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ORIGINAL ARTICLE

Association of common *PAX9* variants with permanent tooth size variation in non-syndromic East Asian populations

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Studies on the heredity of dental characteristics in humans have indicated that the variance in many dental traits results from genetic variation. However, the genetic factors that influence commonly occurring dental variants are poorly understood. *Paired domain box 9* (*PAX9*) codes a transcription factor that is important in tooth development. We investigated whether *PAX9* polymorphisms are associated with normal variations in tooth agenesis and morphology. The study subjects were 273 Japanese and 223 Korean adults. Single-nucleotide polymorphisms (SNPs) in *PAX9* (rs2295222, rs4904155, rs2073244, rs12881240 and rs4904210) were genotyped, and third molar agenesis and mesiodistal and buccolingual diameters were measured. We found that four of the five SNPs were significantly associated with the crown size. However, no SNP was associated with third molar agenesis. In additional analyses on non-metric dental traits, we found significant associations of *PAX9* SNPs with shoveling of upper first incisors. In summary, common variants in *PAX9* contributed to morphological variation in permanent teeth in humans.

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INTRODUCTION

Mammalian dentition is a segmented system, organized as a series of homologous elements that share a similar structure, but have differences in shape and size.[1] Recent findings on molecular aspects of odontogenesis indicate that the development of teeth is under strict genetic control. This genetic system determines the positions, numbers and shapes of different types of teeth.[2] Many proteins can have different functions during the various processes of organogenesis, during the development of the different kinds of teeth, or during the development of primary and permanent dentitions.[3] Genetic linkage and molecular biology studies have allowed the identification of mutations responsible for some patterns of syndromic and non-syndromic tooth agenesis and coincidently occurring alterations in shape, size and position of the other remaining teeth.[2,4–7] The discovery of several genetic mutations has verified that certain genes have key roles in the development of dentition; the genes that encode the transcription factors, MSX1, PAX9 and PITX2, the signaling protein, EDA, and its receptor, EDAR, are among these key genes.[8,9]

PAX9 is located on chromosome 14 and belongs to the *PAX* gene family. These genes encode transcription factors that have roles in early development. PAX proteins are defined by the presence of a specific type of DNA-binding domain, the 'paired-domain'.[10,11] Roles of *PAX9* and its homologs have been indicated by messenger RNA and protein expression patterns, by the phenotype of transgenic mice lacking copies of the mouse homolog, and by phenotypes associated with *PAX9* mutations in humans. *Pax9* expression in mice is highly specific during embryogenesis; it is expressed in derivatives of the foregut endoderm, somites, limb mesenchyme, midbrain and the cephalic neural crest.[12] In the dental mesenchyme, *Pax9* is expressed before the first morphological manifestation of odontogenesis.[13] As in *Msx1*-knockout mice, tooth development in the embryos of homozygous *Pax9*-deficient mice is arrested at the bud stage, indicating that tooth development beyond this stage requires *Pax9* expression.[12,14] In humans, dominant mutations in *PAX9* have been identified as a cause of congenital absence of some posterior (and occasionally anterior) teeth. Frame-shift,[15–17] insertion,[18,19]

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missense[18] and non-sense[20,21] mutations, as well as whole-gene deletion,[18,22,23] have been described in families exhibiting hypodontia, primarily with absence of molar teeth. In addition, it has been shown that common polymorphisms in *PAX9* are associated with 3rd molar (M3) agenesis.[24]

The genetic origins of common dental variations, unlike those of dental anomalies, are poorly understood. While a common non-synonymous variant in *EDAR* (Val370Ala) is associated with normal variation in dental traits, such as crown size, shoveling and double shoveling of incisors,[25,26] this *EDAR* polymorphism alone cannot account for the degree of heritability of such dental traits. Common variations in dental traits are thought to be controlled by multiple factors. Reportedly, tooth agenesis is associated with the size of the extant teeth,[27,28] therefore, it is reasonable to suppose that common polymorphisms in *PAX9* contribute to variation in tooth size. The aim of this study was to determine whether polymorphisms in *PAX9* are associated with dental variation, especially variation in third molar agenesis and crown size.

MATERIALS AND METHODS

Study subjects

As described in a previous report,[26] plaster casts of permanent dentition and blood, buccal mucosa or saliva specimens for DNA preparation were obtained from 223 Korean individuals at Pusan National University, Pusan, and from 273 Japanese individuals at Showa University Dental Hospital, Tokyo. The Korean subjects were 130 male and 93 female healthy individuals with 20–40 years of age (mean: 28.1). The Japanese subjects included 61 male and 212 female individuals with 12–57 years of age (mean: 24.2), who underwent orthodontic treatments. Therefore, these Japanese individuals are not to be considered members of the general population with regard to dentition although no individual with oligodontia was included. All the subjects gave informed consent for their participation in this study. This study was conducted with the approval of Ethical Committees of Pusan National University, Showa University and University of the Ryukyus.

Analysis of tooth agenesis and morphology

M3 agenesis was examined only in the Korean subjects. These subjects were asked about their experience of extraction of M3 and evaluated for congenital M3 agenesis using dental casts and panoramic radiographs.

The detailed methods for the measurement of metric traits and the determination of non-metric traits were explained previously.[26] In the Korean and Japanese subjects, the mesiodistal (MD) and buccolingual (BL) crown diameters of upper (U) and lower (L) teeth, that is, 1st and 2nd incisors (I1 and I2), canines (C), 1st and 2nd premolars (P1 and P2), and 1st and 2nd molars (M1 and M2), except for M3, were measured with a sliding caliper. The geometric mean (GM) of the MD and BL diameters was calculated for each tooth and used as an index of crown size. The length and width of the maxillary and mandibular dental arches were also measured. To correct for differences between the sexes and between populations, we standardized the metric data into *z* scores for each sex in each population, and then merged the scores of males and females. The *z* scores of MD and BL diameters and GM for each individual were averaged to evaluate a measure of crown size for all teeth and that for subsets of teeth, that is, upper/lower and anterior (I1, I2, and C)/posterior (P1, P2, M1, and M2). We also evaluated 10 non-metric dental traits (shoveling and double shoveling in UI1, tuberculum in UI1, UI2 and UC, hypocone in UM2, Carabelli's cusp in UM1 and UM2, and hypoconulid and groove pattern in LM2), as previously reported.[26]

SNP genotyping

DNA samples were prepared from blood, buccal mucosa or saliva specimens using standard methods. We genotyped five single-nucleotide polymorphisms (SNPs; rs2295222 in the 5′-flanking region; rs4904155 in the 5′-untranslated region; rs2073244 in intron 1; and rs12881240 and rs4904210 in exon 4) that are located in the *PAX9* region by using the Digitag2 assay,[29] the Taqman genotyping assay or the PCR-direct sequencing method (Table 1). The two SNPs in exon 4, rs12881240 and rs4904210, are synonymous and non-synonymous, respectively, and these SNPs are adjacent nucleotide sites. The χ^2-test was used to identify any deviation from Hardy–Weinberg equilibrium in genotype distributions. Haplotype frequencies were estimated using PHASE,[30] and the linkage disequilibrium (LD) plot was drawn using Haploview.[31]

Association analyses

The odds ratio in each locus was calculated to examine the effect of a copy of the derived allele (D) on lacking one or more M3. Phenotype–phenotype and genotype–phenotype association analyses were also performed by testing the significance of correlation; the Pearson's correlation coefficient (*r*) was used for metric traits and Spearman's rank correlation coefficient (rho) was used for non-metric traits. The genotypes were denoted by the number of copies of the derived allele, that is, 0: homozygotes for the ancestral allele (AA), 1: heterozygotes (AD) and 2: homozygotes for the derived allele (DD) in the additive (codominant) model; 0: AA, and 1: AD and DD in the dominant model; 0: AA and AD, and 1: DD in the recessive model. These statistical analyses were performed separately for the Korean and Japanese subjects. Then, the outcomes from the two populations were combined in meta-analyses, where the correlation coefficients were converted using Fisher's r-to-z transformation and then a weighted average by the inverse variance was calculated.[32] The significance level was set at 0.05, unless otherwise stated. To correct for multiple SNP testing, the false discovery rate was controlled at a level of 0.05 using the Benjamini and Hochberg method.[33] We also performed the permutation test for multiple testing of SNPs as they are in LD. Merged

Table 1 Genotype and allele frequencies for SNPs in the *PAX9* region in the Korean and Japanese subjects

								Genotype frequency			*Allele frequency (%)*	
Population	*SNP ID*	*Chr: Position*	*Location*	*Ancestral (A)*	*Derived (D)*	*Amino acid*	*Call*	*AA*	*AD*	*DD*	*A*	*D*
Korean	rs2295222	Chr14: 37126308	5′-flanking	C	A		222	81	117	24	62.8	37.2
	rs4904155	Chr14: 37127044	5′-UTR	G	C		223	56	123	44	52.7	47.3
	rs2073244	Chr14: 37129874	Intron 1	A	G		221	44	121	56	47.3	52.7
	rs12881240	Chr14: 37135752	Exon 4	C	T	His239His	219	123	86	10	75.8	24.2
	rs4904210	Chr14: 37135753	Exon 4	G	C	Ala240Pro	219	46	120	53	48.4	51.6
Japanese	rs2295222	Chr14: 37126308	5′-flanking	C	A		271	122	126	23	68.3	31.7
	rs4904155	Chr14: 37127044	5′-UTR	G	C		269	53	126	90	43.1	56.9
	rs2073244	Chr14: 37129874	Intron 1	A	G		267	90	125	52	57.1	42.9
	rs12881240	Chr14: 37135752	Exon 4	C	T	His239His	270	134	109	27	69.8	30.2
	rs4904210	Chr14: 37135753	Exon 4	G	C	Ala240Pro	270	91	132	47	58.1	41.9

Abbreviation: SNPs, single-nucleotide polymorphisms.

data of the Korean and Japanese subjects were applied to a step-wise multiple regression analysis (F-in = 2, F-out = 2); all the SNPs were set as explanatory variables and population (Korea = 0 and Japan = 1) as a covariable.

RESULTS

Five SNPs in the *PAX9* region were genotyped in this study (Table 1). The differences in the allele frequencies between the Korean group and the Japanese group ranged from 5.5 to 9.8%. The genotype distribution for these SNPs did not deviate significantly from the expectation of the Hardy–Weinberg equilibrium. Estimated haplotype frequencies are shown in Table 2. We found that all five SNPs are in strong LD ($D' > 0.8$), and genotypes for rs4904155, rs2073244 and rs4904210 are highly correlated with one another ($r^2 > 0.8$) (Figure 1).

Among the Korean subjects, 31.8% of the individuals were missing one or more M3 (Supplementary Table 1). There was no significant difference between males and females in the frequency of M3 agenesis. The number of congenitally present M3 was negatively correlated with diameters of premolars and molars (Supplementary Table 2), but the number did not show any correlation with the length or width of dental arches or non-metric traits. We did not find any correlation between the number of M3 and the number of derived alleles at *PAX9* SNPs (Table 3). The odds ratio between alleles showed no significant association of the SNPs with one or more M3 agenesis.

Then, we examined whether *PAX9* polymorphisms are associated with crown sizes. We calculated the correlation coefficient between the number of derived alleles at each SNP and the average of *z* scores of GM over all the teeth (Table 4). Of the five SNPs, four showed significant correlations with the measure of crown size for all teeth in the meta-analysis. These associations were also significant after correction for multiple testing via the Benjamini and Hochberg method and the permutation test ($P < 0.05$).

When data from the Korean and Japanese subjects were merged and a stepwise multiple regression analysis was performed, only one SNP, rs2295222, was included in the regression equation; all other variables were excluded from the equation:

$$Y = 0.139X - 0.089$$

where Y is the average of *z* scores of GM over all teeth and X is the number of derived alleles at rs2295222. These results indicated that the derived allele had the effect of increasing the *z* scores by 0.139 and that the correlations of these SNPs with the crown size were not independent.

Subsequently, we tested the dominant and recessive models for the derived allele at each SNP. The dominant model was as good as the additive model for rs2295222, rs2073244 and rs4904210, whereas the recessive model good for rs4904155. When we separately evaluated MD and BL diameters, upper and lower teeth, and anterior and posterior teeth, there was neither directional nor regional effects of the SNPs on tooth size (Supplementary Table 3). However, the effects of the SNPs on each tooth varied: UP2, LI2 and LC were the most affected teeth (Table 5). The largest absolute value of any correlation coefficient was observed when the recessive model for the derived allele at rs4904155 was applied to UC ($r = -0.150$, $P = 0.0020$).

These SNPs were also tested for associations with the length or width of dental arches or non-metric dental traits (Supplementary Tables 3 and 4) because these traits are related with the crown size.[26] Of the four SNPs that showed significant correlations with the crown size, three SNPs (rs4904155, rs2073244 and rs4904210) were significantly correlated with shoveling of UI1 ($P = 0.026$, 0.033 and 0.0092, respectively) in the additive model (Supplementary Table 4). The other traits were not correlated with any of the four SNPs.

Table 2 Haplotype frequencies for *PAX9* SNPs

	Korea (N = *446*)		*Japan* (N = *546*)	
Haplotype[a]	n	%	n	%
AGGCC	163	36.55	164	30.04
CCATG	105	23.54	154	28.21
CCACG	103	23.09	149	27.29
CGGCC	65	14.57	56	10.26
CCACC	3	0.67	9	1.65
AGGTG	0	0.00	8	1.47
CGGTG	3	0.67	3	0.55
CGGCG	2	0.45	1	0.18
AGGCG	2	0.45	1	0.18
CGACC	0	0.00	1	0.18

Abbreviation: SNPs, single-nucleotide polymorphisms.
[a]In order of rs2295222, rs4904155, rs2073244, rs12881240 and rs4904210.

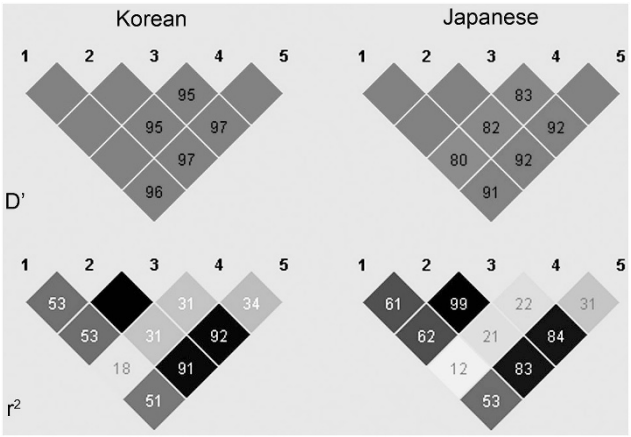

Figure 1 Haploview images of linkage disequilibrium coefficients for PAX9 SNPs in the Korean and Japanese subjects. 1: rs2295222, 2: rs4904155, 3: rs2073244, 4: rs12881240 and 5: rs4904210.

DISCUSSION

Reportedly, common polymorphisms in *PAX9* (rs2073244, rs2073246 and rs2073247), which are located in the same LD block, were significantly associated with hypodontia including M3 agenesis in individuals of European ancestry;[24,34] the derived (G) allele of rs2073244 showed increased odds of M3 agenesis (odds ratio: 1.75) in an analysis of 106 healthy control individuals and 72 individuals with M3 agenesis. Although these three SNPs were previously considered to be located in the 5′-flanking region of *PAX9*, a recent update of annotation information has added one more non-coding exon at the top of this gene, and these SNPs are thus located in intron 1. In the present study, therefore, we examined rs2295222 and rs4904155 that are located in the 5′-flanking region and in the untranslated exon 1, respectively, in addition to rs2073244. Furthermore, we added a common non-synonymous SNP in exon 4, rs4904210, and its adjoining synonymous SNP, rs12881240, to our analysis. As results, we did not observe any significant correlation between *PAX9* SNPs and the numbers of congenitally present M3 in the Korean subjects. Other studies have also failed to show any association between *PAX9* SNPs and sporadic tooth agenesis in the Polish and Chinese subjects.[35,36]

Table 3 Association between PAX9 SNPs and the number of third molars in the Korean subjects

			Without M3 agenesis		*With one or more M3 agenesis*			
SNP	*Rho*	P	*Allele A*	*Allele D*	*Allele A*	*Allele D*	*Odds ratio*	*(95% CI)*
rs2295222	0.033	8.1.E−01	90 (64%)	50 (36%)	189 (62%)	115 (38%)	0.91	(0.60–1.38)
rs4904155	0.048	8.4.E−01	74 (52%)	68 (48%)	161 (53%)	143 (47%)	1.03	(0.69–1.54)
rs2073244	0.016	8.5.E−01	67 (48%)	73 (52%)	142 (47%)	160 (53%)	0.97	(0.64–1.44)
rs12881240	−0.013	2.1.E−01	97 (71%)	39 (29%)	235 (78%)	67 (22%)	1.41	(0.89–2.23)
rs4904210	0.013	3.7.E−01	70 (51%)	66 (49%)	142 (47%)	160 (53%)	0.84	(0.55–1.25)

Abbreviations: M3, 3rd molar; SNPs, single-nucleotide polymorphisms.
Positive and negative values of Spearman's rank correlation coefficient (rho) mean that the number of the derived allele is associated with larger and smaller number of M3, respectively. The odds ratio denotes the effect of a copy of the derived allele (D) on lacking one or more M3.

Table 4 Correlation between PAX9 SNPs and the average standardized crown size for all teeth

		Korean		*Japanese*		*Meta-analysis*	
Model	*SNP*	r	P	r	P	r	P
Additive	rs2295222	0.065	3.3.E−01	**0.147**	**1.6.E−02**	**0.110**	**1.5.E−02**
	rs4904155	**−0.133**	**4.8.E−02**	−0.089	1.5.E−01	**−0.109**	**1.6.E−02**
	rs2073244	**0.134**	**4.7.E−02**	0.078	2.1.E−01	**0.103**	**2.3.E−02**
	rs12881240	−0.015	8.3.E−01	−0.006	9.2.E−01	−0.010	8.2.E−01
	rs4904210	0.117	8.5.E−02	0.077	2.1.E−01	**0.095**	**3.6.E−02**
Dominant	rs2295222	0.079	2.4.E−01	**0.179**	**3.3.E−03**	**0.134**	**3.0.E−03**
	rs4904155	−0.087	2.0.E−01	−0.017	7.9.E−01	−0.049	2.9.E−01
	rs2073244	0.125	6.4.E−02	0.100	1.1.E−01	**0.111**	**1.4.E−02**
	rs12881240	−0.001	9.9.E−01	0.007	9.0.E−01	0.004	9.4.E−01
	rs4904210	0.084	2.2.E−01	0.119	5.2.E−02	**0.103**	**2.3.E−02**
Recessive	rs2295222	0.012	8.6.E−01	0.013	8.3.E−01	0.013	7.8.E−01
	rs4904155	−0.128	5.7.E−02	**−0.120**	**5.0.E−02**	**−0.124**	**6.3.E−03**
	rs2073244	0.092	1.8.E−01	0.021	7.3.E−01	0.053	2.4.E−01
	rs12881240	−0.039	5.7.E−01	−0.027	6.6.E−01	−0.032	4.8.E−01
	rs4904210	0.104	1.3.E−01	−0.006	9.2.E−01	0.043	3.4.E−01

Abbreviation: SNPs, single-nucleotide polymorphisms.
Bold: $P<0.05$. The additive, dominant and recessive denote models for derived allele. Positive and negative values of Pearson's correlation coefficient (r) mean that the derived allele is associated with larger and smaller size, respectively.

Table 5 Meta-analysis of the correlation between PAX9 SNPs and the crown size (GM) of each tooth

SNP	*Model*	*UI1*	*UI2*	*UC*	*UP1*	*UP2*	*UM1*	*UM2*	*LI1*	*LI2*	*LC*	*LP1*	*LP2*	*LM1*	*LM2*
rs2295222	Additive	0.044	0.005	0.110*	0.097*	0.106*	0.094*	0.038	0.059	0.109*	0.131†	0.117*	0.103*	0.021	0.078
	Dominant	0.039	0.032	0.115*	0.089	0.128†	0.130†	0.065	0.068	0.135†	0.131†	0.112*	0.106*	0.058	0.119*
rs4904155	Additive	−0.011	−0.033	−0.061	−0.058	−0.139†	−0.086	−0.086	−0.065	−0.117*	−0.136†	−0.093*	−0.079	−0.068	−0.080
	Recessive	−0.014	−0.074	−0.077	−0.059	−0.150†	−0.106*	−0.090	−0.061	−0.101*	−0.142†	−0.110*	−0.087	−0.080	−0.084
rs2073244	Additive	0.002	0.018	0.058	0.055	0.140†	0.084	0.076	0.059	0.113*	0.131†	0.092*	0.075	0.060	0.081
	Dominant	0.000	0.055	0.069	0.049	0.140†	0.095	0.077	0.054	0.090	0.133†	0.104*	0.077	0.067	0.078
rs4904210	Additive	0.027	0.018	0.038	0.052	0.111*	0.056	0.068	0.092	0.146†	0.131†	0.066	0.038	0.037	0.064
	Dominant	0.017	0.052	0.044	0.050	0.104*	0.068	0.064	0.078	0.113*	0.131†	0.084	0.048	0.049	0.065

Abbreviations: GM, geometric mean; SNPs, single-nucleotide polymorphisms.
Pearson's correlation coefficients (r) were shown. *$P<0.05$, †$P<0.01$.

If we assume the previously observed value (1.75) of the odds ratio, the estimated power for the sample size of our test is calculated to be 76%. As the sample sizes were relatively small in both the previous and present studies, statistical error may explain the aforementioned difference in outcomes between the studies. Another possibility is that the difference in outcomes occurred owing to difference in the studied populations. Agenesis of one or more M3 occurs with variable frequency in different human populations; the frequencies reported vary from 0.2 to 11% in Africans, 9 to 25% in Europeans and 27 to 42% in East Asians,[37–43] and our observation in the Korean population (31.8%) was consistent with previous studies. Moreover, M3 agenesis is associated with crown-size reduction of the extant teeth in people of European ancestry, whereas M3 agenesis is associated with large crown size of molars in Japanese.[27,28,44] Similarly, we found that, among the Korean subjects, individuals lacking one or more M3 had a significantly larger crown size for

premolars and molars than did individual with four M3. Therefore, factors causing M3 agenesis are thought to be quite complicated and to differ between populations. It was suggested that late formation of tooth germs is associated with congenital absence of LM3.[45] It is thus possible that the timing of teeth formation varies among populations. To clarify the mechanism for causing M3 agenesis, further studies are indispensable.

Of the five *PAX9* SNPs examined in this study, four SNPs were significantly correlated with crown size. The strong LD between these SNPs and the results of the stepwise multiple regression analysis indicated that the effects of the examined SNPs on tooth morphology are not independent. However, the present association study did not identify the causative SNP. The human *PAX9* gene is composed of one non-coding exon (exon 1) and four coding exons (exon 2–5). *PAX9* mutations that cause oligodontia have been most frequently found in the exon 3 (denoted as the exon 2 in some previous reports), which bears the conserved paired-box sequence, a specific DNA-binding domain that is necessary for *PAX9* to function as a transcription factor.[46,47] One of the SNPs that showed significant association with crown size in our study, rs4904210, is a non-synonymous SNP located in a region of exon 4 that is highly conserved among mammals.[35] In addition, it has been predicted using computer programs that the Ala-to-Pro amino-acid change affects the protein structure.[35] However, these pieces of indirect evidence cannot be an adequate explanation for the functional change of the PAX9 protein. As the LD block that includes the examined SNPs ranges widely, it would be difficult without functional assays to determine which SNP is responsible.

The patterns of tooth agenesis caused by *MSX1* and *PAX9* mutations have been studied.[9] These studies have demonstrated that the pattern of missing teeth is bilaterally symmetrical, but can be different between the maxillary and mandibular teeth. The typical feature of *MSX1*-associated tooth agenesis is frequent absence of UP1, UP2 and/or LP2, whereas the typical feature of *PAX9*-associated tooth agenesis is frequent absence of UM2 and/or LM2.[48] Some individuals with a *PAX9* mutation lack UPs, LPs and/or LI1.[9] *PAX9* is a dosage-sensitive gene in humans, and its function appears to be most relevant in the posterior teeth, especially in those derived from the proliferation of the dental lamina from which permanent molars originate.[3] In our study, however, the teeth most strongly affected by *PAX9* SNPs were UP2, LI2 and LC, which do not match the positions of *PAX9*-associated tooth agenesis. It has been shown that hypodontia-affected family members with a *PAX9* mutation have significantly smaller crown dimensions; this finding indicates that the effects of *PAX9* mutations are manifested not only as the congenital absence of some teeth, but also as smaller crown sizes throughout the dentition.[49] Reportedly, missense mutations provide a milder phenotype than do non-sense or frame-shift mutants.[9] These facts suggest that *PAX9* is involved in the morphogenesis of the entire dentition, rather than in the positioning and development of certain teeth.[5]

A common non-synonymous variant in *EDAR*, which is specific to the Asian populations, has been shown to be associated with larger crown size, greater degree of shoveling and double shoveling of UI1, and the presence of hypoconulids of LM2.[25,26] Similarly, *PAX9* polymorphisms have been shown to be associated with the shoveling grade of UI1, as well as with the crown size. This fact is suggestive when we consider the developmental process of shovel-shaped incisors. As the volume of teeth becomes greater, the degree of shoveling may become larger.

In summary, our study indicated that *PAX9* variants have a role in the crown size and shape of permanent teeth in the East Asian populations, but failed to show any association between *PAX9* SNPs and M3 agenesis. However, the biological functions of these *PAX9* SNPs remain to be elucidated. Likewise, other genes (for example, *MSX1*) that are related with genetic disorders that result in malformation of teeth may also influence common dental characteristics. Further studies will be required to understand the variation in human dentition.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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