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The role of polymorphisms associated with early tooth eruption in dental and occlusal traits in East Asian populations

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Objective: A recent study suggested that rs6504340, a polymorphism within the homeobox B (*HOXB*) gene cluster, is associated with the susceptibility for malocclusions in Europeans. The resulting malocclusions require orthodontic treatment. The aim of this study was to investigate the association of rs6504340 and other dentition-implicated polymorphisms with dental and occlusal traits in Korean and Japanese populations. **Methods:** The study participants included 223 unrelated Koreans from the Busan area and 256 unrelated Japanese individuals from the Tokyo metropolitan area. DNA samples were extracted from saliva specimens. Genotyping for rs6504340 and four single nucleotide polymorphisms (SNPs) that have been shown to be associated with the timing of first tooth eruption and the number of teeth at 1 year of age (rs10506525, rs1956529, rs9674544, and rs8079702) was performed using TaqMan assays. The Index of Orthodontic Treatment Need (IOTN), overjet, overbite, arch length discrepancy, crown sizes, and length and width of the dental arches were measured. Spearman's correlation coefficients were calculated to evaluate relationships between rs6504340 and these dental/occlusal traits. **Results:** We evaluated the aesthetic components and dental health components of the IOTN in the Korean and Japanese populations and found that neither rs6504340 nor the other four SNPs showed any association with dental and occlusal traits in these East Asian populations. **Conclusions:** These negative results suggest that further research is needed to identify the genetic determinants of malocclusions in order to reach a consensus.
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INTRODUCTION

Malocclusions, such as crowding (arch length discrepancy), are a dental health issue of global significance. Surveys conducted by the United States Public Health Service in the 1960s on a sample of 8,000 people found that 40% of children aged 6–11 years and 85% of youths aged 12–17 years had experienced dental crowding. Issues with crowding and irregularity also continued into adulthood.[1] Crowding is the most prevalent component of malocclusions in dental patients.

Genetic factors are suggested to contribute to the etiology of crowding.[2] A recent study provided evidence for associations between the genes *EDA* and *XEDAR* and dental crowding.[3] Class III malocclusions (negative overjet) have also been the focus of human genetic linkage analyses[4-7] and association studies.[8-11] In addition, several studies have provided increasing evidence for associations between genetic factors and common dental traits, such as tooth size.[12-14]

Homeobox (*HOX*) genes regulate normal development and cell differentiation, in addition to controlling other cellular processes.[15] The *HOX* network determines the phenotypic identity of epithelial and ectomesenchymal cells that interact in the generation of human tooth germs.[15] Moreover, *HOX* genes are important components of an ancient dental gene-regulatory circuit and can be used to pinpoint subsequent modifications of this gene network that accompanied the evolution of toothed jaws.[16] Recently, Pillas et al.[17] conducted a general population-based genome-wide association study (GWAS) among European populations and reported that several single nucleotide polymorphisms (SNPs) were associated with the timing of first tooth eruption and the number of teeth present at 1 year of age. Among these polymorphisms, a SNP within the homeobox B (*HOXB*) gene cluster, rs6504340, also had an association with malocclusions requiring orthodontic treatment in Europeans. The prevalence and distribution of malocclusions vary between different populations. Therefore, further studies on other populations are required to confirm the applicability of this association with malocclusions requiring orthodontic treatment to other ethnic groups.[18]

In the present study, rs6504340 in the *HOXB* gene cluster and four SNPs (rs10506525, rs1956529, rs9674544, and rs8079702) that have been shown to be associated with the timing of first tooth eruption and the number of teeth at 1 year of age were genotyped in Japanese and Korean populations, and the potential correlations with dental/occlusal traits were evaluated. The results of the present study contribute to understanding of the genetic contribution of these polymorphisms to malocclusions.

MATERIALS AND METHODS

Study participants

Informed consent was obtained from all individuals who participated in the study, and ethical approval was obtained from the ethics committees of Showa University, Pusan National University, and the University of the Ryukyus.

The study participants included 256 unrelated Japanese individuals who were from the Tokyo metropolitan area and had attended the orthodontic clinics of Showa University Dental Hospital and 223 unrelated Korean healthy volunteers from the Busan area. Individuals with congenital diseases, such as ectodermal dysplasia, cleft lip or palate, and Down syndrome were excluded from the study. The Japanese subjects were patients who visited orthodontic clinics for orthodontic treatment, and therefore, these individuals might not be considered members of the general population with regard to dentition. Patients who had undergone orthodontic treatment before coming to our clinic were also excluded.

Determination of dental and occlusal traits

Plaster casts of the permanent dentition were collected from the Korean and Japanese individuals. The dentitions of the individuals were evaluated according to the two components of the Index of Orthodontic Treatment Need (IOTN): the dental health component (DHC)[19] and the aesthetic component (AC).[20]

The degrees of overjet and overbite and the length (L) and width (W) of the maxillary and mandibular dental arches (L_{MAXI}, W_{MAXI}, L_{MAND}, and W_{MAND}) were measured with a sliding caliper. The crown sizes were evaluated as described previously.[13] The crown diameters in the buccolingual (BL) and mesiodistal (MD) directions were measured for all teeth, except for the third molars. The geometric means (GMs) of the MD and BL diameters were calculated as another index of crown size. After removal of abnormal, outlier values (≥3SD or ≤3SD), the values for the left and right homologous teeth (e.g., left and right canines) were averaged, unless there was only a single standing tooth available for measurement. If the teeth on both sides were absent, the value was considered missing. The space available in the dental arch was assessed by measuring the arch length (AL) from the left second premolar to the right second premolar (AL[5-5]). Arch length discrepancy (ALD) in the maxilla and the mandible (ALD_{MAXI} and ALD_{MAND}) were calculated by subtracting the sum of the MD diameters of each tooth in the arch (between and including the left and right second premolars) from the arch length.[21]

To correct for sex-based differences in the metric measurements, including the MD, BL, and GM diameters of each tooth and the L_{MAXI}, W_{MAXI}, L_{MAND}, and W_{MAND}, we standardized the data into Z-scores for each sex in each population and then merged the Z-scores of the male and female individuals.[13] The standardized values of the crown sizes and diameters for all teeth in each individual were averaged for collective evaluation (average Z[MD], average Z[BL], and average Z[GM]).

SNP genotyping

DNA samples were extracted from saliva specimens using standard methods. We genotyped rs6504340 in the *HOXB* gene cluster, which was suggested by Pillas et al.,[17] to be associated with the number of teeth present at 1 year of age and with malocclusions requiring orthodontic treatment in Europeans.

In addition, Pillas et al.[17] reported six SNPs showing association, at the genome-wide significance level, with the timing of first tooth eruption and/or the number of teeth present at 1 year of age. Though occlusion status in individuals with mixed dentition is influenced by several factors, it is predominantly determined by the primary dentition[22] and as teeth largely acquire their final form at a very early age,[23] we selected four of these SNPs (rs8079702 near *KCNJ2*, rs10506525 in *MSRB3*, rs9674544 in *IGF2BP1*, and rs1956529 in *RAD5IL1*), which are polymorphic in the Japanese and Han Chinese populations according to the HapMap database (http://www.hapmap.org). The two remaining SNPs in *EDA* on the X-chromosome are monomorphic in these Asian populations and were therefore rejected from the analysis. Genotyping was performed using TaqMan assays (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Deviation of the observed genotype distribution from the Hardy–Weinberg equilibrium was tested using the chi-square test. The chi-square test and odds ratio were used to test the association of the SNPs with malocclusions, where the grades of AC were dichotomized into AC ≤ 4 and AC > 4. The correlation

Table 1. Evaluation of occlusion status parameters

	Korean						Japanese					
	Male			Female			Male			Female		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
AC (grades 1–10)	3.75	2.05	130	3.73	1.89	93	6.19	2.50	43	6.30	2.46	172
DHC (grades 1–5)	2.72	1.11	130	2.58	1.04	93	3.60	1.16	43	3.74	1.06	172
Overjet (mm)	2.67	1.72	130	2.51	1.46	93	1.92	4.37	45	1.87	4.33	164
Overbite (mm)	2.28	1.64	130	1.83	1.48	93	0.60	2.30	45	0.98	2.17	164
ALD_{MAXI} (mm)	–4.13	3.19	111	–3.88	2.66	75	–3.39	5.03	50	–6.45	6.52	200
ALD_{MAND} (mm)	–2.53	3.23	113	–2.90	3.24	73	–2.74	6.30	47	0.76	7.08	204

AC, Aesthetic component; DHC, dental health component; ALD_{MAXI}, arch length discrepancy (maxilla); ALD_{MAND}, arch length discrepancy (mandible); SD, standard deviation.

Table 2. Summary information for the single nucleotide polymorphisms (SNPs) evaluated in this study

SNP ID	Chr	Position	Gene(s)	Location	Alleles		Frequency in Koreans		Frequency in Japanese	
					A/D*	Effective[†]	A	D	A	D
rs6504340	17	43972018	*HOXB3*; *HOXB1*; *HOXB2*	Intergenic	A/G	G	0.042	0.958	0.040	0.960
rs10506525	12	64069645	*MSRB3*	Intron	A/G	G	0.423	0.577	0.425	0.575
rs1956529	14	67858677	*RAD51L1*	Intron	G/A	A	0.627	0.373	0.540	0.460
rs9674544	17	44439710	*IGF2BP1*	Intron	A/G	G	0.217	0.783	0.213	0.787
rs8079702	17	65702421	near *KCNJ2*	Intergenic	A/G	G	0.619	0.381	0.559	0.441

*The ancestral (A) and derived (D) alleles are shown by nucleotides.
[†]The effective allele denotes the allele positively associated with later eruption or smaller number of teeth during infancy in a previous study.
Chr, Chromosome.

Table 3. Association of the single nucleotide polymorphisms with the aesthetic component (AC) examined in this study

		Korean subjects							Japanese subjects						
		Genotype frequencies*			Chi² p-value	Allele frequencies*		OR (95% CI)	Genotype frequencies*			Chi² p-value	Allele frequencies*		OR (95% CI)
		AA	AD	DD		A (%)	D (%)		AA	AD	DD		A (%)	D (%)	
rs6504340 (*HOXB*)	AC ≤ 4	1	12	134		14 (4.8)	280 (95.2)	1.56	1	5	64		7 (5.0)	133 (95.0)	1.60
	AC > 4	0	4	62	0.685	4 (3.0)	128 (97.0)	(0.57–4.28)	0	9	129	0.364	9 (3.3)	267 (96.7)	(0.52–4.96)
rs10506525 (MSRB3)	AC ≤ 4	26	75	53		127 (41.2)	181 (58.8)	0.94	16	28	28		60 (41.7)	84 (58.3)	0.86
	AC > 4	16	29	23	0.478	61 (44.9)	75 (55.1)	(0.62–1.41)	28	65	47	0.574	121 (43.2)	159 (56.8)	(0.57–1.30)
rs1956529 (RAD51L1)	AC ≤ 4	63	66	21		192 (64.0)	108 (36.0)	1.48	29	29	14		87 (60.4)	57 (39.6)	1.20
	AC > 4	22	36	9	0.380	80 (59.7)	54 (40.3)	(0.98–2.23)	34	74	32	0.052	142 (50.7)	138 (49.3)	(0.79–1.82)
rs9674544 (IGF2BP1)	AC ≤ 4	6	55	89		67 (22.3)	233 (77.7)	1.09	4	24	44		32 (22.2)	112 (77.8)	1.14
	AC > 4	4	19	44	0.442	27 (20.1)	107 (79.9)	(0.67–1.77)	14	31	97	0.143	59 (20.8)	225 (79.2)	(0.69–1.88)
rs8079702 (near KCNJ2)	AC ≤ 4	59	66	30		184 (59.4)	126 (40.6)	0.94	21	36	15		78 (54.2)	66 (45.8)	0.70
	AC > 4	35	22	11	0.171	92 (67.6)	44 (32.4)	(0.63–1.41)	43	73	27	0.943	159 (55.6)	127 (44.4)	(0.46–1.07)

*A and D denote the ancestral and derived alleles, respectively.
OR, Odds ratio; CI, confidence interval.

between genotype (i.e., the count of the derived allele) and phenotype was also examined using Spearman's rank correlation coefficient in each population. A meta-analysis was subsequently performed to combine the correlation analyses in these two populations. The statistical significance of the correlation was evaluated while controlling for the false discovery rate at the 0.05 level using the Benjamini–Hochberg method.

RESULTS

To evaluate occlusal traits, we determined the DHC, AC, overjet, overbite, ALD_{MAXI}, and ALD_{MAND} parameters (Table 1). The Japanese individuals, which are orthodontic patients, had higher AC and DHC scores than the Korean healthy volunteers.

The selected SNPs were genotyped successfully. Table 2 shows the allele frequencies of the examined SNPs in the Korean and Japanese individuals, and demonstrates that the frequencies were very similar between the two populations. However, the genotypic frequency of one SNP, rs9674544, deviated significantly from the Hardy–Weinberg equilibrium ($p = 0.00046$) with an excess of homozygotes only in the Japanese subjects.

To examine the association of the SNPs with malocclusions, the grades of AC were dichotomized (AC ≤ 4 and AC > 4). Then, chi-square tests for genotypic frequencies were performed and odds ratios for alleles were calculated (Table 3). We did not detect any significant association between rs6504340 and malocclusions or between the other SNPs and malocclusions. The genotypic frequencies of rs9674544 in the Japanese subjects with AC > 4 deviated significantly from the Hardy–Weinberg equilibrium ($p = 0.00006$), but not in those with AC ≤ 4 ($p = 0.76$).

Spearman's correlation coefficients were calculated and statistically analyzed, but did not reveal any relationships between the rs6504340 polymorphism and dental/occlusal traits (Table 4). The rs1956529 polymorphism showed a trend toward a positive association ($p < 0.05$) with the DHC in the meta-analysis. However, after controlling the false discovery rate using the Benjamini–Hochberg method to correct for multiple SNP testing, this correlation was not significant.

DISCUSSION

A previous study suggested that the rs6504340 polymorphism within the *HOXB* gene cluster is associated not only with the number of teeth present at 1 year of age, but also with the need for corrective orthodontic treatment for malocclusions (odds ratio = 1.35).[17] Since malocclusions are a complex phenotype caused by multiple genetic and environmental factors, it

Table 4. Spearman's rank correlation coefficients between the single nucleotide polymorphisms and dental/occlusal traits

Dental/occlusal traits	rs6504340		rs10506525		rs1956529		rs9674544		rs8079702	
	Rho	*p*-value*	Rho	*p*-value*	Rho	*p*-value*	Rho	*p*-value*	Rho	*p*-value*
AC (grades 1–10)	0.057	0.244	–0.040	0.409	0.084	0.084	0.069	0.155	–0.031	0.520
DHC (grades 1–5)	–0.002	0.970	–0.034	0.479	0.099	0.041[†]	0.081	0.093	–0.005	0.912
Overjet (mm)	–0.037	0.459	–0.056	0.255	–0.013	0.789	–0.021	0.667	–0.030	0.532
Overbite (mm)	–0.012	0.803	–0.017	0.725	–0.044	0.366	0.036	0.465	–0.052	0.279
ALD_{MAXI} (mm)	0.058	0.253	0.037	0.462	–0.001	0.983	–0.051	0.310	–0.029	0.555
ALD_{MAND} (mm)	0.076	0.119	0.010	0.829	–0.017	0.728	0.061	0.204	0.038	0.430
Z (L_{MAXI})	0.038	0.424	–0.055	0.245	–0.028	0.562	–0.018	0.711	0.041	0.380
Z (W_{MAXI})	–0.037	0.451	–0.010	0.840	–0.021	0.661	0.012	0.800	–0.029	0.544
Z (L_{MAND})	0.076	0.106	–0.071	0.127	0.012	0.795	–0.035	0.450	0.055	0.237
Z (W_{MAND})	0.014	0.771	–0.081	0.087	0.041	0.388	0.042	0.373	0.028	0.557
Avg. Z (GM)	0.017	0.712	–0.057	0.395	–0.009	0.900	0.009	0.591	–0.048	0.306
Avg. Z (MD)	–0.021	0.648	–0.079	0.088	0.008	0.858	–0.002	0.970	–0.024	0.608
Avg. Z (BL)	0.066	0.162	–0.060	0.198	–0.016	0.733	0.027	0.556	–0.034	0.464

Rho denotes the Spearman's correlation coefficient combined between Korean and Japanese subjects by meta-analysis.
*p-value for the correlation.
Genotypes for a SNP are represented by the number of the derived alleles (D): 0, 1, or 2.
[†]$0.01 \leq p\text{-value} < 0.05$.
AC, Aesthetic component; DHC, dental health component; ALD_{MAXI}, arch length discrepancy (maxilla); ALD_{MAND}, arch length discrepancy (mandible); Z, Z score; Avg., average; L_{MAXI}, arch length (maxilla); W_{MAXI}, arch width (maxilla); L_{MAND}, arch length (mandible); W_{MAND}, arch width (mandible); GM, geometric mean of diameters; MD, mesiodistal diameter; BL, buccolingual diameter.

is essential to obtain more data on different populations to elucidate the role of rs6504340 and other factors in dental and occlusal traits. In the present study, however, we did not obtain any significant associations between this SNP and dental/occlusal traits. Since our sample size was small, this study has limited statistical power. The odds ratio (95% confidence interval) was 1.56 (0.57–4.28) in the Japanese subjects and 1.60 (0.52–4.96) in the Korean subjects. These effect sizes were comparable to those observed in the previous study.

Examination of the allele frequencies for rs6504340 in the Korean and Japanese individuals demonstrated that the frequencies were very low in both populations (ancestral allele frequencies: Korean, 4.2%; Japanese, 4.0%). The lack of evidence for association could therefore also be attributable to the low frequencies of the rs6504340 "G" allele in the East Asian populations compared with those in the European populations (20.9–22.4%),[17] as statistical power decreases when the minor allele frequency is low. In addition, the difference in allele frequencies indicates heterogeneity between Asians and Europeans in the genetic factors influencing

dental traits. To confirm the association of rs6504340 to malocclusions, studies with more extensive data are required.

A study reported that there were no significant differences in the IOTN grades of adult male individuals (army recruits) among Chinese, Malay, and Indian groups, with an average DHC of 3.3.[24] The Japanese individuals in the current study were orthodontic patients and thus showed higher mean DHC scores (3.60 for male individuals, 3.74 for female individuals), whereas the Koreans were healthy volunteers (mean DHC scores: 2.72 for male individuals, 2.58 for female individuals).

The genotypic distribution of rs9674544 in the Japanese subjects did not conform to the Hardy-Weinberg equilibrium with an excess of homozygotes. However, when the Japanese subjects were divided into two groups according to the grades of AC, only those with AC > 4 strongly deviated from Hardy–Weinberg equilibrium. It could be speculated that the deviation from the Hardy–Weinberg equilibrium observed for rs9674544 may result from the presence of a deletion polymorphism or a SNP affecting the results of the

Taqman assay and that such a polymorphism may be more frequent in individuals with malocclusions. To validate this speculation, a further sequencing study on the *IGF2BP1* region will be required.

The features of the primary occlusion may improve or worsen as an individual moves from the primary to the permanent dentition. These features include the following: MD crown diameter of unerupted teeth; the relationship between the crown size of permanent teeth and their deciduous predecessors; the direction of tooth eruption, as well as its sequence and timing; the rhythm and direction of jaw development; the development of the alveolar processes; and the balance within the masticatory musculature.[25] The occlusion status in individuals with mixed dentitions is influenced by several factors, but is predominantly determined by the primary dentition.[22] Teeth largely acquire their final form at a very early age.[23] Dental traits, such as tooth shoveling, show marked regional differences, being frequent in Asia, yet rare or absent in Africa and Europe. Likewise, crowding is more often found in European children than in African children, and its incidence has recently increased in Europeans, while this trend is not consistent in Africans.[26] Korean, Japanese, and Taiwanese Chinese populations consistently exhibit a high prevalence of crowding.[27] The heterogeneity of genetic factors influencing dental and occlusal traits between Asian and European populations may lead to inconsistencies in results.

The genetic factors influencing a number of dental traits should still be explored. Such further studies will provide knowledge about the associations between genotypes and phenotypes of malocclusions and dental traits[12-14]; however, a larger number of participants is needed to define the real role of these genes in malocclusions.

CONCLUSION

In summary, to our knowledge, the present study is the first to investigate the association between the rs6504340 polymorphism in the *HOXB* gene cluster and dental/occlusal traits in East Asian populations. The negative results obtained in the present study, in combination with the evidence available in the literature, suggest that there is still no consensus on the influence of this polymorphism, and that more studies should be performed.

ACKNOWLEDGMENTS

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