ORAL DISEASES

Oral Diseases (2011) 17, 399–406 doi:10.1111/j.1601-0825.2010.01766.x

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ORIGINAL ARTICLE

Association between *AMELX* polymorphisms and dental caries in Koreans

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OBJECTIVES: Dental caries is greatly influenced disease by environmental factors, but recently there are increasing evidences for a genetic component in caries susceptibility. *AMELX* is the gene coding amelogenin, which is the most important factor for normal enamel development. The aim of this study was to examine the relationship between dental caries and single nucleotide polymorphisms (SNPs) in *AMELX*.

SUBJECTS AND METHODS: For this study, we used DNA samples collected from 120 unrelated individuals older than 12 years of age. All of them were examined for their oral and dental status under the WHO recommended criteria, and clinical information such as DMFT and DMFS were evaluated. Individuals whose DMFT and DMFS index lower than 2 were designated 'very low caries experience' and higher than 3 were designated 'higher caries experience'. Genomic DNA was extracted from hair samples, and single nucleotide polymorphisms of *AMELX* were genotyped. Genotyping of three SNPs (rs17878486, rs5933871, rs5934997, intron) in *AMELX* gene was determined by direct sequencing and analyzed with SNPStats.

RESULTS: There were significant associations between rs5933871 and rs5934997 SNP and caries susceptibility in the water fluoridation group.

CONCLUSIONS: These results suggest that SNPs of *AMELX* might be associated with dental caries susceptibility in Korean population.

Oral Diseases (2011) **17**, 399–406

Keywords: amelogenin (*AMELX*); dental caries; Korean; single nucleotide polymorphism (SNP)

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Received 23 April 2010; revised 13 September 2010; accepted 1 October 2010

Introduction

Dental caries is simply defined as a process that causes a loss of mineral from the tooth structure and also synonymously known as tooth decay or cavity. It is one of the most common bacterial diseases throughout the world (Kristiansen and Rohde, 1991). The disease can cause pain, tooth loss, infection and more severe diseases (Mattila *et al*, 1995). Most people are susceptible to this disease throughout their lifetime. Dental caries is widely known that it is mostly influenced disease by environmental factors such as bacteria and diet, but there are also increased evidences for a genetic component in caries susceptibility. Many molecular genetic studies in humans focused on saliva and microbacterial attachment. Association of polymorphism in CA6 gene related with salivary buffer and caries was reported (Peres *et al*, 2009), and some studies showed relation between salivary protein and dental caries (Roa *et al*, 2008). In addition, association between enamel formation gene itself and dental caries was proposed. Contribution to caries susceptibility of variation in amelogenin, ameloblastin and tuftelin was reported (Patir *et al*, 2008), tuftelin-1 genotypes appeared to interact with levels of *S. mutans* infection in children with early childhood caries (Slayton *et al*, 2005), and possible association of amelogenin to high caries incidence was proposed (Deeley *et al*, 2008).

AMELX is the gene coding amelogenin, which is involved in biomineralization during tooth enamel development. Amelogenin is important for normal enamel formation, and aberration of *AMELX* appears to cause predominantly mineralization defects and X-linked amelogenesis imperfecta (Greene *et al*, 2002; Paine *et al*, 2002). Therefore, some authors suggested that genetic variation contributes to structural alterations of the enamel that may cause higher levels of mineral losses, bacterial attachment or biofilm deposition (Patir *et al*, 2008).

We hypothesized that single nucleotide polymorphisms (SNPs) in *AMELX* may have a relation with dental caries. The aim of this study was to examine the

relationship between dental caries and single nucleotide polymorphisms (SNPs) in *AMELX*.

Materials and methods

Study subjects

This study was approved by the Institutional Review Board of School of Dentistry, Kyung Hee University, Seoul, Republic of Korea. Written informed consent was obtained from all subjects. Genomic DNA was prepared from hair samples using a Qiagen® DNA Micro kit (Qiagen) and stored at −20°C before use. The caries group included 120 patients (86 male and 34 female patients; mean ± s.d., 22.7 ± 7.8 years). All individuals were examined for the oral and dental status with the WHO criteria. Decayed, missing, filled Tooth (DMFT) and decayed, missing, filled surface (DMFS) were scored and most individuals reported that they brushed their teeth at least once a day with a toothbrush and toothpaste. Drinking water in the region was also examined by questionnaire. We hypothesized that individuals who had more caries lesion would be genetically weak to caries. Therefore, the study subjects were divided into two groups according to the DMFT and DMFS index (Deeley *et al*, 2008). Individuals whose DMFT or DMFS index was two or less indicated 'very low caries experience' and three or more indicated 'higher caries experience' (Table 1). The 'very low caries experience' group was regarded as control group and 'higher caries experience' as experimental group.

Table 1 Demographic characteristics of the sample

	Very low caries experience		*Higher caries experience*	
Characteristics	*DMFT scores*	*DMFS scores*	*DMFT scores*	*DMFS scores*
Number	33	28	87	92
Males	23	19	63	67
Females	10	9	24	25
Age	21.3 ± 7.8	20.4 ± 7.6	23.2 ± 7.8	23.4 ± 7.8
Age range	13–33	13–33	13–39	13–39
Scores				
0	11	12	–	–
1	12	9	–	–
2	10	7	–	–
3	–	–	9	9
4	–	–	11	4
5	–	–	12	4
6	–	–	11	8
7	–	–	8	8
8	–	–	11	7
9	–	–	8	6
10	–	–	2	3
11	–	–	7	4
12	–	–	4	3
13	–	–	3	2
14	–	–	1	4
15	–	–	0	3
16	–	–	0	2
17	–	–	0	2
18	–	–	0	4
19	–	–	0	3
20	–	–	0	16

SNP selection and genotyping

We selected three SNPs within *AMELX* using the following methods: (1) According to the reported association by other authors (Deeley *et al*, 2008; Patir *et al*, 2008) (2) known heterozygosity and minor allele frequency (MAF) >0.05 and (3) validation reported. Three SNPs in intron of the *AMELX* gene were studied for the association with dental caries. The genotypes were determined by direct sequencing. Genomic DNA was amplified using polymerase chain reaction. (sense, 5′- ATCCTGCCTACACCCAATAC -3′; antisense, 5′- GGCCACCTGAATGATGATCT -3′, 604 bp) Sequence data were analyzed using the SeqManII software (DNASTAR Inc., Madison, WI, USA).

Statistical analysis

Hardy-Weinberg equilibrium (HWE) for three SNPs was assessed using SNPStats (http://bioinfo.iconcologia.net/index.php). A linkage disequilibrium (LD) block of polymorphisms was tested using Haploview 4.2. Multiple logistic regression models were calculated for the odds ratio (OR), 95% confidence interval (CI) and corresponding *P*-values, considering for water fluoridation as a co-variable. Fisher's exact test was also used for analysis. We used SNPStats (http://bioinfo.iconcologia.net/index.php), Haploview 4.2 (http://www.broadinstitute.org/haploview), and SPSS 17.0 (SPSS, Chicago, IL, USA).

Results

Allelic association and haplotypes in "higher caries experience" group and "very low caries experience" group

All genotypes were in Hardy-Weinberg equilibrium range. In order to investigate the relationship between the haplotypes within the *AMELX* genes and dental caries susceptibility, LD and haplotype were analyzed by Haploview software version 4.2. LD data were calculated for all SNP pairs between the "higher caries experience" group and "very low caries experience" group.

It appeared that LD block was not noted between three SNPs within *AMELX* gene by the Gabriel method. There were no significant differences between "higher caries experience" group and "very low caries experience" (Table 2).

AMELX single nucleotide polymorphism in experiments and controls

The clinical characteristics of "higher caries experience" group and "very low caries experience" group are shown in Table 1. The mean age in "higher caries experience" group was 23.2 ± 7.8 in DMFT score and 23.4 ± 7.8 in DMFS score, and the mean age in "very low caries experience" group was 21.3 ± 7.8 in DMFT score and 20.4 ± 7.6 in DMFS score. The genetic association between of the *AMELX* polymorphisms and susceptibility to dental caries was analyzed. The genotype distributions and allele frequencies of *AMELX* gene polymorphisms in the "higher caries experience" group and "very low caries experience" groups are shown in Table 3.

It was noted that there were no significant differences in genotype, phenotype and gene frequency between

Table 2 Haplotype analysis in DMFT and DMFS score

				Higher caries experience		*Very low caries experience*			
	Block	*Haplotype*	*Freq.*	+	−	+	−	*Chi Square*	P-*value*
DMFT	Block 1	TTT	0.920	161.9	12.1	58.9	7.1	0.913	0.3394
		CCT	0.042	6.0	4.0	4.0	62.0	0.818	0.3657
		TTC	0.026	169.9	2.1	2.1	63.9	0.120	0.7294
DMFS	Block 1	TTT	0.920	171.9	12.1	48.9	7.1	2.129	0.1446
		CCT	0.042	0.042	178.0	4.0	52.0	1.621	0.2030
		TTC	0.026	0.026	179.9	2.1	53.9	0.376	0.5395

Each haplotype with a frequency of more than 0.1 is shown. *P*-values of haplotype association were calculated using haploview 4.1. Freq, frequency.

''higher caries experience'' group and ''very low caries experience'' group using the analysis of codominant, dominant and recessive models, and Fisher's exact test. The results showed no association between the dental caries susceptibility and SNPs of *AMELX* gene in Korean population.

Difference in caries experience between male and female genders

As AMELX is located in X-chromosome, the DMFT and DMFS index was analyzed divided by gender. Neither of them showed significance (Table 4).

Association in water fluoridation and caries

To determine the effects of water fluoridation on caries experience, we studied the DMFT and DMFS index among each genotype divided into water fluoridation and non-fluoridation groups. TC and CC genotype was so small that we could not get the *P*-value; rs17878486, TC:4, CC:1; rs5933871, TC:4, CC:4; rs5934997, TC:3, CC:4. In TT genotype, although there was no significance in DMFT index, DMFS index showed difference significantly (Table 5). The subjects who lived or were living in fluoridation area showed lower caries experience.

AMELX single nucleotide polymorphism and the effect of fluoridation

The experiment group was divided into two groups who lived or are living in fluoridized area and non-fluoridized area. Moreover, each group was divided into ''higher caries experience'' group and ''very low caries experience'' group in the same manner. The genetic association between the *AMELX* polymorphisms and susceptibility to dental caries was analyzed using DMFT and DMFS index in the groups. The results are shown in Tables 6 and 7.

Moreover, the analysis of codominant, dominant and recessive models, and Fisher's exact test have shown rare significant differences in genotype, phenotype and gene frequency. However, in those subjects who lived or living in fluoridized area, the rs5933871 and rs5934997 showed a difference. Logistic regression analysis of rs5933871 and rs5934997 in DMFT and DMFS score lived in fluoridized area was not significant. However, performing the Fisher's exact test, there was a significance difference between the ''higher caries experience'' group and ''very low caries experience'' groups. The results showed a correlation between the dental caries susceptibility and SNPs of *AMELX* gene considering water fluoridation in Korean population.

Discussion

Dental caries is a complex multifactorial disease process that afflicts a large proportion of the world's population. It is a severe problem because it can cause many health problems and tooth loss. There are many causes that induce caries. Microorganism, time, substrates and host are well-known causes of caries. Of these factors, host factor includes tooth structure and morphology, salivary flow rate and buffering capacity, genetic factors, and so on (Leone and Oppenheim, 2001). Some previous articles reported evidence that genetic factors could be related to the structure of dental enamel and the immunologic response to cariogenic bacteria (Shuler, 2001) and have a significant contribution to dental caries, whereas microbial acid production appears to be modulated by the environment (Slayton, 2006). Recently, some studies focused on the enamel structure and role of gene. These studies suggest that the genes involved in the enamel formation in caries susceptibility in humans. Genetic variation in these genes contributes to structural alterations of the enamel that may cause higher levels of mineral losses (Deeley *et al*, 2008; Patir *et al*, 2008).

Enamel is the hardest tissue covering of teeth in human and most extracellular matrix protein of developing enamel is amelogenin (Moradian-Oldak *et al*, 1998). *AMELX* is the gene coding amelogenin and particular isoforms of amelogenin formed by alternative splicing serve critical signaling functions during the development of odontogenic and other tissues (Hu *et al*, 2005). Mutations in the human *AMELX* gene cause a phenotypically diverse set of inherited enamel malformations (Kim *et al*, 2004). Enamel phenotype is associated with *AMELX* gene mutation (Ravassipour *et al*, 2000). Moreover, mutations in the *AMELX* gene result in phenotypical and genetical diversity of X-linked amelogenesis imperfectas (Wright *et al*, 2003). As many

Table 3 Logistic regression analysis and Fisher's exact test of *AMELX* polymorphisms in DMFT and DMFS score

Index	*SNP Locus*	*Genotype*	*Higher caries experience* n *(%)*	*Very low caries experience* n *(%)*	*Codominant OR (95% CI)*	*P-value*	*Dominant OR (95% CI)*	P-*value*	*Recessive OR (95% CI)*	P-*value*	*Fisher's exact test*
DMFT index	rs17878486	TT	82 (94.3%)	29 (87.9%)	0.35 (0.05–.63)	0.44	0.53 (0.08–3.34)	0.51	NA (0.00-NA)	0.43	0.17
	intron	CT	2 (2.3%)	2 (6.1%)							
		CC	1 (1.1%)	0 (0%)							
	rs5933871	TT	79 (90.8%)	31 (93.9%)	NA (0.00-NA)	0.17	1.18 (0.23–6.15)	0.84	0.37 (0.05–2.77)	0.34	0.07
	intron	CT	4 (4.6%)	0 (0%)							
		CC	2 (2.3%)	2 (6.1%)							
	rs5934997	TT	83 (95.4%)	30 (90.9%)	0.75 (0.07–.59)	0.6	0.48 (0.10–2.28)	0.37	0.36 (0.05–2.70)	0.33	0.11
	intron	CT	2 (2.3%)	1 (3.0%)							
		CC	2 (2.3%)	2 (6.1%)							
DMFS index	rs17878486	TT	87 (94.6%)	24 (85.7%)	0.27 (0.04–.04)	0.37	0.41 (0.07–2.62)	0.37	NA (0.00-NA)	0.48	0.14
	intron	CT	2 (2.2%)	2 (7.1%)							
		CC	1 (1.1%)	0 (0%)							
	rs5933871	TT	84 (91.3%)	26 (92.9%)	NA (0.00-NA)	0.18	0.93 (0.18–4.88)	0.93	0.30 (0.04–2.20)	0.25	0.07
	intron	CT	4 (4.3%)	0 (0%)							
		CC	2 (2.2%)	2 (7.1%)							
	rs5934997	TT	88 (95.7%)	25 (89.3%)	0.60 (0.05–.88)	0.45	0.38 (0.08–1.81)	0.24	0.29 (0.04–2.15)	0.24	0.08
	intron	CT	2 (2.2%)	1 (3.6%)							
		CC	2 (2.2%)	2 (7.1%)							

n = number of subject.
Differences in genotype frequency of rs17878486, rs5933871 and 5934997 in logistic regression model were not significant.
Genotype distributions are shown as number (%). Odd ratio (OR), 95% confidence interval (CI) and *P*-values were from logistic regression analysis with codominant, dominant and recessive models. SNP, single nucleotide polymorphism.
Total number of each SNP is different because genotypes of some SNPs are unreadable.

Table 4 Gender distribution for DMFT and DMFS index

		Male (n = *86*)	*Female* (n = *34*)
DMFT	Mean	5.8 ± 4.0	4.5 ± 3.0
	t	1.921	
	P-value	0.058	
DMFS		10.7 ± 9.7	7.5 ± 7.6
	t	1.868	
	P-value	0.066	

Data shows no significancy.

previous studies showed the importance of *AMELX* gene in enamel formation, some authors proposed the relation between dental caries and *AMELX* gene polymorphism. Amelogenin was associated with the high caries experience in a Guatemalan-Mayan population (Deeley *et al*, 2008) and enamel formation genes are associated with high caries experience in Turkish Children (Patir *et al*, 2008).

We investigated whether the SNPs in *AMELX* gene are related to dental caries by genotyping. The rs5933871 (C > T) SNP with 0.368 of heterozygosity is located in the intron region of the *AMELX* gene, located on chromosome Xp22.31-22.1. The CC, CT and TT genotype frequencies have been reported to be 0.133, 0.150 and 0.717 in European, TT genotype frequencies have been reported to be 1.000 in Chinese, CC and TT genotype frequencies have been reported to be 0.023 and 0.977 in Japanese, and the CC, CT and TT genotype frequencies have been reported to be 0.500, 0.233 and 0.267 in Sub-Saharan African populations, respectively. In the Korean population, the CC, CT and TT genotype frequencies were 0.033, 0.033 and 0.917, which are similar to those seen in Asian populations. The rs5934997 (C > T) SNP with 0.414 of heterozygosity is located in the intron region of the *AMELX* gene. The TT genotype frequencies have been reported to be 1.000 in Chinese; CC and TT genotype frequencies have been reported to be 0.025 and 0.975 in Japanese; and the CC, CT and TT genotype frequencies have been reported to be 0.559, 0.259 and 0.185 in Sub-Saharan African populations, respectively. In the Korean population, the CC, CT and TT genotype frequencies were 0.033, 0.025 and 0.942, which are similar to those seen in Asian populations. Moreover, the rs17878486 (C > T) SNP with 0.270 of heterozygosity is also located in the intron region of the *AMELX* gene. The C and T allele frequencies have been reported to be 0.050 and 0.950 in African American, and 0.270 and 0.730 in Caucasian, respectively. In the Korean population, the C and T allele frequencies were 0.026 and 0.974, which are similar to those seen in African American populations (Figure 1).

In these SNPs, rs17878486 has reported that is associated with high caries experience (Deeley *et al*, 2008). However, it was performed in a Guatemalan-Mayan population, and genotype frequency of them might be different from that of Koreans. As mentioned above, the genotype frequency of rs17878486 shows a difference between different populations. There are many reports about the different caries frequency between races. The risk for early childhood caries was more than three times higher in Asians and African Americans compared with those in whites controlling for socioeconomic status variables (Shiboski *et al*, 2003). Oral health status of San Francisco public school kindergarteners 2000–2005 showed that caries prevalence in Asian children was greater than that in others (Chung *et al*, 2006). The prevalence of dental caries in young children was higher in South Asian children and associated with ethnicity in the controlled data for deprivation and fluoridation status (Gray *et al*, 2000). The ethnicity influences the prevalence of dental caries and Asian or African-American have more dental caries than Caucasian. In this study, the findings showed that dental caries susceptibility was not associated with the genotype frequency of rs17878486, which were different from previous studies. The results of this present study showed a significancy in other *AMELX* SNPs.

As AMELX is located in X-chromosome, we studied the DMFT and DMFS index divided by gender. However, the result of present study showed no association between DMFT and DMFS index and gender. Although we divided the subjects by genotype, area which they lived or living and topical fluoridation, we could not find any relation at least in our study.

Fluoride plays an important role in caries incidence and prevalence. Maintaining both pre- and post-eruptive fluoride at surface of the teeth is important in development of an early carious lesion such as demineralized area of the tooth. Although it does not prevent the initiation of the disease, it controls its progression (Groeneveld *et al*, 1990). Fluoride inhibits bacterial

Table 5 DMFT and DMFS index of TT genotype subject divided by water fluoridation or non-fluoridation area in SNPs of AMELX

	rs17878486			*rs5933871*			*rs5934997*		
	Water fluoridation area (n = *53*)	*Non-fluoridation area* (n = *51*)	P-*value*	*Water fluoridation area* (n = *52*)	*Non-fluoridation area* (n = *51*)	P-*value*	*Water fluoridation area* (n = *53*)	*Non-fluoridation area* (n = *53*)	P-*value*
DMFT (mean ± s.d.)	4.9 ± 3.5	5.7 ± 3.9	0.242	4.8 ± 3.6	6.1 ± 4.0	0.084	4.8 ± 3.5	6.2 ± 4.1	0.059
DMFS (mean ± s.d.)	6.8 ± 5.4	12.1 ± 11.4	**0.003**	6.7 ± 5.6	12.7 ± 11.3	**0.001**	6.8 ± 5.5	13.1 ± 11.3	**0.000**

Total number of each SNP is different because genotypes of some SNPs are unreadable.

Table 6 Logistic regression analysis and Fisher's exact test of *AMELX* polymorphisms in DMFT and DMFS score lived in fluoridized area

Index	*SNP Locus*	*Geno type*	*Higher caries experience* n *(%)*	*Very low caries experience* n *(%)*	*Codominant OR (95% CI)*	P-*value*	*Dominant OR (95% CI)*	P-*value*	*Recessive OR (95% CI)*	P-*value*	*Fisher's exact test*
DMFT index	rs17878486*	TT	37 (97%)	16 (100%)			0.70 (0.58–0.83)*	0.51*			0.70
	intron	CT	1 (3%)	0 (0%)							
	rs5933871	TT	38 (97%)	14 (82%)	0.38 (0.07–1.96)	0.02	0.12 (0.01–1.28)	0.06	NA (0.00-NA)	0.39	**0.02**
	intron	CT	0 (0%)	3 (18%)							
		CC	1 (3%)	0 (0%)							
	rs5934997	TT	39 (98%)	14 (82%)	0.37 (0.07–1.93)	0.02	0.12 (0.01–1.25)	0.05	NA (0.00-NA)	0.4	**0.02**
	intron	CT	0 (0%)	3 (18%)							
		CC	1 (2%)	0 (0%)							
DMFS index	rs17878486*	TT	39 (98%)	14 (100%)			0.73 (0.63–0.86)*	0.55*			0.74
	intron	CT	1 (2%)	0 (0%)							
	rs5933871	TT	39 (95%)	13 (87%)	0.63 (0.13–3.05)	0.25	0.33 (0.04–2.61)	0.30	NA (0.00-NA)	0.43	0.12
	intron	CT	1 (2%)	2 (13%)							
		CC	1 (2%)	0 (0%)							
	rs5934997	TT	40 (95%)	13 (87%)	0.62 (0.13–2.99)	0.24	0.33 (0.04–2.54)	0.29	NA (0.00-NA)	0.43	0.11
	intron	CT	1 (2%)	2 (13%)							
		CC	1 (2%)	0 (0%)							

*chi-square test.
Differences in genotype frequency of rs17878486, rs5933871 and 5934997 in logistic regression model were not significant. However, Fisher's exact test showed a significance (P-value < 0.05). Total number of each SNP is different because genotypes of some SNPs are unreadable.

Table 7 Logistic regression analysis and Fisher's exact test of *AMELX* polymorphisms in DMFT and DMFS score lived in non-fluoridized area

Index	*SNP Locus*	*Geno type*	*Higher caries experience* n *(%)*	*Very low caries experience* n *(%)*	*Codominant OR (95% CI)*	P-*value*	*Dominant OR (95% CI)*	P-*value*	*Recessive OR (95% CI)*	P-*value*	*Fisher's exact test*
DMFT index	rs17878486	TT	37 (92%)	14 (93%)	1.37 (0.20–9.51)	0.71	1.14 (0.11–11.85)	0.91	NA (0.00-NA)	0.42	0.33
	intron	CT	2 (5%)	1 (7%)							
		CC	1 (2%)	0 (0%)							
	rs5933871	TT	38 (95%)	13 (87%)	0.47 (0.15–1.51)	0.25	0.34 (0.04–2.68)	0.32	0.17 (0.01–1.99)	0.14	0.12
	intron	CT	1 (2%)	0 (0%)							
		CC	1 (2%)	2 (13%)							
	rs5934997*	TT	40 (98%)	13 (87%)			6.15 (0.52–73.53)*	0.16*			0.16
	intron	CT	1 (2%)	2 (13%)							
DMFS index	rs17878486	TT	40 (93%)	11 (92%)	1.09 (0.16–7.42)	0.70	0.82 (0.08–8.73)	0.87	NA (0.00-NA)	0.48	0.33
	intron	CT	2 (5%)	1 (8%)							
		CC	1 (2%)	0 (0%)							
	rs5933871	TT	41 (95%)	10 (83%)	0.40 (0.12–1.28)	0.18	0.24 (0.03–1.95)	0.2	0.12 (0.01–1.45)	0.09	0.09
	intron	CT	1 (2%)	0 (0%)							
		CC	1 (2%)	2 (17%)							
	rs5934997*	TT	43 (98%)	10 (83%)			8.60 (0.71–104.47)*	0.05*			0.10
	intron	CT	1 (2%)	2 (17%)							

*chi-square test.
Total number of each SNP is different because genotypes of some SNPs are unreadable.

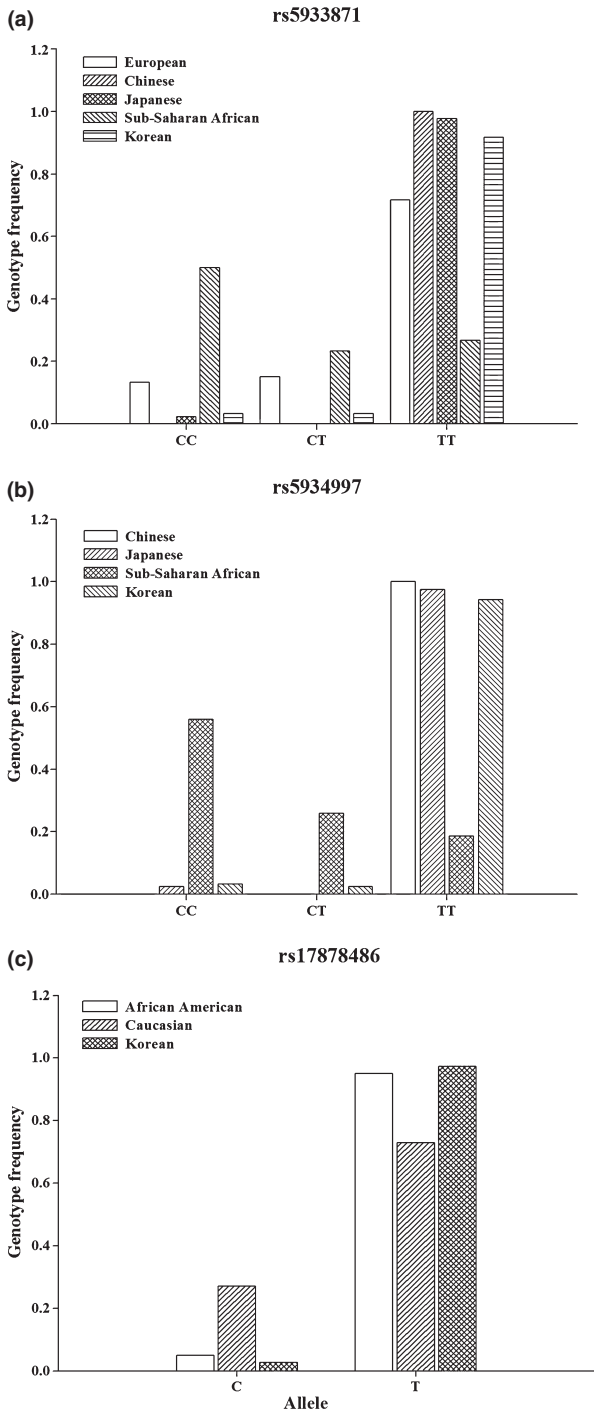

Figure 1 (a) The genotype frequencies of rs5933871 in various populations. The results in Korean population were similar to those seen in Asian populations. **(b)** The genotype frequencies of rs5934997. In the Korean population, the genotype frequencies were also similar to Asian populations. **(c)** The allele frequencies of rs17878486. The Korean population has been analogous to African American rather than Caucasian, respectively

enzymes by acidifying the bacterial cells, inhibits demineralization at the crystal surfaces inside the tooth through replacing hydroxyapatite by fluorapatite, and enhances remineralization by speeding up the growth of a new crystal surface. Through these mechanisms, fluoride in drinking water and fluoride-containing products reduces dental caries (Featherstone, 1999). The American Academy of Pediatric Dentistry (AAPD) said that fluoride is effective in reducing the risk of caries and reversing enamel demineralization (AAPD, http://www.aapd.org). Preventive fluoride can be used systemically. Systemic preventive fluoride includes the community water fluoridation or dietary fluoride supplements. The adjustment of the fluoride level in community water supplies to optimal concentration is a beneficial and inexpensive method of reducing the occurrence of caries (Horowitz, 2003). In non-fluoridation water areas, fluoride is used as supplements like tablets, drops, salt, milk or lozenges for children. In Korea, some areas have been supplied with fluoridated water, and the others with non-fluoridated water. Therefore, we examined all the study subjects whether they had lived in fluoridation area in childhood and whether they had been supplied the fluoride supplements or not using questionnaire. In our study, there was no significant difference in DMFT index between water fluoridation area and non-fluoridation area (rs17878486, $P = 0.242$; rs5933871, $P = 0.084$; rs5934997, $P = 0.059$). However, there were considerable significancy in DMFS index in each SNPs (rs17878486, $P = 0.003$; rs5933871, $P = 0.001$; rs5934997, $P = 0.000$). Therefore, we divided the subjects whether they lived or are living in water fluoridation area. Only for the subject who lived in water fluoridation area during childhood, the SNPs of rs5933871 and rs5934997 were significantly associated with caries susceptibility by Fisher's exact test.

In conclusion, rs5933871 and rs5934997 SNPs of *AMELX* gene might be associated with susceptibility to dental caries in the Korean population. However, our study has some limitations. We did not have radiographs, so interproximal lesions or secondary caries underneath restorative material may have not been detected in both experimental and control groups. Moreover, number of experiments and controls were not perfectly matched. Considering the limited sample size, these results need to be replicated in other and larger populations.

Acknowledgements

This work was supported by a grant from Kyung Hee University in 2009 (KHU-20090595).

Author contributions

Dr. Kang designed the study, collected, analyzed and interpreted the data. Dr. Lee helped with analysis and interpretation of the data and writing the report. Dr. Yoon helped with collection, analysis and interpretation of data. Dr. Cho was involved with designing the study, revision of the manuscript and took the decision to submit the article for publication.

Conflict of interest

None.

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