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Genomewide association study on the severity of periodontitis in Korean population: results from the Yangpyeong health cohort

한국인의 치주질환에 대한 전장유전체연관분석

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-ABSTRACT-

Genomewide association study on the severity of periodontitis in Korean population: results from the Yangpyeong health cohort

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Objective: This study aims to locate the genes related to periodontitis through genome-wide association study (GWAS) in Korean population.

Methods: Total of 677 adults aged 44-88 years were recruited from the Yangpyeong cohort in Korea. The participants did not have self-reported metabolic diseases, cardiovascular diseases or cancer. Periodontitis was assessed using alveolar bone loss from a digital panoramic radiograph and classified into three groups: normal to mild, moderate and severe periodontitis. DNA from blood samples were genotyped using the Illumina Human 1M-duo Beadchip. Multivariable logistic regression analysis in PLINK was applied to examine the SNPs related to periodontitis after controlling for various confounders.

Results: Associations of three SNPs suggested *TENM2* (rs4242220) and *LDLRAD4* (rs12969041, rs2027756) as putative risk genes of chronic periodontitis (p-values$< 1 \times 10^{-5}$). The odds ratio (95% confidence interval [CI]) of *TENM2* was 0.53 (0.40-0.70) for moderate periodontitis and that of *LDLRAD4* was 2.86 (1.92-4.27) for severe periodontitis. Two nonsynonymous SNPs of protein coding region and seven SNPs selected from previous reports

showed nominal association.

Conclusion: Our GWAS supports a previously reported gene of *TENM2* and newly suggests *LDLRAD4*. These two genes' role on lipid metabolism may play a part in the molecular etiology of periodontitis.

Keywords: Periodontitis, GWAS, epidemiology, SNP, Korean

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Contents

I. INTRODUCTION

Periodontitis is a chronic inflammatory disease caused by bacterial infection of the supporting tissues around the teeth (Haffajee and Socransky, 1994, Page et al., 1997). Pathologic destruction of alveolar bone due to periodontitis is an irreversible process and periodontitis is one of the major causes of tooth loss in adults. Severe periodontitis, which may result in tooth loss, is found in 5–20% of most adult populations worldwide (Albandar, 2005, Richards, 2014). The prevalence of periodontitis in Korea is reported to be 10.2% to 55.7% among different age groups (Kim et al., 2014).

Periodontitis is a multifactorial disease and the risk factors for periodontitis includes periodontal microorganisms (Socransky et al., 1998, Socransky and Haffajee, 2005), smoking (Gelskey, 1999), systemic conditions such as cardiovascular diseases (Kim et al., 2010, Sim et al., 2008), and metabolic syndrome (Han et al., 2012). It is also associated with age, sex, socioeconomic status and lifestyle (Genco and Borgnakke, 2013).

Twin studies suggested that genetic factor plays an important role on the development of chronic periodontitis (Michalowicz et al., 2000, Michalowicz et al., 1991). Various candidate genes were selected based on their putative role in the etiology of periodontitis and candidate single-nucleotide polymorphisms (SNPs) were investigated for association with an increased disease risk (Laine et al., 2012). These candidate SNP studies, which were often conducted with small sample sizes, could not provide convincing evidence for the presence of risk alleles in these genes by repeated replication in independent, large case-control populations (Divaris et al., 2013, Schaefer et al., 2013). Genome-wide testing of SNP associations provides an unbiased approach for the identification of risk variants. Thus, further genome-wide association studies (GWAS) in different populations are needed to clarify the association

between genetic factors and periodontitis.

Previously, four GWASs on chronic periodontitis of Europeans and Japanese have been reported (Divaris et al., 2013, Teumer et al., 2013, Rhodin et al., 2014, Shimizu et al., 2015). They suggested more than 30 loci as promising loci and candidate genes for periodontal health and diseases. Risk variants often exert their effects only in a specific situation, such as a specific environmental context or specific combination of given alleles. Moreover, some genetic variation is distinct according to populations with different ancestry, and effect sizes may be larger in certain populations (McCarthy and Hirschhorn, 2008). The Korean population lives in a different environmental context and has a different genetic background compared with North-West Europeans. Thus, other variants than those identified in Europeans may be relevant.

Most risk alleles were identified in North-West Europeans and it is of interest to validate the relevance of the reported risk alleles and risk genes for different ethnicities. Hence, we conducted a GWAS in a Korean cohort.

II.MATERIALS AND METHODS

Study design and ethical consideration

This study was a GWAS for periodontitis. The outcome variable was periodontitis and the main explanatory variable was SNPs.

This study was approved by the Institutional Review Board for Human Subjects of the Seoul National University Dental Hospital (approval number: ERI14001). All participants joined this study voluntarily and provided their written informed consent.

Study subjects

Participants were selected from a cohort of the ongoing project named Korean Genome and Epidemiologic Study (KoGES). Participants were recruited from the cohort of Yangpyeong city in Korea. Initially, a total of 3,262 residents were enrolled as a part of the Yangpyeong health cohort of KoGES from 2004 to 2008. Among them, 2,117 residents were followed up and they received medical and dental examinations from 2010 to 2013, which is the third phase of KoGES. When selecting the participants for DNA analysis, the priority was set to healthy residents without significant systemic diseases. Among them, only 732 participants were enrolled in the GWAS bank of Korea and underwent DNA genotyping. These 732 participants from the initial cohort were adults who did not have self-reported metabolic diseases (type 2 diabetes, hypertension, or hyperlipidemia), cardiovascular diseases (myocardial infarction or stroke), or cancers. They had a normal blood pressure range (SBP <130mmHg and DBP <90mmHg) and fasting glucose level (<126 mg/dL), as measured during health examinations. Finally, 677 participants remained in the final cohort for the GWAS analysis according to the exclusion criteria; 1) participants with any missing variable, 2) remaining natural teeth less than two. In terms of age, sex, smoking, drinking and body mass index characteristics, the final cohort was the same as the initial cohort (Table 1). However, education level differed between the initial and final cohorts.

Population characteristics

Out of 677 participants, those without periodontitis were 60, those with mild, moderate and severe periodontitis were 203, 314, and 100, respectively. Controls (those without periodontitis or mild periodontitis) were 263, while moderate cases were 314 and severe cases were 100

(Table 1). Compared to controls, moderate and severe cases showed greater proportion of males, smokers and drinkers.

Periodontitis assessment

History of periodontitis was assessed by panoramic radiographs obtained from a digital panoramic X-ray machine (Pax-Primo, Vatech Global, Seoul, Korea). Radiographic alveolar bone loss (RABL) which refers to the distance from cement enamel junction (CEJ) to alveolar bone crest was measured on the mesial and distal side of all teeth in 0.1 millimeter level by trained dentists. All measurements were automatically adjusted for the magnification scale by an image viewer program. If there were findings of any unapparent CEJ margins due to prostheses or overlapping of teeth, arbitrary CEJ was applied by referring to the CEJ of adjacent teeth. In the presence of vertical bone defects or differences in buccal and lingual bone levels, the lower level of bone loss was adopted. In terms of the test-retest reliability, RABL showed a kappa value of 0.69 according to the cut-off value of 6mm. Compared to the gold standard of alveolar bone loss measured during periodontal flap surgery, the sensitivity and specificity of RABL was 0.62 and 0.79, respectively (Korea Centers for Disease Control and Prevention, 2011).

Based on the CDC/AAP criteria, we divided the participants into four categories; normal, mild periodontitis (2 or more interproximal sites with 3mm≤RABL<4mm), moderate periodontitis (2 or more interproximal sites with 4mm≤RABL<6mm [not on same tooth]) and severe periodontitis (2 or more interproximal sites with RABL≥6mm [not on same tooth]) (Eke et al., 2012). For GWAS for periodontitis, we defined normal and mild periodontitis as controls, and moderate and severe periodontitis as cases.

Genotyping and quality control

Genomic DNA samples, isolated from peripheral blood drawn from Yangpyeong health cohort participants, were genotyped using the Illumina Human 1M-duo Beadchip (Illumina Inc., San Diego, CA, USA). The Bayesian Robust Linear Modeling using Mahalanobis Distance (BRLMM) genotyping algorithm was used for genotype calling of 1,010,624 SNPs (Rabbee and Speed, 2006). Subjects with genotype accuracies below 98% or high missing genotype call rates (≥4%), high heterozygosity (>30%), or inconsistencies in sex were excluded from subsequent analyses. Pairwise identity-by-state (IBS) was calculated using the PLINK (version 1.07) (Purcell et al., 2007), and highly related individuals with IBS values > 0.80 were excluded. Low minor allele frequency (MAF; <0.01) or significant deviation from Hardy–Weinberg equilibrium (*p-value* <0.01) were excluded, leaving a total of 723,056 SNPs to be examined in 677 individuals.

Statistical analysis

723,056 SNPs were examined for three case-control datasets (both moderate and severe cases versus [vs] controls [414 vs 263], moderate cases vs controls [314 vs 263] and severe cases vs controls [100 vs 263]), as done previously (Divaris et al., 2013).

Each case-control set was analyzed by multivariable logistic regression controlling for age, sex, smoking, drinking, education and body mass index as the covariates. Statistical analyses were performed using PLINK (version 1.07) (Purcell et al., 2007).

Since we didn’t have a chance to do replication, we applied a two-step analysis to increase the confidence to report a true positive association (Schaefer et al., 2010): 1) the first "hypothesis-generating explorative step" in the total population including moderate cases,

severe cases and controls, 2) the subsequent validation step using SNPs that pass the pre-assigned significance threshold in each sub-populations of moderate cases plus controls and severe cases plus controls. Since we could not identify genome-wide significant SNPs (*p*-value $< 5 \times 10^{-8}$), we used a suggestive criterion: *p*-value$< 5 \times 10^{-5}$ for the total population and *p*-value$< 1 \times 10^{-5}$ for sub-populations. We also excluded genes with MAF<0.2 to reduce false positive associations. Sample size for 80% power at alpha = 0.05 is based on YPC parameters including MAFs, effect size and Korean population prevalence of the chronic periodontitis. The effect size applied odds ratios for each gene in our data (Table 2) and the prevalence of the chronic periodontitis was 10.2-55.7% (Kim et al., 2014). Hence, the sample size of each SNP was calculated retrospectively after the analysis.

For more information, nonsynonymous SNPs with amino acid change (*p*-values< 0.01) were also isolated from the GWAS results (Hong et al., 2010). The nonsynonymous SNP is a nucleotide change from major to minor allele that replaced the amino acid residue. It generally assumed that the nonsynonymous SNP may influence on the protein function. Moreover, we performed an association study from selected SNPs. We tested the 42 SNPs from previously reported GWASs (Divaris et al., 2013, Rhodin et al., 2014, Teumer et al., 2013, Shimizu et al., 2015) and two candidate gene SNP studies (Laine et al., 2012, Nikolopoulos et al., 2008). For this, we applied the *p*-value criteria < 0.05 (Hong and Oh, 2012).

Minor allele frequency comparison

We compared the MAFs for our suggestive SNPs with the MAF of the 1000 genome populations of Asians to evaluate the MAF similarity between our GWAS and the 1000 genome populations of Asians.

III.RESULTS

Genome-wide association study

We found no SNP with genome-wide significance (p-value < 5×10^{-8}). Yet, 9 SNPs passed the hypothesis-generating explorative step at p-values < 5×10^{-5} and MAF>0.2. The 9 SNPs (7 genes) were rs4242220 (TENM2), rs12969041 and rs2027756 (LDLRAD4), rs4704706 (RNUSE-1), rs4783276 (CCDH13), rs10219568 (SLC15A5), rs7257867 (NLRP12) rs6876160 and rs6890619 (RASGRF2). However, none of the SNPs showed a statistical significance at p-values <0.05 after the Bonferroni correction.

In the next step, to increase confidence in our finding, we verified if the nine SNPs were also associated in severe and moderate chronic periodontitis independently. In the next sub-population analyses, we used a suggestive criterion at p-values < 1×10^{-5}. Three SNPs passed the criterion (Table 2). Of them, one SNP (rs4242220) was associated with moderate chronic periodontitis and two SNPs (rs12969041, rs2027756) with severe chronic periodontitis. According to Manhattan plots (Figure 1), symbol genes with the suggestive association (p-values < 1×10^{-5}) were two: *TENM2* (teneurin transmembrane protein-2) for moderate cases and *LDLRAD4* (low density lipoprotein receptor class A domain containing 4) for severe cases.

In terms of moderate periodontitis, one SNP (rs4242220 on TENM2) was nominally significantly associated with reduced risk (adjusted odds ratio [AOR] of 0.53, 95% confidence interval [CI]: 0.40-0.70, p-value = 8.97×10^{-6}) (Table 2) which was similar to the crude association (Supplementary 1).

In terms of severe periodontitis, two SNPs (rs12969041 and rs2027756 on LDLRAD4) were nominally significantly associated with increased risk (AOR: 2.86, 95% CI: 1.92-4.27, p-value = 2.79×10^{-7}) (Table 2) which was also similar to the crude association (Supplementary

1). The two SNPs were located on the 5' upstream region of a low density lipoprotein receptor gene (*LDLRAD4*, low density lipoprotein receptor class A domain containing 4) and its R (r square) was 0.963, indicating both SNPs are in complete linkage disequilibrium and show the same association.

Nonsynonymous SNPs

Among three nonsynonymous SNPs with the *p*-values from 1×10^{-5} to 1×10^{-2}, two SNPs had MAF > 0.20. The first SNP (rs16846206) was located on a transporter gene (*SLC9C2* or *NHE-11*, sodium/hydrogen exchanger 11), and the nucleotide substitution from cytosine to guanine replaced the 505^{th} amino acid residue Alanine by Glycine (Table 2). The polyphen-2 predicted the amino acid changes to be benign with the polyphen score 0.167. This SNP was associated with the subpopulation severe periodontitis (AOR: 2.02, 95% CI: 1.42– 2.86, *p*-value = 7.66×10^{-5}), but not with the subpopulation of moderate periodontitis. The other SNP (rs892055) was located on a Rasguanyl nucleotide exchange factor (*RASGRP4*, RasGuanyl Releasing Protein 4), and the substitution from guanine to adenine replaced the 18^{th} Isoleucine by Threonine. The polyphen-2 predicted the amino acid changes to be benign with the polyphen score 0. This SNP was also associated with the subpopulation severe periodontitis (AOR: 0.49, 95% CI: 0.34-0.71, *p*-value = 1.23×10^{-4}), but not with the subpopulation of moderate periodontitis.

Association study of selected candidate SNPs

Out of the 42 SNPs from the previous GWASs and candidate studies, five GWAS SNPs and two candidate gene SNPs showed weak association (*p*-value <0.05) in our study. Among them, interleukin 4 (IL4), B Lymphoid Tyrosine Kinase (BLK) and G protein-coupled receptor 141 (*GPR141*) genes were related with immune response (Table 2).

Minor allele frequency comparison

We compared the minor allele frequencies for our suggestive SNPs with the minor allele frequency of the 1000 genome populations of Asians (Supplementary 2). Allele frequencies for the controls were similar to those of Asians.

IV. DISCUSSION

Our GWAS explored genes associated with periodontitis defined by panoramic alveolar bone loss instead of clinical attachment level and probing depth. Although we found no SNP with genome-wide significance, we found three SNPs on two genes with suggestive association ($1\times10^{-8} < p\text{-value} < 1\times10^{-5}$). One SNP (rs4242220 on TENM2) showed a tendency of reduced periodontitis risk with adjusted odds ratio (AOR) of 0.53 (p-value = 8.97×10^{-6}); remaining two SNPs (rs12969041 and rs2027756 on LDLRAD4) showed a tendency of increased periodontitis risk with AOR of 2.86 (p-value = 2.79×10^{-7}). These candidate genes showed association with lipid regulation. Considering that dyslipidemia is associated with higher risk of periodontitis, our findings can explain the possible genetic factors for the underlying mechanism.

TENM2 gene encodes teneurintransmembraneprotein-2 which is a membrane-bound transcriptional regulator. A transcription factor zic-1 is influenced by the intracellular domain of *TENM2*, and zic-mediated transcription from the apolipoprotein E (APOE) promoter can be inhibited (Bagutti et al., 2003). The APOE is well-known for its role in lipoprotein metabolism (Viiri et al., 2006). A knockout mice study of *APOE* gene showed that hyperlipidemia disrupted cytokine production pattern in response to *Porphyromonas gingivalis* (Lei et al., 2013). Our

results showed that the minor allele of rs4242220 located on *TENM2* intron was associated with decreased risk of moderate periodontitis. A possible mechanism is that the minor allele decreases the activity of *TENM2* gene and promotes the *APOE* gene expression, which increases the immunity against *P. gingivalis* and subsequently, decreases periodontitis. An American GWAS also showed that subgingival *Aggregatibacter actinomycetemcomitans,* which is known as one of periodontal pathogens, was associated with *TENM2* (a synonym of '*ODZ2'*) (Divaris et al., 2012). Hence, our data supports the previous GWAS that *TENM2* is a periodontal risk factor. Further studies for *TENM2* are indicated to elucidate the mechanism of its link on chronic periodontitis.

On the other hand, the *LDLRAD4* gene encodes the low density lipoprotein receptor (LDLR) class A domain containing 4. The LDL receptor binds LDL and transports it into cells by acidic endocytosis. The LDLR class A domains form the binding sites for LDL and calcium. Numerous familial hypercholesterolemia mutations of the LDL receptor can alter the calcium coordinating residue of LDL-A domains (Yamamoto et al., 1984). Therefore, the *LDLRAD4* gene might act to regulate cholesterol homeostasis in mammalian cells. This gene is also important in the regulation of serum lipid levels, and periodontal infection of *P. gingivalis* in mice was associated with elevated LDLR expression and circulatory LDL cholesterol (Miyazawa et al., 2012). Our results suggest that the SNP rs12969041 or rs2027756 may tag a variant of *LDLRAD4* gene and leads to a high concentration of serum lipids. Thus, individuals with these minor alleles were more likely to have severe periodontitis.

Previous GWASs found suggestive association ($1x10^{-5}$< *p*-value <$1x10^{-8}$) of genes on periodontitis or periodontal microorganisms. Among them, some genes such as *NIN, EMR1, KCNK1, UHRF2, FBXO38, HTR4* and *JDP2* were replicated among different populations with similar ethnicity (Divaris et al., 2012, Divaris et al., 2013, Rhodin et al., 2014). However, our

data found suggestive association on only *TENM2 (ODZ2)* which was associated with *A. actinomycetemcomitans* (Divaris et al., 2012). Moreover, a Japanese GWAS (Shimizu et al., 2015) did not replicate any significant gene of the Western GWAS results. Hence, it is speculated that there would be an ethnic difference in GWAS for periodontitis.

According to the analysis of nonsynonymous SNPs from this study, *SLC9C2* gene encodes solute carrier family 9, member C2 and is also known as sodium/hydrogen exchanger 11 (NHE11). It is well established that activation of the Na-H exchanger NHE11 and increased intracellular pH (pHi) are early and universal responses to mitogens and have permissive effects in promoting cell proliferation (Putney and Barber, 2003). Our results indicate that individuals with minor allele of the SNP rs16846206 located on *SLC9C2* gene were more likely to have severe periodontitis. Notwithstanding these results, the role of *SLC9C2* gene on periodontitis is still unclear.

In our association study of selected SNPs, *IL4*, *GPR141* and *BLK* were nominally associated with chronic periodontitis and the association was not statistically significant at *p*-value < $1x10^{-5}$. *IL4* encodes the interleukin which is a pleiotropic cytokine involved in various immune responses, inflammatory processes, and hematopoiesis. *BLK* encodes B lymphoid tyrosine kinase which is a nonreceptor tyrosine-kinase of the *src* family of proto-oncogenes that are typically involved in cell proliferation and differentiation (Delgado-Vega et al., 2012). The protein has a role in B-cell receptor signaling and B-cell development. Our results suggest that those with minor allele located on *IL 4*or *BLK* genes were associated with the suppression of periodontitis. Our result showed increased risk for periodontitis with the *GPR141* gene, which supports the result of Japanese study (Shimizu et al., 2015). *GPR141* in bone marrow and cancer cells (Fredriksson et al., 2003) is a member of the rhodopsin family of G protein–coupled receptors that include chemokine-like receptors. A recent study showed that *GPR141*

was down-regulated in peripheral arterial disease patients (Masud et al., 2012). Thus, *GPR141* might modify susceptibility to periodontitis by impairing immunologic responses against periodontal pathogens (Shimizu et al., 2015). More studies are needed to clarify this mechanism.

Our study had some limitations: since the population size was relatively smaller than the conventional GWAS sample size (n ~ 10000) for complex traits, we assumed that the suggestive results ($p < 1 \times 10^{-5}$) might be interpreted as having genome-wide significance. Our data did not include the replication sample for the confirmation of genes associated with periodontitis. Replication is thought to be a gold standard to reduce false positive errors for GWAS (Chanock et al., 2007). Some difficulties of replication have arisen alternative methods such as studies of multiple level/sources (Liu et al., 2008). Though our results are from two step analyses to reduce false positive results, further replication or a study on different level (RNA, protein, etc.) is indicated.

To the best of our knowledge, our GWAS for chronic periodontitis is the first report for Korean population. In terms of MAFs, the consistent allele frequency between Korean controls and Asians imply that our association results can be explored in other Asian populations. We hope that our report triggers further genetic studies on oral diseases among Asian researchers. Recent genome-wide exploration found and validated the interaction between gene and sex (Freitag-Wolf et al., 2014) on periodontitis. We think further studies on the interaction between genes and systemic health-related factors such as obesity and atherosclerosis could expand the knowledge on the relation between systemic health and periodontitis.

V. CONCLUSION

Overall, our GWAS for periodontitis showed that some suggestive genes were independently associated with periodontitis for the first time in Korean population. Our data add evidence to the previously reported GWAS association of *TENM2* and support its role in the etiology of CP. Moreover, our data suggests that the process of lipid metabolism could play an important role in the pathogenesis of CP. However, further replication and functional studies are indicated to clarify the genes related to periodontitis and their mechanism.

Table 1. Characteristics of chronic periodontitis cases and controls

Variables	Initial cohort (n=732)	Final cohort (n=677)	*p*-value	Control[§] (n=263)	Cases[¶]		*p*-value
					Moderate (n=314)	Severe (n=100)	
Age, year (range)	63.0 (44 - 91)	62.5 (44 - 88)	0.944[*]	61.6 (45 - 85)	62.7 (44 - 88)	64.3 (44 - 83)	0.053[‡]
Sex, n (%)			0.373[†]				**<0.001**[†]
Male	267 (36.5)	250 (37.0)		62 (23.6)	138 (43.9)	50 (50)	
Female	465 (63.5)	427 (63.0)		201 (76.4)	176 (56.1)	50 (50)	
Smoking, n (%)			0.982[†]				**<0.001**[†]
No	520 (71.0)	481 (71.0)		217 (82.5)	240 (65.0)	60 (60.0)	
Yes	212 (29.0)	196 (92.5)		46 (17.5)	110 (35.0)	40 (40.0)	
Drinking, n (%)			0.309[†]				**0.021**[†]
No	351 (48.0)	321 (47.4)		141 (53.6)	141 (44.9)	39 (39.0)	
Yes	381 (52.0)	356 (62.6)		122 (46.4)	173 (55.1)	61 (61.0)	
Education, n (%)			**<0.001**[†]				0.576[†]
<Middle school	514 (70.2)	464 (68.5)		179 (68.1)	212 (67.5)	73 (73.0)	
>High school	218 (29.8)	213 (31.5)		84 (31.9)	102 (32.5)	27 (27.0)	
BMI, mean (standard deviation)	24.4 (3.0)	24.5 (3.0)	0.468[*]	61.6 (10.0)	62.7 (9.2)	64.3 (9.8)	0.053[‡]

Values were obtained from T-test[*], chi-square test[†], and ANOVA[‡].

[§]Control denotes normal or mild periodontitis.

[¶]Cases denote chronic periodontitis defined by CDC/AAP criteria.

BMI denotes body mass index (kg/m^2).

Bold denotes statistical significant at *p*-value<0.05.

Table 2. Adjusted association of genome-wide association study for chronic periodontitis cases and controls: GWAS top signal SNPs (*p*-value< 1×10^{-5}), nonsynonymous SNPs (*p*-value< 0.01), association study of selected SNPs (*p*-value< 0.05)

Chr	SNP	BP	Remarks	Gene Symbol	Function	MAF†	Alleles		Moderate + Severe				Sample Size	Moderate				Severe			
							Major	Minor	OR	L95	U95	P		OR	L95	U95	P	OR	L95	U95	P
GWAS top Signal SNPs ($p < 1 \times 10^{-5}$)																					
5	rs4242220	166677319		TENM2	Intron	0.24	A	C	**0.53**	**0.41**	**0.69**	**2.84E-06**	96 -105	**0.53**	**0.40**	**0.70**	**8.97E-06**	0.54	0.36	0.81	0.003
18	rs12969041	13181184		LDLRAD4	5' upstream	0.28	G	A	1.79	1.36	2.35	2.81E-05	128-151	1.56	1.16	2.08	0.003	**2.86**	**1.92**	**4.27**	**2.79E-07**
18	rs2027756	13180107		LDLRAD4	5' upstream	0.28	G	A	1.79	1.36	2.35	3.06E-05	128-151	1.55	1.16	2.08	0.003	**2.86**	**1.92**	**4.27**	**2.79E-07**
Nonsynonymous SNPs (p<0.01)																					
1	rs16846206	171783494	c. C1514G p. Ala505Gly*	SLC9C2	exon (coding)	0.38	C	G	1.35	1.07	1.71	0.011	379-397	1.20	0.93	1.54	0.153	2.02	1.42	2.86	7.66E-05
19	rs892055	43604604	c. G53A p. Ile18Thr*	RASGRP4	exon (coding)	0.44	G	A	0.74	0.59	0.93	0.010	351-360	0.83	0.65	1.05	0.123	0.49	0.34	0.71	1.23E-04
Association study from selected SNPs																					
3	rs1346834	159973114	Teumer et al., 2013	MFSD1	5' upstream	0.34	A	G	0.71	0.55	0.91	0.007	376-388	0.74	0.57	0.96	0.024	0.63	0.42	0.94	0.022
5	rs2243250	132037053	Laine et al., 2012	IL4	5' upstream	0.18	A	G	0.65	0.49	0.87	0.004	279-307	0.68	0.50	0.93	0.015	0.56	0.35	0.90	0.016
5	rs2070874	132037609	Laine et al., 2012	IL4	5' UTR	0.18	A	G	0.66	0.50	0.89	0.006	300-329	0.69	0.51	0.94	0.020	0.58	0.36	0.92	0.021
5	rs294958	151548292	Teumer et al., 2013	NMUR2	3' downstream	0.36	G	A	1.29	1.02	1.63	0.034	534-556	1.28	1.00	1.65	0.051	1.35	0.92	1.88	0.134
7	rs2392510	37746569	Shimizu et al., 2015	GPR141-NME8	Intron	0.46	C	T	1.48	1.17	1.87	9.48E-4	321-351	1.51	1.18	1.93	0.001	1.37	0.97	1.92	0.070
8	rs2243407	11517866	Teumer et al., 2013	BLK	3' downstream	0.34	G	A	0.73	0.58	0.93	0.010	372-383	0.74	0.58	0.95	0.019	0.60	0.48	0.99	0.046
16	rs11866781	7079174	Teumer et al., 2013	A2BP1 (RBFOX1)	Intron	0.28	G	A	1.26	0.98	1.62	0.077	810-854	1.32	1.01	1.72	0.045	1.08	0.74	1.59	0.688

Chr: chromosome, SNP: dbSNPrsID, BP: base pair, OR: odds ratio, L95: 95% confidence interval lower boundary, U95: 95% confidence interval upper boundary, P: *p*-value.

OR: adjusted for age, sex, smoking, drinking, education and body mass index.

Bold denotes statistically significant at *p*-value < 1×10^{-5}.

*: Amino acid changes

† Minor allele frequency of total (n=677) population

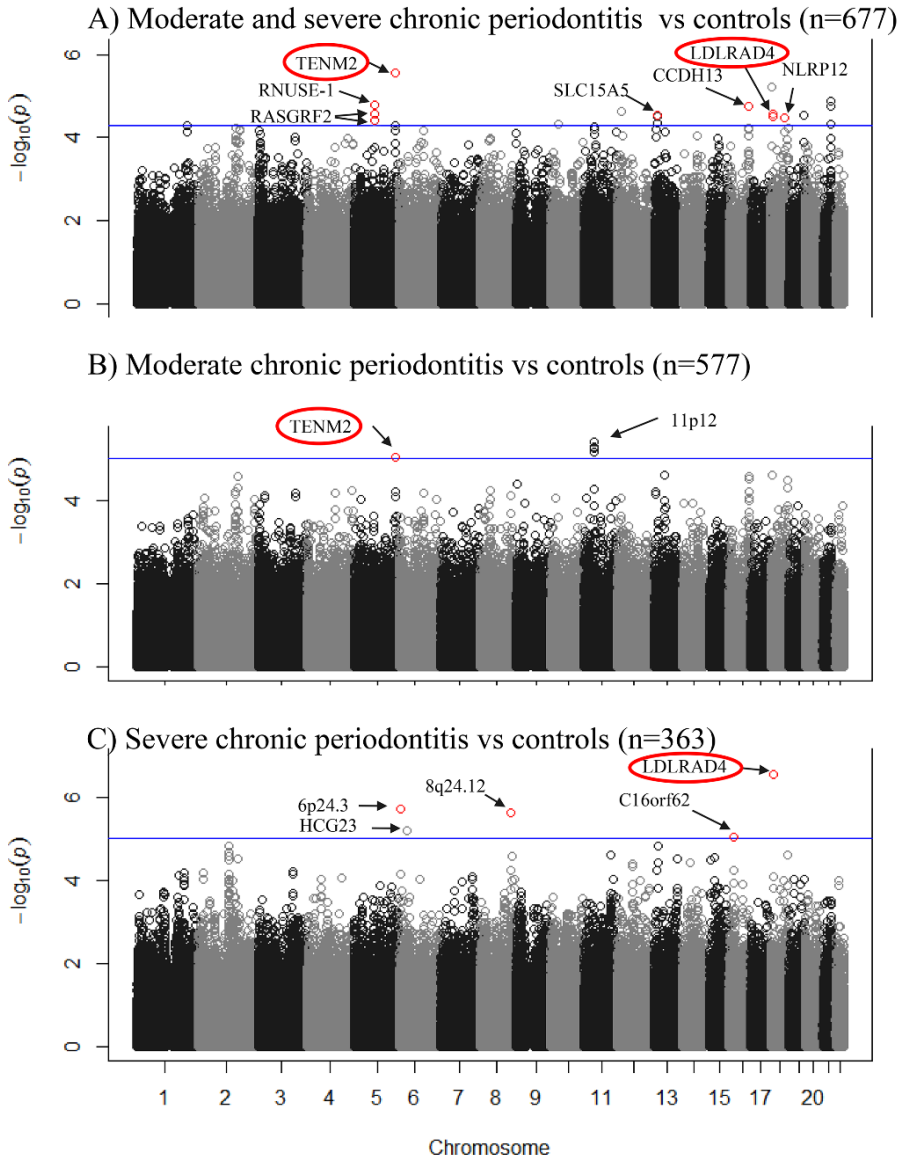

Figure 1. Manhattan plots of GWASs for chronic periodontitis cases and controls. A) moderate plus severe cases results, B) moderate cases results, C) severe cases results. SNPs are plotted based on their physical chromosomal positions (horizontal axis) together with their $-\log_{10}$ (*p*-values) in the GWAS (vertical axis). The blue line indicates the suggestive association threshold at *p*-value= 5×10^{-5} for moderate and severe periodontitis (A), and *p*-value = 1×10^{-5} for moderate or severe periodontitis (B and C). The red dotted gene denotes MAF>0.2. The red circled gene denotes the validated genes by two-step analysis.

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국문초록

한국인의 치주질환에 대한 전장유전체연관분석

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연구목적

본 연구는 전장유전체분석을 통하여 한국인에서 치주질환과 연관 있는 유전자를 발굴하는 것을 목적으로 한다.

연구대상 및 방법

본 연구의 대상자는 양평 지역의 농촌 코호트 연구에 참여한 732명 중에서 결측치가 있는 자를 제외한 677명이 선정 되었고, 대상자의 연령은 44세에서 88세까지 분포하였다. 대상자는 대사증후군, 심혈관질환, 암과 같은 전신질환이 없는 건강한 자로 선정되었다. 치주질환은 디지털 파노라마 방사선사진을 통해 치조골소실을 평가함으로 정의되었고, 정상, 경증, 중등도, 중증 치주질환으로 분류되었다. 혈액 시료에서 DNA가 추출되어 Illumina Human 1M-duo Beadchip 를 통해 전장유전체분석을 시행하였다. 치주질환과 연관 있는 SNP를 발굴하기 위해 PLINK 프로그램을 이용하여 다변수 로지스틱 회귀분석을 시행하였고, 연령, 성별, 교육수준, 흡연, 체질량지수로 보정하였다.

결 과

두 가지 유전자에 있는 세 SNP인 *TENM2* (rs4242220) 와 *LDLRAD4*

(rs12969041, rs2027756)가 치주질환과 연관 있는 후보유전자로 분석되었다. (p value< 1×10^{-5}) 각 유전자의 교차비 (95% 신뢰구간 [CI])는 중등도 치주질환에 대하여 TENM2 는 0.53 (0.40−0.70) 이었고 중증 치주질환에 대하여 LDLRAD4 은 2.86 (1.92−4.27) 이었다. 기존에 알려져 있는 치주질환 연관 유전자 중, 단백질을 발현시키는 두 개의 nonsynonymous SNP에서 명목적 연관성이 나타났다.

결론

본 연구 결과, TENM2가 치주질환과 연관 있다는 기존의 전장유전체연구를 확인하였고, 또한 LDLRAD4와 같은 새로운 유전자를 치주질환과 연관 있는 후보유전자로 제시하였다. 두 가지 유전자는 지질 대사에 관여하여 치주질환 발생에 연관된 것으로 사료된다.

주요어: 치주질환, 전장유전체연구, 역학, SNP, 한국인

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