Single nucleotide polymorphisms of complement component 5 and periodontitis

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Background and Objective: Polymorphisms of host defence genes might increase one's risks for periodontitis. This study investigated whether tagging single-nucleotide polymorphisms (SNPs) of the gene encoding complement component 5 (C5) are associated with periodontitis in a Hong Kong Chinese population.

Material and Methods: Eleven tagging SNPs of 229 patients with at least moderate periodontitis and 207 control subjects without periodontitis were genotyped with an i-plexGOLD MassARRAY mass-spectrometry system (Sequenom, San Diego, CA, USA).

Results: Genotype AG of SNP rs17611 was more prevalent in the periodontitis patient group than in the controls (54.6% vs. 41.7%, $p = 0.007$). The haplotype CGCA of the haplotype block consisting of rs1035029, rs17611, rs25681, and rs992670 was significantly associated with periodontitis in a dominant model ($p = 0.001$). The SNP rs17611 showed high linkage disequilibrium with rs1035029, rs25681, and rs992670. Smoking was also significantly associated with periodontitis ($p = 0.006$).

Conclusion: The tagging SNP rs17611 of the C5 gene and smoking may be associated with periodontitis among the Hong Kong Chinese population.

Periodontitis is a complex human inflammatory disease that is caused by dental plaque, and its clinical manifestations are determined by both environmental influences on and genetic makeup of affected individuals. Polymorphisms of host defence genes might also increase the risk of periodontitis (1).

The complement system is a lytic effector system that protects the host against microbial pathogens and acts as a key link between innate and specific immune responses (2, 3), and has been implicated in the pathogenesis of periodontitis. The complement components have been detected in the gingival cervical fluid collected from the periodontitis patients (4, 5). It has been reported that the increased complement cleavage was associated with increased severity of inflammation and periodontal destruction (6). The complement component deficiencies seemed associated with severe chronic periodontitis (7). Complement component 5 (C5) is a pivotal element in the complement system therefore could take part in the pathogenesis of periodontitis. It is cleaved by C5 convertase to yield the C5b fragment and the anaphylatoxin C5a. The latter binds to the G protein-coupled receptor C5aR to trigger intracellular signalling, which results in chemotaxis, respiratory burst, and release of proinflammatory mediators from granulocytes (8, 9). The C5b fragment combines with complement components C6 and C7 to initiate the formation of the membrane attack complex (MAC) in the membrane of invading microorganisms (10).

Because haplotype and tagging polymorphisms of C5 may be associated with some chronic inflammatory diseases such as liver fibrosis, rheumatoid arthritis, and bronchial asthma (11-16), C5 polymorphisms may also be important in chronic inflammatory oral diseases such as periodontitis. Yet, published data on the relevance of C5 polymorphisms in periodontitis are not yet available.

The objective of this study was to screen tagging single-nucleotide polymorphisms (SNPs) of the C5 gene in Hong Kong Chinese patients with moderate to severe periodontitis and in periodontitis-free controls. We investigated whether there were any genetic variations in the C5 genes of these two groups and tested whether such variations, together with other possible risk factors such as smoking status, age and sex, were associated with periodontitis.

Materials and methods

This case-control study was approved by the Ethics Committee of the Faculty of Dentistry, The University of Hong Kong. Written informed consent was obtained from all participants.

Study participants

Participants were recruited from new patients attending the Primary Care Clinic, Prince Philip Dental Hospital (PPDH), Faculty of Dentistry, The University of Hong Kong, from May 2005 to August 2007. Patients' records and radiographs were screened within 1 month of first attendance and potentially eligible patients were invited to attend a clinical examination. Demographic information and medical and dental histories were obtained from patients' records, supplemented by information obtained during the day of the clinical examination. Race and ethnicity were self-reported, with a participant being considered Chinese if his or her biological parents, grandparents, and great grandparents were all reported to be ethnic Chinese. Smoking history was self-reported; patients who currently smoked or who had quit within 12 months were considered to be smokers and those who had never smoked or who had quit for more than 12 months were considered to be non-smokers. All participants needed to be systemically healthy, so those with systemic conditions including cardiovascular diseases, hypertension, liver diseases, kidney diseases, blood disorders, diabetes mellitus, autoimmune diseases, and malignant tumours were excluded, as were pregnant females.

Periodontitis-free and periodontitis groups were defined on the basis of both radiographs and findings of clinical examinations. Cases or periodontitis subjects

were Chinese patients aged 18 to 60 years with radiographic evidence of at least moderate periodontitis, according to the following criteria: the orthopantomogram (OPG) taken at the first visit to PPDH before recruitment showed more than 50% alveolar bone loss at more than 30% of sites, as measured with a Schei ruler without using the 1mm space (each tooth contributed to a mesial and a distal site) (17), and subsequent clinical periodontal examination showed at least two teeth in each quadrant that had a probing depth of ≥5 mm and that bled on probing. All the periodontitis subjects showed clinical and radiographic signs of attachment loss and bone loss, respectively, and were further classified into aggressive periodontitis or chronic periodontitis as described follows. According to the 1999 periodontal disease classification criteria (18), patients ≤35 years of age and systemic healthy but experiencing over 30% sites with over 5 mm clinical attachment loss were classified as aggressive periodontitis (AP) while the remaining subjects over 35 years old who fulfilled our case recruitment criteria and self-reported nil periodontal disease history before 35 years were classified as chronic periodontitis (CP). Controls were Chinese patients aged 18 to 60 years without periodontitis, according to the following criteria: the OPG taken at recruitment showed no sites with more than 15% bone loss or any radiographic evidence of furcation involvement (each tooth contributed to a mesial and a distal site), subsequent clinical periodontal examination confirmed there were

no sites with a probing depth of more than 4 mm and gingival recession of more than 2 mm, and there was no history of tooth loss due to periodontal diseases. Control or periodontitis-free subjects included individuals periodontally health and those with gingivitis. Bleeding on probing (BOP) was not recorded in the control group.

DNA isolation and genotyping

Ten millilitres of venous blood was obtained from each participant and stored in ethylene-diamine-tetra-acetic acid at -70°C until DNA extraction. QIAamp DNA blood mini-kits (Qiagen, Hilden, Germany) were used to extract genomic DNA. Tagging SNPs of the C5 gene were selected by using the SNP Tagging Wizard of SNPbrowser software version 3.5 (Applied Biosystems, Foster City, CA, USA). The reason to choose tagging SNPs was to select a minimum informative subset of SNPs and eliminate redundant information due to strong linkage disequilibrium among the SNPs in the region. Therefore the SNPs studied here can provide some level of information of the SNPs not selected in this study. Tagging SNPs were those left after eliminating all the SNPs with reported minor allele frequencies under 10% and reported 100% linked with other SNPs in the same region. SNP sequences were checked in the RealSNP Assay Database (Sequenom, San Diego, CA, USA). Genotyping was performed with the i-plexGOLD genotyping assay of the

MassARRAY mass-spectrometry system, following the protocol recommended by the manufacturer (Sequenom).

Quality control

As recommended in the genotyping protocol, all template DNA samples needed to have a ratio of spectrophotometer readings at 260 nm and 280 nm (A_{260}/A_{280}) of between 1.7 and 2.0. Samples were then aliquoted in 96-well plates. For each well of DNA to be tested, five duplicate wells and one water well served as internal and negative controls, respectively. Before genotyping, DNA samples and randomly selected positive controls were subjected to electrophoresis in 1% agarose gel to confirm the quality and quantity of genomic DNA. After genotyping by iPLEX, 25 random samples of selected SNPs were subjected to concordance testing by direct sequencing to ensure that the genotyping data were reliable.

Statistical analyses

Statistical analysis was performed with the Statistical Package for the Social Sciences 15.0 (SPSS, Chicago, IL, USA). Differences between the distributions of sex, and reported smoking habit among periodontitis and periodontitis-free individuals were tested with the chi-square test. Differences between cases and controls in number of

standing teeth and age were assessed with the *t* test.

Three screening steps were adopted for genetic data processing. First, the genotype distribution and allele-type of each SNP among AP or CP and periodontitis-free subjects were calculated. Secondly, those SNPs with call rate over 80% and minor allele frequencies (MAFs) over 10% were put into the second step: Hardy-Weinberg Equilibrium (HWE) test. Thirdly, those SNPs in the periodontitis-free group with HWE test p values ≥ 0.01 were subjected to final statistical analysis. Multiple comparison of genotype and allele-type of AP, CP and control groups were initially screened by Chi square test. SNPs fulfilling criteria for final statistical analysis was put under stepwise logistic regression analysis together with other possible risk indicators of periodontitis—namely, age, sex, and reported smoking habit. The alpha level was set at 0.05, unless otherwise specified. Risk alleles, odds ratios (ORs), and 95% confidence intervals for each SNP were also determined. Linkage disequilibrium (LD) analysis and haplotype association analysis were performed with Haploview 3.32 (http://www.broad.mit.edu/mpg/haploview/). Haplotype effects in different genetic models were tested by HAPSTAT 3.0 (http://www.bios.unc.edu/~lin/hapstat/).

Results

A total of 436 participants were recruited: 229 patients with at least moderate periodontitis defined earlier and 207 periodontitis-free controls. The mean age (±SD) age of the participants was 43.1 ± 7.7 years; 249 (57.1%) subjects were women. There were no differences in mean age and sex distributions between patient and control groups. The case group had about twice the proportion of smokers as did the control group and significantly fewer standing teeth (Table 1). Clinical parameters such as probing pocket depth (PPD) ≥5 mm, probing attachment level (PAL) ≥5 mm, and BOP% were also summarized in Table 1.

Eleven tagging SNPs of the C5 gene were identified as candidate SNPs by the SNP Tagging Wizard of SNPbrowser 3.5. Most of them were intronic SNPs. Ten of the 11 SNPs were genotyped successfully by the iPLEX assay and seven were found to have more than one genotype (minor allele frequency > 0.001) with an average call rate of 99.2%. One SNP (rs10818491) was found not fulfilling the Hardy-Weinberg Equilibrium ($p < 0.01$) (Table 2), hence analyze of the genotype and allele-type distribution differences of the remaining six SNPs among subgroups like AP, CP and controls was carried out. None of the 6 SNPs showed significant allele or genotype distribution difference between either patient group vs control group (adjusted p-value 0.008) (Table 3), which indicated there was no difference between AP, CP and control groups. Therefore, in the following stepwise logistic regression analysis, AP and CP

data were combined.

The six C5 SNPs fulfilling criteria for genetic analysis (Table 3), together with age, sex and smoking habit were selected for stepwise logistic regression analysis regarding the association between these possible risk indicators and periodontitis.

After the adjustment of age, sex and smoking, only rs17611 and smoking habit remained to be significantly associated with periodontitis (p = 0.023 and 0.006, respectively, Table 4), genotype AG of rs17611 seemed more frequent in periodontitis (p = 0.007, OR = 6.08, **95% CI = 1.31-28.22).** Smoking was also significantly associated with periodontitis in this Hong Kong Chinese sample with OR = 2.84 (**95% CI = 1.31-6.14,** Table 4).

Haploview 3.32 was used to detect any LD block and the association between haplotypes and periodontitis (19) for the six SNPs that had a Hardy-Weinberg Equilibrium test p value of larger than 10^{-5}, a call rate of > 0.8, and a minor allele frequency of > 0.001. The pairwise comparisons were designed to identify markers within 500 kb from each other, and one LD block including SNPs rs1035029, rs17611, rs25681 and rs992670 were detected (Figure 1). The haplotype CGCA in this block was found to be marginal significantly associated with periodontitis (p = 0.038; Table 5). Then HAPSTAT 3.0 was used to estimate haplotype effects and haplotype–environment interactions (age, sex and smoking habit in particular in this

study) under different genetic models such as dominant and additive models (20). While dominant genetic model means heterozygote has the same increased risk as minor homozygous genotypes (21), additive model is a statistical model modified from several regression models (22). No environment or haplotype – environmental interaction was found significantly associated with periodontitis. Haplotype CGCA was found significantly associated with periodontitis in the dominant model (p = 0.001, OR = 4.85, **95% CI = 1.85-12.71**) (Table 5). Other genetic models were not suitable for our dataset therefore were not calculated.

Discussion

It is well-known that complement system is a biochemical cascade helping clear pathogens from an organism. Humoral activation of the complement system by the classical, alternative, or lectin pathway results in the cleavage of C3 into C3a and C3b fragments. Subsequent downstream cleavage of C5 generates the anaphylatoxin C5a and fragment C5b, which acts as a nucleus for the MAC (23) by anchoring the assembly of a molecule each of C6, C7, and C8, to guide the polymerization of C9 into a membrane channel in the target cell (24). Not only does pore formation cause direct cell injury and necrosis (25), but it may also amplify the inflammatory response by promoting the expression of pro-inflammatory mediators (26). Additionally, the

MAC can influence the recruitment of inflammatory cells and leukocyte adhesion to endothelium (27, 28), thereby promoting the release of cell stimulants, such as hydrolytic enzymes, reactive oxygen species, and cytokines (29, 30). Hence, mutations in any of the MAC complement components might modify this inflammatory response to pathogens. In this study of Hong Kong Chinese patients with and without periodontitis, genotype AG of non-synonymous SNP rs17611 in the C5 gene and the haplotype containing rs17611 were found to be significantly associated with periodontitis, indicating that this genetic variation and haplotype might play a role in the pathogenesis of periodontitis.

The C5 gene is located on chromosome 9q34.1. Eleven SNPs were selected in this study. **Although most of the SNPs selected were in intron and their biological function was unclear, but nature of other regional SNPs can be obtained through investigation of their linkage and haplotype status with those tagging SNPs. Therefore information about SNPs searching range and hence the true causative variation(s) could potentially be identified in subsequent experiments.** The haplotype and tagging polymorphisms of C5 have been studied in diseases such as liver fibrosis, rheumatoid arthritis, and asthma (11-16). Haplotype included the non-synonymous SNP rs17611 (A/G) in exon 19 was reported to associate with bronchial asthma and liver fibrosis (12, 14). The A to G variation that was found to be

associated with periodontitis in this study causes an amino acid change from isoleucine to valine at position 802. Because these two amino acids are both aliphatic and their structures are similar except for an extra center of asymmetry in the isoleucine side chain, the change from isoleucine to valine can be considered to be a conservative one.

Classically, it has been assumed that a mutant protein is unstable and leads to a functional defect, so subjects carrying a homozygous mutant genotype would be more susceptible than others to disease. However, a mutated gene may also encode a stable mutant protein that interferes with the formation of a functional form of the wild-type protein especially for structure proteins or proteins forming dimers (31). An individual carrying a heterozygous genotype in this situation would be more susceptible than one carrying homozygous genotypes (31, 32) because products from both genotypes are stable. In this way, the protein with the conservative mutation for valine in position 802, which is expected to be stable, might theoretically affect the wild-type protein. The amino acid mutation in rs17611 is in the MG6 domain of C5, which forms a conserved large cavity with other domains (33). Although the amino acid is far from the cleavage site, this cavity area may be involved in recognition of C5 by the cleavage enzyme C5 convertase, which is a large macromolecular complex that could conceivably interact with this domain (34). The alteration in rs17611 could also

potentially affect the formation of the C5b-9 MAC because the substituted amino acid would theoretically be situated within the C5b peptide after cleavage. Further studies on the functions of this gene variant and its effect on periodontitis risk are required. Additionally, the significant association in dominant model of haplotype consisting of SNPs rs1035029, rs17611, rs25681, and rs992670 indicates that SNPs within this gene region could be associated with periodontitis, and further investigation should focus on this particular region.

Smoking is a well-established risk factor in the incidence and progression of periodontal diseases (35-39). One community study conducted among the Hong Kong population showed that heavy smoking is the major risk factor of periodontitis (odds ratio, OR = 4.61), and that moderate and light smoking are also significant risk factors (OR = 2.69 and 2.33, respectively) (40). These values are similar to the OR (95% CI) of 2.84 (1.31-6.14) calculated in our study. Nevertheless, we could not subcategorize the small number of smokers as heavy, moderate, light, and very light smokers, which may explain why the OR for smoking is lower than the published value for heavy smoking. The value is also lower than the OR (95% CI) of 6.08 (1.31-28.22) calculated for the rs17611 genetic variant in this study. It must be noted, however, that our strict criterion for a case was any patient with at least moderate periodontitis, and smoking and the rs17611 genetic variant could have a different influence on mild to

moderate periodontitis. Finally, other factors such as age and sex have been reported to be associated with periodontitis in Hong Kong Chinese patients (40), but the effects of these two factors were removed by our case-control study design.

It is noticed that in our study we used extra-oral radiographs instead of intraoral radiograph although OPG had only a sensitivity of 79% for periodontal pathology (41). Nevertheless, OPG has reported a specificity of 92% for periodontal pathology, and studies have confirmed that bone loss measured on OPG is in close correlated with periodontal status (42, 43). In our study, a simple and robust method must be adopted in order to screen a large number of subjects and to eliminate any ambiguous subjects whose periodontal condition did not fulfill recruitment criteria of either groups, therefore OPG was selected for assessing bone loss level of the subjects. We used Schei ruler without using the 1 mm space or what was referred to be the "normal" distance from alveolar crest to CEJ, instead a certain level or fraction of "bone loss" was allowed for the control group, reason being that OPG typically magnify tooth length ranges from 15-30% (44, 45). Considering average permanent tooth root length is about 11 mm to 17 mm (46), 1 mm equals to about 5-9% root length from CEJ to radiographic apex. In order to provide some leeway for measurement, we used 15% root length as the cut off when some other study considered 2 mm as the "normal" distance between the alveolar crest to CEJ (47). All

these subjects selected by OPG screening were subsequently periodontally examined to confirm their eligibility as per our inclusion criteria.

In conclusion, our study showed that genotype AG of non-synonymous SNP rs17611 of the C5 gene was more prevalent in the periodontitis patient group than in periodontitis-free controls and together with smoking may be associated with periodontitis among the Hong Kong Chinese population. In addition, one haplotype including SNP rs17611 was associated with periodontitis. An association study with a large sample size and other ethnic populations are needed to confirm this finding. Further studies should also investigate how genetic variation may alter the structure and biological function of C5 and its fragments, and how these alterations modify humoral immune responses and susceptibility to periodontitis among the Hong Kong Chinese population, and possibly other populations.

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Legend

Fig. 1. Pairwise linkage disequilibrium (LD) in the C5 gene. All SNPs of C5 assessed in this study. The horizontal bar represents the relative location of each SNP along the chromosome 9, 9q33-q34, and the numbers above the bar are corresponding chromosome positions; SNPs highlighted in green are coding SNPs. Diamonds in the haplotype blocks represent pairwise linkage disequilibrium between all SNPs assessed; the darker the diamond is, the stronger the linkage disequilibrium is between the two SNPs.

Table 1. Demography and clinical profile of study participants

Characteristic	Categories	Periodontitis-free (n=207)	Periodontitis (n=229)	Test	Statistics	*p*-value
Age (years)	Mean ± SD	42.4 ± 8.1	43.7 ± 7.4	t	-1.69	NS
Gender	Male	87 (42.0)	100 (43.7)	χ^2	0.27	NS
	Female	120 (58.0)	129 (56.3)			
Smoking habit	Non-smoker	191 (92.3)	193 (84.3)	χ^2	7.15	<0.005
	Smoker	16 (7.7)	36 (15.7)			
	Pack-year (mean ± SD)	4.7 ± 6.7	16.0 ± 10.2	t	-3.80	<0.05
Teeth remain	Mean ± SD	27.0 ± 1.8	24.7 ± 3.4	t	5.46	<0.001
% sites PPD≥ 5mm	Mean ± SD	0 ± 0	30.3 ± 18.1			
% sites PAL≥ 5mm	Mean ± SD	0 ± 0	47.2 ± 27.2			
BOP %	Mean ± SD	ND	72.6 ± 25.0			

Results are no. (%) unless otherwise indicated. ND: not determined.

Table 2. Candidate C5 single nucleotide polymorphisms (SNPs) of study participants

SNP ID	Type	Variation	Call rate (%)	MAF[a]	HWE *p*-value[b]
rs2300930	Intronic	A/C	98.9	0.197	0.73
rs10818491	Intronic	C/T	98.7	0.406	$<10^{-5}$
rs7035682	Intronic	C/T	99.3	single genotype detected	
rs7026551	Intronic	A/C	98.4	single genotype detected	
rs2269066	Intronic	A/G	98.2	0.237	0.20
rs2269067	Intronic	C/G	99.1	single genotype detected	
rs1035029	Intronic	C/T	99.1	0.409	0.93
rs17611	Non-synonymous	A/G	99.8	0.409	0.73
rs25681	Synonymous	C/T	99.8	0.409	0.96
rs992670	Intronic	A/G	99.1	0.230	0.24
rs2300934	Intronic	A/C	0	ND	

HWE: Hardy-Weinberg Equilibrium; MAF: Minor Allele Frequency; ND: not determined

[a]MAF not determined when call rate <80.0% or only one genotype detected.

[b]For periodontitis-free group; HWE *p*-value not determined if call rate <80.0% or only one genotype detected; only SNPs with HWE *p*-value ≥0.01 are subjected to further analysis.

Table 3. Genotype and allele-type of C5 SNPs selected for stepwise logistic regression analysis

SNP ID	Genotype/ Allele-type	Count[a] (%)			*p*-value[b]
		Periodontitis-free (n = 207)	AP (n = 32)	CP (n = 197)	
rs2300930	AA	7 (3.4)	1 (3.1)	7 (3.6)	0.278
	AC	57 (27.9)	8 (25.0)	73 (37.4)	
	CC	140 (68.6)	23 (71.9)	115 (59.0)	
	A	71 (17.4)	10(15.6)	87 (22.3)	0.156
	C	337 (82.6)	54(84.4)	303 (79.7)	
rs2269066	AA	118 (57.6)	19 (59.4)	110 (56.4)	0.487
	AG	75 (36.6)	11 (34.4)	80 (41.0)	
	GG	12 (5.9)	2 (6.2)	5 (2.6)	
	A	311 (75.9)	49 (76.6)	300 (76.9)	0.938
	G	99 (24.1)	15 (23.4)	90 (23.1)	
rs1035029	CC	36 (17.6)	5 (15.6)	30 (15.4)	0.055
	CT	87 (42.6)	13 (40.6)	110 (56.4)	
	TT	81 (39.7)	14 (43.8)	55 (28.2)	
	C	159 (39.0)	23 (35.9)	170 (43.6)	0.294
	T	249 (61.0)	41 (64.1)	220 (56.4)	
rs17611	AA	82 (40.2)	13 (40.6)	56 (28.7)	0.053
	AG	85 (41.7)	14 (43.8)	110 (56.4)	
	GG	37 (18.1)	5 (15.6)	29 (14.9)	
	A	249 (61.0)	40 (62.5)	222 (56.9)	0.430
	G	159 (39.0)	24 (37.5)	168 (43.1)	
rs25681	CC	37 (18.1)	6 (18.8)	28 (14.4)	0.033
	CT	86 (42.2)	12 (37.5)	111 (56.9)	
	TT	81 (39.7)	14 (43.8)	56 (28.7)	

	C	160 (39.2)	24 (37.5)	167 (42.8)	0.685
	T	248 (61.8)	40 (62.5)	223 (57.2)	
rs992670	AA	117 (57.1)	19 (59.4)	115 (59.0)	0.178
	AG	75 (36.6)	11 (34.4)	77 (39.5)	
	A	309 (75.4)	49 (76.6)	307 (78.7)	0.529
	G	101 (24.6)	15 (23.4)	83 (21.3)	

AP: Aggressive periodontitis; CP: Chronic periodontitis

[a]Total not adding up because call rates are <100%.

[b]The level of significance was set at 0.008 after adjustment for multiple comparison

*Table 4***.** Odds ratios for study participants with periodontitis

		Unadjusted (univariate)[a]			Adjusted[b]		
Variable	Categories	OR	*p*-value	95% CI	OR	*p*-value	95% CI
Age		1.02	0.072	0.99 - 1.05			
Gender	Female	1.00	0.601				
	Male	0.90		0.62 - 1.32			
Smoking habit	Non-smoker	1.00	0.003		1.00	0.006	
	Smoker	2.57		1.34 - 4.92	2.84		1.31 - 6.14
Genotype (SNP)							
rs2300930	AA	1.00	0.220				
	AC	1.24	0.690	0.43 - 3.62			
	CC	0.86	0.781	0.30 - 2.44			
rs2269066	AA	1.00	0.338				
	AG	1.11	0.605	0.75 - 1.65			
	GG	0.53	0.202	0.20 - 1.40			
rs1035029	TT	1.00	0.051		NS		
	CT	1.66	0.024	1.09 - 2.53			
	CC	1.14	0.667	0.68 - 2.01			
rs17611	AA	1.00	0.026		1.00	0.023	
	AG	1.73	0.014	1.14 - 2.65	6.08	0.007	1.31 - 28.22
	GG	1.09	0.775	0.62 - 1.92	1.15	0.707	0.63 - 1.97
rs25681	TT	1.00	0.044			NS	

	CT	1.65	0.024	1.08	-	2.52
	CC	1.06	0.886	0.60	-	1.87
rs992670	AA	1.00	0.120			
	AG	1.02	0.905	0.69	-	1.52
	GG	0.34	0.044	0.12	-	0.97
Allele-type						
rs2300930	C	1.00				
	A	1.28	0.147	0.91	-	1.8
rs2269066	G	1.00				
	A	0.93	0.663	0.68	-	1.28
rs1035029	C	1.00				
	T	0.86	0.291	0.66	-	1.13
rs17611	A	1.00				
	G	1.15	0.307	0.88	-	1.51
rs25681	C	1.00				
	T	0.92	0.531	0.70	-	1.20
rs992670	A	1.00				
	G	0.84	0.288	0.61	-	1.16

OR = odds ratio; CI = confident interval; NS = not significant.
[a]OR for each factor was computed from logistic regression
[b]Adjusted ORs were computed with stepwise logistic regression with variables that were significant in the univariate analysis

Table 5. Haplotype association results in different genetic models (n = 872)

Haplotype[a]	Count Ratio[b] (Frequencies)		χ^2 *p*-value[c]	Haplotype effects and haplotype–environment interactions[c,d]	
	Periodontitis-free	Periodontitis		Additive model	Dominant model
TATA	240.9/169.1 (0.588)	245.9/201.1 (0.599)	0.395	0.194	0.078
CGCG	95.7/314.3 (0.233)	93.6/362.4 (0.205)	0.319	0.308	0.323
CGCA	59.3/350.7 (0.145)	90.3/365.7 (0.198)	0.038	0.029	0.001[e]
TACA	4.8/405.2 (0.012)	5.1/450.9 (0.011)	0.950	0.216	0.845

[a]Two haplotypes are not listed due to low frequencies (fractional likelihood frequency < 0.01)

[b]Counts were obtained by summing the fractional likelihoods of each individual for each haplotype.

[c]The level of significance was set at 0.0125 after adjustment for multiple comparison

[d]Logistic regression analysis, shown are *p*-values

[e]Odds Ratio and 95% confident interval: 4.85 (1.85-12.71)

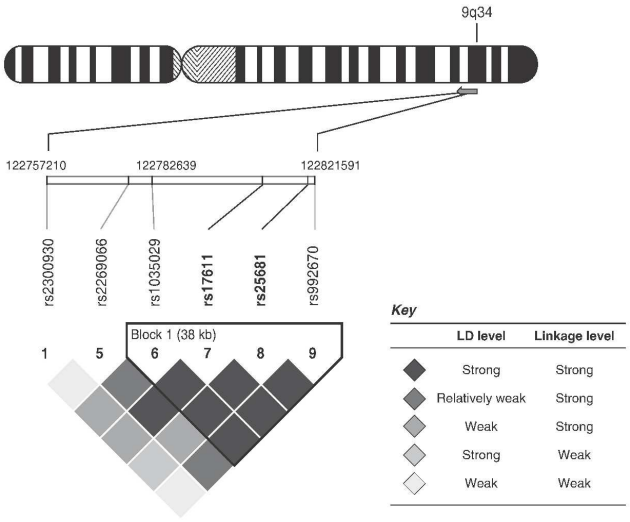

Figure 1
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