelial-mesenchymal reciprocal interactions mediated by multiple signaling molecules and their receptors, such as the transforming growth factor β (*TGFβ*), fibroblast growth factors (*FGF*), the hedgehog (*Hh*) and wingless (*WNT*) families[3]. *BMP4* is an important member of the *TGFβ* superfamily and participates in the tooth development[4]. It is expressed at the particular stages of development in the specific regions of the epithelium of the nasal, as well as the maxillary and mandibular processes[5]. It is important during the bud-cap stage transition and critical in the formation of the enamel knot[6,7]. Mice with reduced *BMP* activity was characterized by the lack of mandibular molars, reduction in size of the second maxillary molars with altered crown shape and reduced number of roots[8]. In addition, in $MSX1^{-/-}$ mutant mice, reduced expression of *BMP4* was observed and tooth development was arrested at the bud stage[9], which could be rescued and restarted by addition of exogenous *BMP4*[10,11]. Furthermore, in 2012, for the

Corresponding Author: Lin Wang, MD; e-mail: lw603@njmu.edu.cn
Yongchu Pan, MD; e-mail: panyongchu@njmu.edu.cn

first time *BMP4* was found to be involved in the development of tooth agenesis in human. Two Mexican families with oligodontia were found to have *BMP4* mutations[12]. Taken together, all of these findings implicate an important role for *BMP4* in tooth development.

Single nucleotide polymorphisms (SNPs), which occur at high frequencies in the human genome, are the most common genetic variants and may affect individual's susceptibility to various diseases, including tooth agenesis. Given the important role of *BMP4* in tooth development, it is quite possible that SNPs in *BMP4* gene may also contribute to tooth agenesis. By reviewing the previously published articles, the polymorphism rs17563 in *BMP4* gene is of great interest. It is a synonymous coding SNP, which might affect the stability and transcription efficacy of mRNA[13,14]. In 2012, Antunes et al[15] found this polymorphism of *BMP4* was associated with tooth agenesis in Brazilian patients, and preferential associations with three or more missing teeth were observed. In addition, this polymorphism had also been found to be associated with the occurrence of nonsyndromic Cleft Lip and Palate[16,17], another type of congenitally anomalies probably sharing a common genetic pathway[18].

Therefore, based on all of the above studies, a hypothesis that this polymorphism in *BMP4* gene (rs17563) may also be associated with tooth agenesis in Chinese populations has been raised. To testify this hypothesis, we deliberately designed a case-control study and recruited 779 subjects (444 controls without tooth agenesis and 335 tooth agenesis cases) in the present study.

Patients and Methods

Study Subjects

This study is an ongoing hospital-based case-control study and approved by the Institutional Review Board of Nanjing Medical University. All subjects were recruited from young patients receiving orthodontic treatment from two affiliated hospitals of Nanjing Medical University: the Stomatological Hospital of Jiangsu Province and Nanjing First People's Hospital, between October 2005 and March 2014[19].

After routine blood test, we collected the rest of the blood sample for further genetic analysis in a standard way. Written informed consent was obtained from the patients enrolled or their guardians.

Tooth agenesis cases (with at least one missing permanent tooth, excluding the third molar) were identified by two dentists based on their own dental treatment records, dental examination and X rays. Exclusion criteria includes: subjects with orofacial clefts or other syndromes; missing tooth attributed to trauma or extraction; mixed dentition or early permanent dentition when the second molar could not be examined. All of the healthy controls have complete dentition (including the third molar), those with a history of dental anomaly or syndromes such as orofacial cleft were excluded.

DNA Extraction

Genomic DNA samples were extracted from peripheral blood samples and then extracted and purified through the QIAamp Spin Procedure including lysis, binding, washing and elution, as suggested by the manufacturer's instructions (QIAmp Blood kit, Qiagen, Germany). Then DNA samples were stored at -80°C for further manipulation after purity and concentration were measured.

Genotyping

SNP rs17563 (T > C) was genotyped by conventional TaqMan MGB procedure in 384-well plates and determined using Sequence Detection Software on an ABI-Prism 7900 analyzer according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Polymerase chain reaction (PCR) was performed using the primers shown in Table I. The majority of subjects were successfully genotyped with a call rate of 99.4% (5 controls and 1 case were failed to be genotyped). Genotyping results were reviewed by two independent investigators blinded to the patient's status. Among the samples, 10% were randomly selected for confirmation and the results were 100% concordant.

Statistical Analysis

Data was subsequently processed and analyzed using the Statistical Package for the Social Sciences (version 16.0, SPSS Inc., Chicago, IL,

Table I. Primers and probes used for genotyping of SNP rs17563.

Name	Sequence 5′ → 3′
Primer	F: TGCTTTTCGTTTCCTCTTTAACCT R: ACGGTGGAAGCCCCTTTC
Probe	T: FAM-CGAGGTGATCTCC C: HEX-TGAGAACGAGGCGAT

USA). Gender distributions between the cases and controls were compared using the χ^2-test. Mean age was compared by Independent-Samples *t*-test. The Hardy-Weinberg equilibrium (HWE) test was assessed among the controls by a goodness-of-fit χ^2-test. Frequencies of alleles and genotypes between the cases and controls were evaluated using the χ^2-test or Fisher's exact *t*-test, while χ^2-test risk analysis was used to obtain odds ratios (OR) and their 95% confidence intervals (95% CI).

Results

Clinical Description

The distributions of gender and age between subjects with tooth agenesis and controls are shown in Table II. There was no significant difference in gender distribution ($p = 0.602$) as well as the average age ($p = 0.295$) between the case and control group, suggesting well matches on the demographic characteristics of our study subjects.

Among all cases, a total of 586 teeth were congenitally absent, ranged from 1 to 11. The overwhelming majority of the cases were congenitally absent of 1-3 teeth (311 cases, 93.1%), followed by 4-6 teeth (17 cases, 5.1%), and then 7 or more (6 cases, 1.8%). Distribution of missing tooth in the maxillary and mandibular is shown in Table III. The mandibular incisor was the most frequently missing tooth, followed by the mandibular premolars.

SNP Analysis

4 controls and 1 case were failed to be genotyped. Eventually, 334 cases and 440 healthy controls were analyzed in the present study. The observed genotype frequencies in the control group were consistent with Hardy-Weinberg equilibrium ($p = 0.16$). The allele and genotype distributions of SNP rs17563 in the case and control groups are presented in Table IV ($p_a = 0.602$, $p_b = 0.295$, respectively).

Table II. Characteristics of tooth agenesis subjects and controls.

	Case (N = 335)	Control (N = 444)	
Variable	n (%)	n (%)	*p*
Gender			
Male	123 (36.7%)	155 (34.9%)	0.602[a]
Female	212 (63.3%)	289 (65.1%)	
Age (mean ± SD)	16.42 ± 6.57	17.04 ± 8.34	0.295[b]

[a]Chi-Square test. [b]Independent-Sample *t*-test.

Table III. Distribution of missing tooth in maxillary and mandible.

Quart	Incisor	Canine	Premolar	Molar
Maxillary	78	50	62	9
Mandibular	267	12	102	6

Table IV. Statistical analysis of rs17563 in controls and tooth agenesis subjects.

rs17563	Control (N = 440, %)	Case (N = 334, %)	OR (95% CI)[a]	*p*[b]
Genotype				
TT	228 (51.8)	183 (54.8)	1.00 (reference)	0.425
TC	185 (42.1)	126 (37.7)	0.85 (0.63-1.14)	0.281
CC	27 (6.1)	25 (7.5)	1.15 (0.65-2.06)	0.628
CC + TC	212 (48.2)	151 (45.2)	0.89 (0.67-1.18)	0.412
Allele				
T	641 (72.8)	492 (73.7)	1.00 (reference)	0.721
C	239 (27.2)	176 (26.3)	0.96 (0.76-1.20)	

[a]OR, odds ratio; 95% CI, 95% confidence interval. [b]Chi-Square test.

As shown in Table IV, the TT, TC, and CC genotypes in control group were 51.8%, 42.1%, and 6.1%, respectively. The distributions of genotypes in tooth agenesis cases were similar to those of the control groups, which were 54.8% (TT), 37.7% (TC), 7.5% (CC), respectively (p = 0.425). Neither the heterozygote (TC) nor mutated homozygote (CC) was associated with tooth agenesis susceptibility, compared with the wild-type homozygote (TT) (p_{het} = 0.281, p_{hom} = 0.628, respectively). When we combined the heterozygote and mutated homozygote together, assuming a dominant model (CC+TC), we did not observe any significant association between the variant genotype and tooth agenesis either (Table IV) (p = 0.412). Furthermore, in subgroup analysis by the type (Table V) or severity of missing tooth (Table VI), no significant association was detected either.

However, when we further divided the case group into anterior tooth agenesis (ATA) group, posterior tooth agenesis (PTA) group and both anterior and posterior tooth agenesis (APTA) group, significant difference was observed in allele distribution between APTA group and the control group (Allele comparison, p = 0.018, OR = 0.37, 95% CI = 0.15-0.87) (Table VII). The heterozygote (TC) and dominant model (CC+TC) were associated with the decreased risk of APTA compared with the wild-type homozygote (TT) (p_{het} = 0.042, OR_{het} = 0.39, 95% CI_{het} = 0.15-0.99; p_{dom} = 0.018, OR_{dom} = 0.34, 95% CI_{dom} = 0.13-0.87).

Discussion

Tooth agenesis is a kind of polygenic diseases. To date, genetic variations in the *MSXI*, *PAX9*, *AXIN2*, *EDA*, *EDARADD* and *WNT10A* genes have been identified to be associated with tooth agenesis[20-28]. *BMP4* mediates epithelial-mesenchymal interactions during early tooth development[29]. As an important signal mediator in the network of tooth development, *BMP4* can interact with multiple growth factors, including *MSX1*, *PAX9*, *OSR2* and *TBX2* to regulate tooth development[9,30-33]. Recently, a research group found that the mutations of *BMP4* gene associated with non-syndromic hypodontia, which further implicates the important role of *BMP4* gene in the occurrence of tooth agenesis among Chinese polulations[34].

Table V. Stratified analysis by the types of missing tooth.

rs17563 (T > C)		Type of tooth agenesis	TT (%)	CC (%)	TC (%)	p^*_{hom}	p^*_{het}	p^*_{dom}	T (%)	C (%)	OR (95% CI)[a]	p^*
Control			228 (51.8)	27 (6.1)	185 (42.1)				641 (72.8)	239 (27.2)	1.00 (refrence)	
Case	U	Incisor	36 (63.6)	3 (5.5)	17 (30.9)	0.779	0.079	0.078	87 (79.1)	23 (20.9)	0.71 (0.44-1.15)	0.161
		Canine	21 (65.6)	0 (0)	11 (34.4)	–	0.253	0.131	53 (82.8)	11 (17.2)	0.56 (0.29-1.08)	0.081
		Premolar	21 (50.0)	2 (4.8)	19 (45.2)	1.000	0.743	0.822	61 (72.6)	23 (27.4)	1.01 (0.61-1.67)	0.965
		Molar	3 (60.0)	0 (0)	2 (40.0)	–	1.000	1.000	8 (80.0)	2 (20.0)	0.67 (0.14-3.18)	1.000
	L	Incisor	110 (56.7)	17 (8.8)	67 (34.5)	0.420	0.118	0.256	287 (74.0)	101 (26.0)	0.94 (0.72-1.24)	0.676
		Canine	5 (55.6)	0 (0)	4 (44.4)	-	1.000	1.000	14 (77.8)	4 (22.2)	0.77 (0.25-2.35)	0.792
		Premolar	36 (52.2)	5 (7.2)	28 (40.6)	0.758	0.876	0.956	100 (72.5)	38 (27.5)	1.02 (0.68-1.52)	0.926
		Molar	2 (50.0)	0 (0)	2 (50.0)	–	1.000	1.000	6 (0.75)	2 (0.25)	0.89 (0.18-4.46)	1.000
		UTA	43 (58.1)	3 (4.1)	28 (37.8)	0.397	0.401	0.316	114 (77.0)	34 (23.0)	0.80 (0.53-1.21)	0.286
		LTA	118 (53.9)	20 (9.1)	81 (37.0)	0.255	0.339	0.617	317 (72.4)	121 (27.6)	1.02 (0.79-1.32)	0.858
		ULTA	22 (53.7)	2 (4.9)	17 (41.4)	1.000	0.885	0.822	61 (74.4)	21 (25.6)	0.92 (0.55-1.55)	0.763

[a]OR: odds ratio; CI: confidence interval; *Chi-Square test or Fisher's Exact Test; control group was used as a reference. U: Upper dentition; L: Lower dentition; UTA: Upper tooth agenesis; LTA: Lower tooth agenesis; ULTA: Upper and Lower tooth agenesis.

Table VI. Statistical analysis by severity of missing tooth.

rs17563 (T > C)	1≤ N*≤ 3 (n = 311)	N* > 3 (n = 23)	Control (n = 440)
Genotype			
TT	169 (54.3 %)	14 (52.9 %)	228 (51.8 %)
TC	117 (37.6 %)	9 (47.1 %)	185 (42.0 %)
CC	25 (8.1 %)	0 (0)	27 (6.2 %)
χ^2 (p, df = 2)	2.059 (0.357)	1.798 (0.407)	
Odds ratio (95% CI) for comparison			
TC vs. TT	0.85 (0.63-1.16)	0.79 (0.34-1.87)	
CC vs. TT	1.25 (0.70-2.23)	–	
CC + TC vs. TT	0.90 (0.68-1.21)	0.69 (0.29-1.63)"	
Allele			
T	455 (73.2 %)	37 (80.4 %)	641 (72.8 %)
C	167 (26.8 %)	9 (19.6 %)	239 (27.2 %)
χ^2 (p, df = 1)	0.018 (0.894)	1.286 (0.257)	

N*: number of tooth agenesis; 95% CI: 95% confidence interval.

Antunes et al[15] found that polymorphism in *BMP4* (rs17563) contributed to tooth agenesis in a Brazilian population. In their study, 46 individuals with tooth agenesis and 88 controls were evaluated using a case-control design. Genotypes were significantly different between the groups (p = 0.047), as the CC genotype occured more frequently in the individuals with 3 or more missing teeth compared with the control group (p < 0.0001). Although their study implicated the association between rs17563 and tooth agenesis susceptibility, two major drawbacks of these two studies should be mentioned. First of all, the studies were conducted based on a relatively small sample size, their statistical power were thereby limited. Secondly, as stratified statistical analysis was not performed, it could not be determined which specific type of missing tooth was associated with *BMP4* (rs17563).

Therefore, in our study, a relative larger sample size with 335 tooth agenesis cases and 444 controls was designed to replicate this association in a Chinese population, and we also conducted stratified analysis to find the possible associations amng the subgroups. No significant association was observed either in the overall or stratified analysis between the types and the severity of missing teeth. However, significant difference was observed between the anterior and posterior tooth agenesis (APTA) cases and con-

Table VII. Statistical analysis by anterior and posterior agenesis in cases and controls.

rs17563 (T > C)	ATA (n = 243)	PTA (n = 67)	APTA (n = 25)	Control (n = 440)
Genotype				
TT	134 (55.1 %)	30 (44.8 %)	19 (76 %)	228 (51.8 %)
TC	88 (36.2 %)	32 (47.8 %)	6 (24 %)	185 (42.0 %)
CC	20 (8.2 %)	5 (7.4 %)	0 (0)	27 (6.2 %)
χ^2 (p, df = 2)	2.657 (0.265)	1.173 (0.556)	–	
Odds ratio (95% CI)				
TC vs. TT	0.81 (0.58-1.13)	1.32 (0.77-2.24)	**0.39 (0.15-0.99)**	
CC vs. TT	1.26 (0.68-2.33)	1.41 (0.50-3.93)	–	
CC + TC vs. TT	0.87 (0.63-1.19)	1.33 (0.79-2. 22)	**0.34 (0.13-0.87)**	
Allele				
T	356 (73.6 %)	92 (68.7%)	44 (88.0 %)	641 (72.8 %)
C	128 (26.4 %)	42 (31.3 %)	6 (12.0 %)	239 (27.2 %)
χ^2 (p, df = 1)	0.081 (0.776)	1.016 (0.313)	**5.603 (0.018)**	
Odds ratio (95% CI)				
C vs. T	0.94 (0.75-1.24)	1.22 (0.83-1.82)	**0.37 (0.15-0.87)**	

ATA: anterior tooth agenesis; PTA: posterior tooth agenesis; APTA: anterior and posterior tooth agenesis; 95% CI: 95% confidence interval.

trols. The heterozygote (TC) and dominant model (CC+TC) were associated with the decreased risk of APTA compared with the wild-type homozygote (TT), suggesting that the C allele might be protective against tooth agenesis. These findings indicate that *BMP4* (rs17563) may modify individual's susceptibility of APTA in Chinese populations.

Conclusions

Our study showed that *BMP4* (rs17563) was associated with APTA in a Chinese Han population. In the future, further studies are required to verify our findings.

Acknowledgements

We gratefully appreciated the support of the patients and dentists who participated in this study. This study was also supported in part by the National Natural Science Foundation of China (81170981,81230022 and 81200808), Ph.D. Programs Foundation of Ministry of Education of China (20113234110003), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD 2014-37), the natural science foundation of Jiangsu Province (BK2012447) and China Post doctoral Science Foundation (2012M521101).

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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