Recapitulation of Genome-wide Association Study on Chronic Periodontitis in a Korean Population

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Periodontitis is the major causation of tooth loss in the elderly population. Multiple risk factors include oral microorganisms, smoking, metabolic syndrome, and genetic factors influence periodontitis development. In this study, we conducted a replication study of using previous Korean GWAS results by examining an independent population. The study population was recruited from Mir Dental Clinic, Daegu, Korea. In total, 93 samples were evaluated from July 2016 to January 2017. The sample groups include relatively older patients (>60 years) with no periodontitis (n = 31) and relatively younger patients (range 40–60 years) with severe periodontitis (n = 62). A total of seven markers which were previously reported to be associated with periodontitis were genotyped. Among the seven SNPs, rs16846206 and rs2392510 showed a significant association by logistic regression analysis and Chi square test, respectively. The former SNP showed significant association with severe periodontitis, whereas this study also showed same tendency in which individuals with the minor allele are significantly more frequent in cases than those in controls. The SNP is located on a coding gene (SLC9C2), where the alanine residue 505 is replaced by glycine (Ala505Gly). The later SNP was significant when differed between case and control groups, but there was no significance by logistic regression analysis when controlled for age and sex as covariant. Although the study population size examined in the current study was relatively smaller compared to previous studies, our results implicated that at least the two SNPs (rs16846206 and rs2392510) might be important candidates for the further genetic study.

Key Words: Periodontitis, GWAS, Allele, SNP

Periodontitis is the major causation of tooth loss in the elderly population (Haffajee and Socransky 1994; Page et al., 1997). Worldwide, 5–10% of the adult population are affected by severe periodontitis (Albandar, 2005; Richards, 2014). In Korea, the prevalence is reported to be between 10–55% among the different age groups (Kim et al., 2014). Multiple risk factors including oral microorganisms, smoking, metabolic syndrome, genetic factors are known to increase periodontitis (Socransky et al., 1998; Gelskey, 1999; Michalowicz et al., 2000; Socransky and Haffajee, 2005; Sim et al., 2008; Kim et al., 2010; Han et al., 2012). In addition, demographic factors such as age, sex, socioeconomic status and lifestyle may also influence periodontitis (Genco and Borgnakke, 2013). Previously, five genome-wide association studies were published from European, Japanese and Korean populations (Divaris et al., 2013; Teumer et al., 2013; Rhodin et al., 2014; Shimizu et al., 2015; Hong et al., 2015). In the Korean study, three genome-
wide suggestive SNPs (1 SNP in TENM2 gene and 2 SNPs in LDLR4A gene), two non-synonymous SNPs (1 SNP in SLC9C2 gene, 1 SNP in RASGRP4 gene) and 7 replicated SNPs were reported as well as from European and Japanese studies (Hong et al., 2015). Although previously published study was the first GWAS of Korean periodontitis, there are no other independent studies that confirmed these findings. In the current report, we conducted a replication study for the previous Korean GWAS results by using an independent population.

The study population was recruited from Mir Dental Clinic, Daegu, Korea. A total of 93 samples were collected from July 2016 to January 2017. The samples were from relatively patients (>60 years old) who have no periodontitis (n = 31) and from relatively younger patients (40~60 years old) who have severe periodontitis (n = 62). History of periodontitis was assessed by panoramic radiographs obtained from a digital panoramic X-ray. More than 50% radiographic alveolar bone loss was designated as chronic periodontitis. All participants agreed to the study and gave a formal written consent. This study was approved by an Institutional Review Board.

For genotyping analysis, oral swab samples were obtained and extracted the DNA by Exgene™ Tissue SV (GeneAll, Seoul, Korea). 125 ng DNA was mixed with 2.5 μl of TaqMan OA GT Master Mix and used for the realtime quantitative PCR by TaqMan assay (Applied Biosystems, Calsbug, CA). The TaqMan probe information is listed in Table 2. The PCR mixture was loaded on the Open Array by using Accufill automated machine (Applied Biosystems, Calsbug, CA). The reaction ready Open Array chip was used in Quant Studio 12K (Applied Biosystems, Calsbug, CA). The realtime PCR reactions were performed at 95°C for 10 min, 40 cycles at 92°C for 15 sec, at 60°C for 1 sec. The genotyping was determined by the Vic and/or FAM fluorescent dye intensity. Chi-square test and logistic regression analysis were used to analyze the association between SNP and periodont-

Table 1. Sample characteristics

| Variables                   | Values                  |
|-----------------------------|-------------------------|
| Total recruited samples     | 93                      |
| Sample size (n)             | 93                      |
| Age (mean ± SD, years old)  | 50±13                   |
| Male (%)                    | 43                      |
| Periodontitis (Case %)      | 67                      |
| Controls (n)                | 31                      |
| Age (mean ± SD, years old)  | 59.3±12.8               |
| Male (%)                    | 38.4                    |
| Case (n)                    | 62                      |
| Age (mean ± SD, years old)  | 46.1±10.24              |
| Male (%)                    | 45.7                    |

Table 2. SNP assay ID and TaqMan probe sequences

| SNP          | Assay ID   | Probe site   | Strand | VIC | FAM | Sequence                  |
|--------------|------------|--------------|--------|-----|-----|---------------------------|
| rs4242220    | C___27106157_10 | Chr5:166744741 | +      | G   | T   | AAGATGTCCCTGTAATGCCCATTTCCTG[G/T] |
| rs12969041   | C___1113968_10 | Chr18:13191184 | +      | C   | T   | GGGAGGCCAGCCGGAGGCTCTCC[T/C][G]   |
| rs16846206   | C___32633727_10 | Chr1:17351687 | +      | C   | G   | ATGCAATCTCGTCTCCCTCCACCA[ATGA]   |
| rs1346834    | C___8238896_20 | Chr3:158490420 | +     | A   | G   | TCGCTATGATTTGAGGAGATTCTGTTT       |
| rs2243250    | C___16176216_10 | Chr5:132009154 | +     | C   | T   | ACACCTAAAACCTGCGGGAGGAAACTTG[T/C]  |
| rs294958     | C___3169965_20 | Chr5:151568099 | +     | A   | G   | CAGCTGTAAGTGTGAATTGGTGAATGGGCA   |
| rs2392510    | C___11835423_10 | Chr7:37746569 | +     | C   | T   | AGAGCAACACCCACCCACATTGTTTG[A/T]   |

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tis case groups and control groups. For logistic regression analysis, age and sex was set as the covariant. A significant association were determined when $P < 0.05$.

The population characteristics of recruited samples are described in Table 1. We hypothesized that elder participants without periodontitis might be protective to the disease, and younger participants with periodontitis might have genetic risk to the disease. The average ages were 59.3 in controls and 46.1 in cases. The male sex proportions were 38.4% in controls, and 45.7% in cases. A total of seven markers which were previously reported were genotyped (see Table 3). The genotype frequency of the current study samples were similar to previously reported Korean results (Hong et al., 2015). These results indicated that, though the sample size used in the current study is smaller, the genotype distribution was similar to the general Korean population frequency.

Among the seven SNPs, rs16846206 and rs2392510 showed a significant association by logistic regression analysis and Chi-square test, respectively (Table 4). The former SNP was previously significantly increased in the severe periodontitis group. The current study also showed the same tendency in which individuals with the minor allele are significantly more frequent in cases than those in controls. The SNP is located on a coding gene (SLC9C2), and alanine residue 505 replaced by glycine (Ala505Gly). The later SNP was significant different from case and control groups, but

### Table 3. SNPs examined

| SNP      | Chr | Pos      | Gene     | Major allele | Minor allele | Minor Allele Frequency | Reference                        |
|----------|-----|----------|----------|--------------|--------------|------------------------|----------------------------------|
| rs4242220 | 5   | 167317736 | TENM2    | T            | G            | 0.24                   | 0.22                             | 0.26                             | 0.6                             | Hong et al., 2015                |
| rs12969041 | 18  | 131911185 | LDLRAD4  | C            | T            | 0.28                   | 0.31                             | 0.26                             | 0.46                             | Hong et al., 2015                |
| rs16846206 | 1   | 173547732 | SLC9C2   | C            | G            | 0.38                   | 0.36                             | 0.35                             | 0.74                             | Hong et al., 2015                |
| rs1346834 | 3   | 158772631 | MFSI1    | A            | G            | 0.34                   | 0.34                             | 0.37                             | 0.67                             | Hong et al., 2015, Teumer et al., 2013 |
| rs2243250 | 5   | 132673462 | IL4      | T            | C            | 0.18                   | 0.21                             | 0.22                             | 0.86                             | Hong et al., 2015, Laine et al., 2012 |
| rs294958  | 5   | 152188538 | NMUR2    | G            | A            | 0.36                   | 0.37                             | 0.33                             | 0.42                             | Hong et al., 2015, Teumer et al., 2013 |
| rs2392510 | 7   | 37706967  | GPR141-NME8 | T         | C            | 0.46                   | 0.44                             | 0.47                             | 0.54                             | Hong et al., 2015, Shimizu et al., 2015 |

### Table 4. Genetic association

| Alleles   | Control n (frequency) | Case n (frequency) | Chi-square test | Logistic regression age and sex adjusted | Odds 95% CI | P-value |
|-----------|-----------------------|--------------------|-----------------|------------------------------------------|-------------|---------|
| rs4242220 | T G                   | 21 (0.68)          | 10 (0.32)       | 0 (0)                                    | 35 (0.56)   | 24 (0.39) | 3 (0.05) | 2.173 | 0.337 | 1.808 | 0.674~4.850 | 0.239 |
| rs12969041| C T                   | 12 (0.39)          | 18 (0.58)       | 1 (0.03)                                  | 32 (0.52)   | 23 (0.37) | 7 (0.11) | 4.351 | 0.114 | 0.816 | 0.368~1.808 | 0.616 |
| rs16846206| C G                   | 15 (0.48)          | 15 (0.48)       | 1 (0.03)                                  | 21 (0.34)   | 32 (0.52) | 9 (0.15) | 3.618 | 0.164 | 0.372 | 1.377~10.970 | 0.01 |
| rs1346834 | A G                   | 13 (0.42)          | 14 (0.45)       | 4 (0.13)                                  | 26 (0.42)   | 31 (0.50) | 5 (0.08) | 0.6   | 0.741 | 1.158 | 0.526~2.551 | 0.715 |
| rs2243250 | T C                   | 22 (0.71)          | 8 (0.26)        | 1 (0.03)                                  | 36 (0.58)   | 23 (0.37) | 3 (0.05) | 1.467 | 0.48 | 1.791 | 0.714~4.493 | 0.214 |
| rs294958  | G A                   | 13 (0.42)          | 16 (0.52)       | 2 (0.06)                                  | 23 (0.37)   | 29 (0.47) | 10 (0.16) | 1.725 | 0.422 | 1.695 | 0.772~3.722 | 0.189 |
| rs2392510 | T C                   | 4 (0.13)           | 18 (0.58)       | 9 (0.29)                                  | 25 (0.40)   | 28 (0.45) | 9 (0.15) | 7.928 | 0.019 | 0.51 | 0.244~1.068 | 0.074 |


the significance disappeared when using logistic regression analysis with controlling age and sex as the covariants. The 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 showed a weak association between GPR141 SNP and periodontitis, and this result supports the previous result in Japanese and Korean studies (Shimizu et al., 2015; Hong et al., 2015). GPR141 is a member of the rhodopsin family of G protein-coupled receptors that include chemokine-like receptors (Fredriksson et al., 2003). A recent study showed that GPR141 was downregulated in peripheral arterial disease patients (Masud et al., 2012). Thus, GPR141 might modify susceptibility to periodontitis by impairing immunological responses against periodontal pathogens (Shimizu et al., 2015). However, further studies are needed to clarify the mechanism of both SLC9C2 and GPR141 at the periodontitis risk. The current study has some limitations as the population size was relatively smaller than previously published studies. However, our results implicated that at least two SNPs (rs16846206 and rs2392510) might be the important candidates for further genetic study.

**CONFlict OF INTEREST**

The authors have no conflicts of interest to disclose.

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http://dx.doi.org/10.15616/BSL.2017.23.2.133

Cite this article as: Park BR, Ma JK, Park KB, Hong KW. Recapitulation of Genome-wide Association Study on Chronic Periodontitis in a Korean Population. Biomedical Science Letters. 2017. 23: 133-137.