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# ORIGINAL

# Exploring the Link: Polymorphisms in Tnf-A, Mmp-2, Mmp-9, Timp-2, And Il-13 Genes and their Association with Chronic Periodontitis Among the Li Ethnic Group in Hainan Province – Implications for Physical Fitness and Mental Health in Athletes

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## ABSTRACT

The prevalence of chronic periodontitis (CP), a pervasive chronic inflammatory condition, is significantly influenced by genetic predisposition. This investigation probes the association between CP and polymorphisms in genes encoding anti-inflammatory and immune response mediators—namely TNF-α, MMP-2, MMP-9, TIMP-2, and IL-13—among the Li ethnic group in Hainan Province. These genes are pivotal in modulating the immune response to periodontal infection, which, beyond oral health, may impact physical fitness and mental well-being, particularly in athletes. Our study encompassed 175 CP patients (75 from the Li ethnic group and 100 from the Han ethnic group) and 122 ethnically matched controls (100 Li and 22 Han individuals), focusing on five single nucleotide polymorphisms (SNPs): TNF-α-308A/G, MMP-2-1306C/T, MMP-9-1562C/T, TIMP-2-418G/C, and IL-13-1112C/T. Genomic DNA was genotyped from buccal swabs using the Snap shot method. Our findings revealed no significant association between the studied SNPs and CP in both the Li and Han populations (P>0.05), indicating these genetic markers may not influence CP susceptibility in these groups.

This suggests that while these SNPs are not directly linked to CP among the Li ethnic group, the broader implications for physical fitness and mental health in athletes warrant further exploration, considering the interconnectedness of systemic and oral health.

**KEYWORDS:** Polymorphisms, TNF-A, MMP-2, MMP-9, TIMP-2, IL-13 genes, Chronic periodontitis

## 1. INTRODUCTION

Chronic periodontitis (CP) represents a significant public health challenge worldwide, manifesting as a persistent inflammatory condition that detrimentally affects the supporting structures of the teeth(Pihlstrom, Michalowicz, & Johnson, 2005). This complex disease is characterized by the destruction of periodontal ligaments and alveolar bone, leading to tooth loss if left untreated. The etiology of CP involves a multifactorial interplay between microbial dental plaque and host immune responses, with genetic predisposition playing a crucial role in modulating susceptibility and disease progression(Baker, 2005; Hart & Kornman, 1997; Kinane & Hart, 2003; Yoshie, Kobayashi, Tai, & Galicia, 2007). Among the myriad of genetic factors implicated in CP, certain polymorphisms within the genes encoding Tumor Necrosis Factor-alpha (TNF-α), Matrix Metalloproteinases (MMP-2, MMP-9), Tissue Inhibitors of Metalloproteinases (TIMP-2), and Interleukin-13 (IL-13) have garnered particular interest(Heidari, Moudi, & Mahmoudzadeh-Sagheb, 2019; Parkhill, Hennig, Chapple, Heasman, & Taylor, 2000). These genes are central to the inflammatory and immune responses, with TNF-α playing a pivotal role in inflammation, MMPs in tissue remodeling and destruction, TIMPs as regulators of MMP activity, and IL-13 as a critical mediator of anti-inflammatory responses(Heidari et al., 2019; Li et al., 2016). The Li ethnic group, residing predominantly in Hainan Province, China, presents a unique population for genetic studies due to its distinct genetic background and lifestyle factors. Investigating the association between CP and specific single nucleotide polymorphisms (SNPs) within these genes in the Li ethnic group could provide insights into the genetic underpinnings of CP and highlight potential targets for therapeutic intervention(Hans, Mehta, & Hans, 2015; Kulshrestha, Srinivasa, & Biswas, 2013; Mattuella et al., 2020; Takashiba, Ohyama, Oyaizu, Kogoe‐Kato, & Murayama, 1999).

Furthermore, the implications of CP extend beyond oral health, potentially affecting physical fitness and mental health(Waterer & Wunderink, 2003), particularly in athletes. Athletes' performance and psychological state can be significantly impacted by systemic inflammation and chronic pain, conditions often exacerbated by CP(Genco, Van Dyke, Levine, Nelson, & Wilson, 1986; Kornman & di Giovine, 1998; Nares, 2003). Understanding the genetic predisposition to CP among athletes, especially those of the Li ethnic

group, could facilitate the development of personalized preventive and treatment strategies, thereby enhancing their overall health, performance, and well-being(Craandijk, Van Krugten, Verweij, Van Der Velden, & Loos, 2002; Kato et al., 1999). This study aims to bridge the gap in knowledge regarding the genetic basis of CP and its broader health implications by exploring the association between CP (Kluknavská et al., 2022)and polymorphisms in TNF-α, MMP-2, MMP-9, TIMP-2, and IL-13 genes among the Li ethnic group in Hainan Province. By elucidating these genetic associations, we seek to contribute to the holistic understanding of CP's impact on physical fitness and mental health, paving the way for targeted interventions that could benefit athletes and the general population alike (Michalowicz et al., 2000).

## 2. Materials and Methods

### 2.1 Study population and Clinical examination

Subjects were recruited from the patients' database of the Department of Periodontology, Haikou people's Hospital, ranging from January 2020 to December 2022. 175 unrelated patients with moderate or severe CP (75 patients in Li nationality and 100 patients in Han nationality) and 122 ethnically matched controls (100 healthy individuals in Li nationality and 22 healthy individuals in Han nationality) were included by random selection. The experiment was conducted with the human subjects' understanding and consent after learning the relevant information regarding the study. The demographic information was shown in Table 1. The protocol was approved by the Ethics Committee of Haikou people's Hospital (2019-（ethics review）-149 and 2020-（ethics review）-089).

**Table 1:** Demographic and clinical characteristics of the study population

| | **Li nationality** | | | **Han nationality** | | |
|---|---|---|---|---|---|---|
| | Healthy controls | CP patients | *P* value | Healthy controls | CP patients | *P* value |
| Age(years; mean ± SD) | 29.69 ± 6.88 | 44.57 ± 5.87 | 0.02 | 31.25 ± 6.75 | 44.96 ± 9.20 | 0.30 |
| Male(N) | 47 | 43 | 0.23 | 14 | 46 | 0.09 |
| Female(N) | 53 | 32 | | 6 | 54 | |
| Total | 100 | 75 | | 22 | 100 | |

The periodontal condition of all subjects was assessed by skilled investigators using the following parameters: probing depth (PD), clinic attachment loss (CAL) and degree of loosening (Ainamo & Bay, 1975; O'LEARY, 1972; Ramfjord, Emslie, Greene, Held, & Waerhaug, 1968). Subjects with systemic diseases, lactation, pregnancy, orthodontic treatment, immunodeficiency diseases, chemotherapy, and smoking were excluded from this study. The healthy control participants included in the present study did

not suffer from any periodontal disease. A recent published report was used to characterize periodontitis (Tonetti, Greenwell, & Kornman, 2018).

### 2.2 DNA Extraction

The buccal swab was a simple, non-invasive and high patient acceptance method to get DNA. Buccal swab was collected from all participants and subsequently used for DNA preparation utilizing a kit (NanoMagBio). The purified DNA was then dissolved in TE buffer (10 mM tris (pH 7.8), 1 mM EDTA) and stored at −70 ℃ until use. Polymerase chain reaction (PCR) was carried out utilizing the purified DNA.

### 2.3 SNP Genotyping

The Snapshot method was applied for genotyping and the primers used in the present study for PCR were shown in Table 2. The genotyping of the five selected SNPs, TNF-A-308A/G, MMP-2-1306C/T, MMP-9-1562C/T, TIMP-2-418G/C and IL-13-1112C/T, was conducted by the method as described previously. A 10 μL PCR mixture containing 20 ng of purified DNA and 2 × Taq PCR master mix (Gene Tech) was used for PCR amplification.

**Table 2:** Primers used for genotyping and extension

| SNPS | Name of primer | Primer sequence |
|---|---|---|
| TNF-A-308A/G rs1800629 | Forward | ACACAGCTTTTCCCTCCAAC |
| | Reverse | ATCAAGGATACCCCTCACAC |
| | Extension | ctgactgactgactgactACCCTGGAGGCTGAACC CCGTCC |
| MMP-2-1306C/T rs243865 | Forward | GCCCCACCTTTTTCAGATAG |
| | Reverse | TTCTCCCTCTCTCAGGAAAG |
| | Extension | ATATTCCCCACCCAGCACTC |
| MMP-9-1562C/T rs3918242 | Forward | ACGTAGTGAAACCCCATCTC |
| | Reverse | ATCGGGCAGGGTCTATATTC |
| | Extension | gactgactgactgactgactGAGTAGCTGGTATT ATAGGC |
| TIMP-2-418G/C rs8179090 | Forward | TACCCTTTCCCCTTCAGCTC |
| | Reverse | TGTTCACAGTAACCACACCC |
| | Extension | ctgactgactGGGGTCCCTTCGAGCCCAGC |
| IL-13-1112C/T rs1143634 | Forward | AGGTGGAGAGCTTTCAGTTC |
| | Reverse | CTTCATCCCTACTGGTGTTG |
| | Extension | gactgactgactCATTTCAGAACCTATCTTCTT |

The PCR cycling conditions consisted of one step at 94 ℃ for 5 min, then 35 cycles of 20 second at 94 ℃, 30 second at 60 ℃, and 30 second at 72 ℃; and a terminal step of 3 min at 72 ℃. The PCR product was purified

with SAP (shrimp alkaline phosphatase) (Chen, Zhang, & Wang, 2016). Snapshot primers were specifically designed for amplifying the site of the target SNPs. The reaction lasted for one base extension, and the products were the loaded onto an ABI 3730XL automatic sequencer for genotype callings of TNF-A-308A/G, MMP-2-1306C/T, MMP-9-1562C/T, TIMP-2-418G/C and IL-13-1112C/T. The genotype callings were then verified manually. All the experiments and data analyses were performed in a double-blind manner and were independently reviewed by two authors. The analyses were also verified by sanger sequencing (Figure 1).

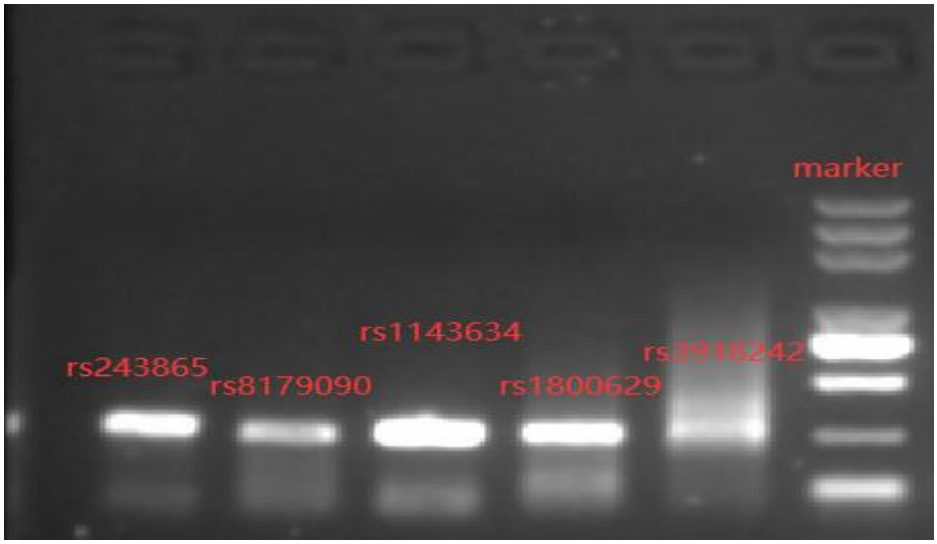


**Figure 1:** Agarose electrophoresis of TNF-A-308A/G, MMP-2-1306C/T, MMP-9-1562C/T, TIMP-2-418G/C and IL-13-1112C/T genotypes by Sanger sequencing.

### 2.4. Statistical Analysis

Quantitative parameters were presented in the form of mean ± standard deviation (SD) unless otherwise indicated. Data analysis was carried out using SPSS version 21. Continuous variables were assessed using an independent t-test, whereas categorical data were assessed using the Chi-square analysis. Odds ratios were determined with a 95% confidence interval, and $P < 0.05$ was considered statistically significant.

## 3. Results

### 3.1 Clinical measurements

Both clinical and demographic data are presented in Table 1. As shown in Table 1, there was no significant difference in the age or sex ratio between the healthy controls and the CP patients of Han Nationality ($P>0.05$). In the Li Nationality, the age of the CP patients was significantly higher than the healthy controls ($P<0.05$), while there was no significant difference in the sex ratio between the healthy controls and the CP patients ($P>0.05$).

### 3.2 Genotyping of TNF-A-308, MMP-2-1306, MMP-9-1562, TIMP-2-418 and IL-13-1112

The genotype frequency distributions of the five SNPs studied in the present work were in accordance with the Hardy-Weinberg equilibrium. Statistics in Table 3 showed the allele frequency and genotype frequency distribution of rs1800629, rs243865, rs3918242, rs8179090 and rs1143634 in the healthy controls and the CP patients of Han Nationality and Li Nationality, respectively. The allele frequency difference and genotype frequency difference of rs1800629, rs243865, rs8179090 and rs1143634 in the healthy controls and the CP patients of Han Nationality and of Li Nationality were less than 0.1. Only the genotype frequency difference of rs3918242 was greater than 0.1 in the healthy controls and the CP patients of Han Nationality.

**Table 3:** Genotype distribution and allele frequencies of TNF-A-308A/G, MMP-2-1306C/T, MMP-9-1562C/T, TIMP-2-418G/C and IL-13-1112C/T polymorphisms in subjects with periodontitis and the control group

| | **Li nationality** | | | **Han nationality** | | |
|---|---|---|---|---|---|---|
| | Healthy controls | CP patients | Diff | Healthy controls | CP patients | Diff |
| TNF-A-308A/G genotypes： | % | % | | % | % | |
| AA | 0.0404 | 0.0781 | 0.0377 | 0.0000 | 0.0000 | 0.0000 |
| GA | 0.3434 | 0.3906 | 0.0472 | 0.1818 | 0.1500 | 0.0318 |
| GG | 0.6162 | 0.5313 | 0.0849 | 0.8182 | 0.8500 | 0.0318 |
| Alleles: | | | | | | |
| A | 0.2121 | 0.2734 | 0.0613 | 0.0909 | 0.0750 | 0.0159 |
| G | 0.7879 | 0.7266 | 0.0613 | 0.9091 | 0.9250 | 0.0159 |
| MMP-2-1306C/T genotypes： | % | % | | % | % | |
| CC | 0.7576 | 0.7344 | 0.0232 | 0.7273 | 0.7300 | 0.0027 |
| CT | 0.2020 | 0.2188 | 0.0167 | 0.2727 | 0.2600 | 0.0127 |
| TT | 0.0404 | 0.0469 | 0.0065 | 0.0000 | 0.0100 | 0.0100 |
| Alleles: | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| C | 0.8586 | 0.8438 | 0.0148 | 0.8636 | 0.8600 | 0.0036 |
| T | 0.1414 | 0.1563 | 0.0148 | 0.1364 | 0.1400 | 0.0036 |
| MMP-9-1562C/T genotypes： | % | % | | % | % | |
| CC | 0.8182 | 0.7344 | 0.0838 | 0.6818 | 0.7900 | 0.1082 |
| CT | 0.1313 | 0.2188 | 0.0874 | 0.3182 | 0.2100 | 0.1082 |
| TT | 0.0505 | 0.0469 | 0.0036 | 0.0000 | 0.0000 | 0.0000 |
| Alleles: | | | | | | |
| C | 0.8838 | 0.8438 | 0.0401 | 0.8409 | 0.8950 | 0.0541 |
| T | 0.1162 | 0.1563 | 0.0401 | 0.1591 | 0.1050 | 0.0541 |
| TIMP-2-418G/C genotypes： | % | % | | % | % | |
| GG | 0.0101 | 0.0625 | 0.0524 | 0.0000 | 0.0700 | 0.0700 |
| GC | 0.2828 | 0.2969 | 0.0140 | 0.2727 | 0.3000 | 0.0273 |
| CC | 0.7071 | 0.6406 | 0.0664 | 0.7273 | 0.6300 | 0.0973 |
| Alleles: | | | | | | |
| G | 0.1515 | 0.2109 | 0.0594 | 0.1364 | 0.2200 | 0.0836 |
| C | 0.8485 | 0.7891 | 0.0594 | 0.8636 | 0.7800 | 0.0836 |
| IL-13-1112G/A genotypes： | % | % | | % | % | |
| GG | 0.9899 | 1.0000 | 0.0101 | 1.0000 | 0.9800 | 0.0200 |
| GA | 0.0101 | 0.0000 | 0.0101 | 0.0000 | 0.0200 | 0.0200 |
| Alleles: | | | | | | |
| G | 0.9949 | 1.0000 | 0.0051 | 1.0000 | 0.9900 | 0.9900 |
| A | 0.0051 | 0.0000 | 0.0051 | 0.0000 | 0.0100 | 0.0100 |

Table 4 summarized details of the odds ratio (Fisher test) and chi-square test of these five SNPs in the healthy controls and the CP patients of Han Nationality and Li Nationality. Among these SNPs, there were no significant differences in the genotypes and alleles of rs1800629, rs243865, rs3918242, rs8179090 and rs1143634 between CP patients and healthy control (P>0.05), suggesting that these SNPs were not significantly associated with the periodontitis phenotype in both ethnic populations.

**Table 4(a):** The odds ratio (Fisher test) and chi-square test of TNF-A-308A/G, MMP-2-1306C/T, MMP-9-1562C/T, TIMP-2-418G/C and IL-13-1112C/T polymorphisms in the healthy controls and the CP patients of Han Nationality and Li Nationality

| **HZ** | **C allele** | **P-value** | **1** | **P-value** | **0.9497** |
|---|---|---|---|---|---|
| | | OR | 0.97003 | X-squared | 0.003977 |
| | TT | p-value | 1 | p-value | 0.9792 |
| | | OR | 1.013782 | X-squared | 0.00068 |
| LZ | C allele | P-value | 0.7501 | P-value | 0.712 |
| | | OR | 0.889737 | X-squared | 0.13628 |
| | TT+CT | p-value | 0.8536 | p-value | 0.7389 |
| | | OR | 0.885378 | X-squared | 0.11114 |
| rs8179090 | | Fisher-Test | | Chi-squared test | |
| HZ | C allele | P-value | 0.3017 | p-value | 0.2134 |
| | | OR | 0.560995 | X-squared | 1.5484 |
| | GG+GC | p-value | 0.4657 | p-value | 0.3872 |
| | | OR | 0.640762 | X-squared | 0.7476 |
| LZ | C allele | P-value | 0.181 | p-value | 0.1678 |
| | | OR | 0.668863 | X-squared | 1.9027 |
| | GG+GC | p-value | 0.3939 | p-value | 0.3741 |
| | | OR | 0.73992 | X-squared | 0.78997 |
| rs1143634 | | Fisher-Test | | Chi-squared test | |
| HZ | C allele | P-value | 1 | p-value | 1 |
| | | OR | 0 | X-squared | 2.95E-30 |
| | GA | p-value | 1 | p-value | 1 |
| | | OR | 0 | X-squared | 2.95E-30 |
| LZ | C allele | P-value | 1 | p-value | 1 |
| | | OR | inf | X-squared | 2.13E-29 |
| | GG | p-value | 1 | p-value | 1 |
| | | OR | inf | X-squared | 2.13E-29 |
| rs1800629 | | Fisher-Test | | Chi-squared test | |
| HZ | C allele | P-value | 0.756 | p-value | 0.7214 |
| | | OR | 1.232213 | X-squared | 0.12712 |
| | GA | p-value | 0.7472 | p-value | 0.7094 |
| | | OR | 1.25676 | X-squared | 0.13885 |
| LZ | C allele | P-value | 0.23 | p-value | 0.2031 |
| | | OR | 0.716155 | X-squared | 1.6201 |
| | AA+GA | p-value | 0.3301 | p-value | 0.283 |
| | | OR | 0.707554 | X-squared | 1.1527 |

**Table 4(b):** The odds ratio (Fisher test) and chi-square test of TNF-A-308A/G, MMP-2-1306C/T, MMP-9-1562C/T, TIMP-2-418G/C and IL-13-1112C/T polymorphisms in the healthy controls and the CP patients of Han Nationality and Li Nationality

| **rs3918242** | | **Fisher-Test** | | **Chi-squared test** | |
|---|---|---|---|---|---|
| HZ | C allele | P-value | 0.3026 | p-value | 0.3081 |
| | | OR | 1.609078 | X-squared | 1.0387 |
| | CC | p-value | 0.2752 | p-value | 0.2746 |
| | | OR | 1.746592 | X-squared | 1.1935 |
| LZ | C allele | P-value | 0.3175 | p-value | 0.2962 |
| | | OR | 0.710499 | X-squared | 1.0911 |
| | TT+CT | p-value | 0.2425 | p-value | 0.2032 |
| | | OR | 0.616301 | X-squared | 1.6191 |

### 3.3 The effect of the multi SNPs combination on the susceptibility of CP

To evaluate the effect of the combination of multi SNPs on the susceptibility of CP, we have selected the locus with a genotype frequency difference greater than 0.02 and clustered the subjects by these loci.rs8179090, rs1800629 and rs3918242 were selected to analyze for Han Nationality, while rs243865, rs8179090, rs1800629 and rs3918242 were selected to analyze for Li Nationality. Clustering is based on the Complete method and pearson distance matrix. The clustering results showed that the genotype rs8179090GG-rs1800629GG-rs3918242CC was unique to the CP group (the sample size was small and not statistically significant) (Figure 2), while the other genotypes did not differ significantly between the CP patients and the heatlthy controls in the Han Nationality. In the Li Nationality, genotypes with a combination of 3 loci or 4 loci did not have significant differences in the CP patients and the healthy controls (Figure 3-7).

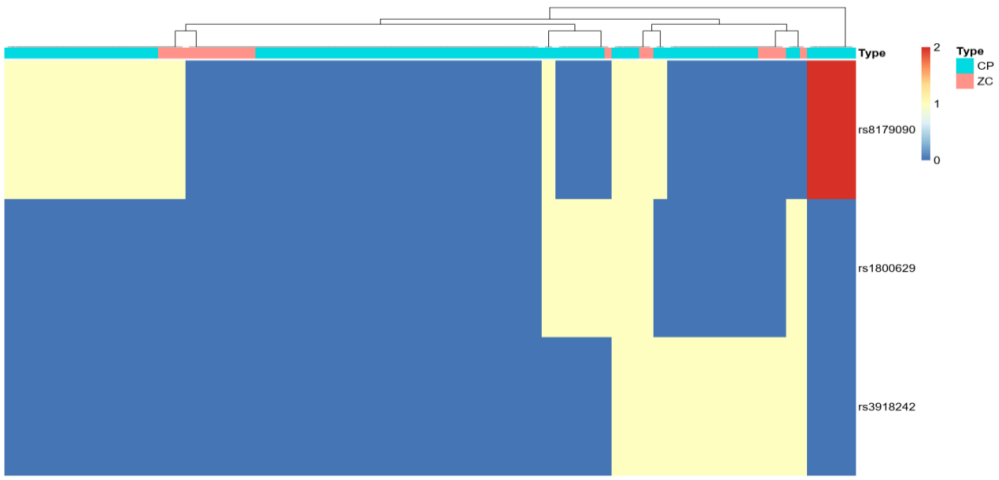


**Figure 2:** Genotype heat map of the Han Nationality based on rs8179090, rs1800629, and rs3918242. 0 is for homozygous major allele, 1 is for heterozygous genotype, and 2 is for homozygous minor allele.

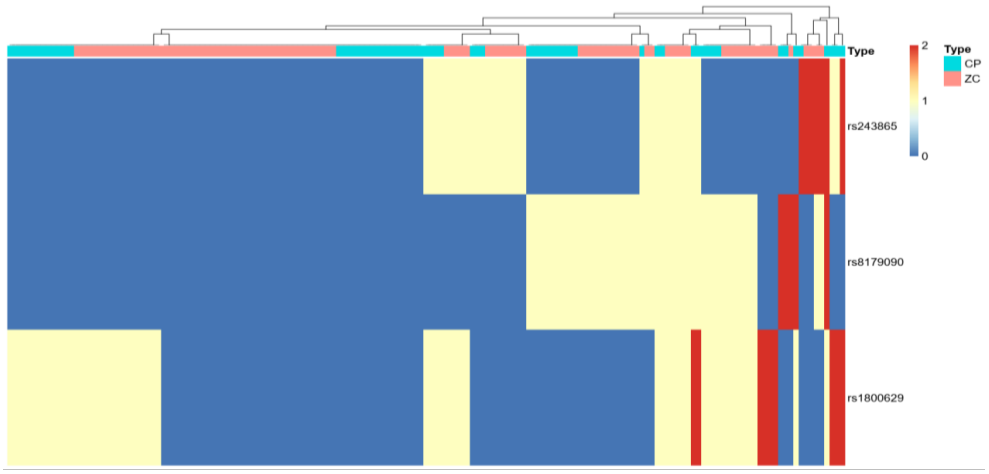


**Figure 3:** Genotype heat map of the Li Nationality based on rs243865, rs8179090 and rs1800629. 0 is for homozygous major allele, 1 is for heterozygous genotype, and 2 is for homozygous minor allele.

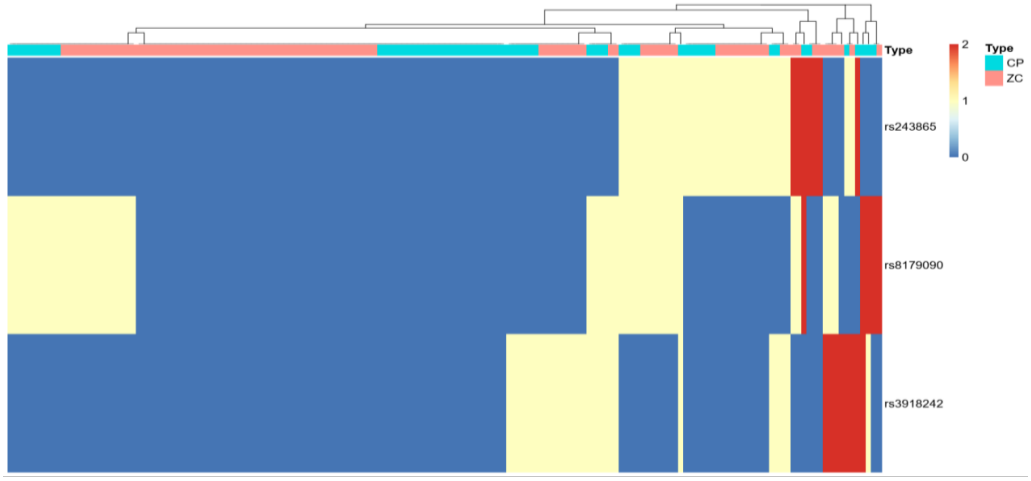


**Figure 4:** Genotype heat map of the Li Nationality based on rs243865, rs8179090 and rs3918242. 0 is for homozygous major allele, 1 is for heterozygous genotype, and 2 is for homozygous minor allele.

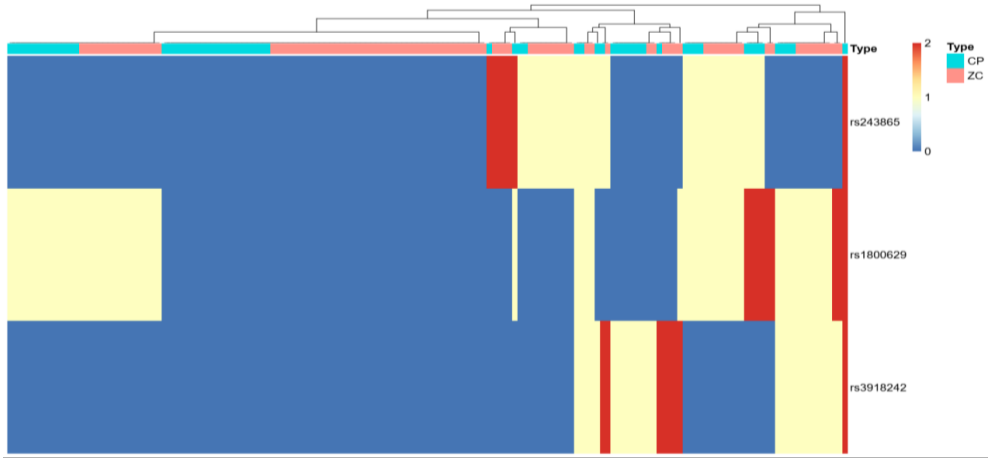


**Figure 5:** Genotype heat map of the Li Nationality based on rs243865, rs1800629 and rs3918242. 0 is for homozygous major allele, 1 is for heterozygous genotype, and 2 is for homozygous minor allele.

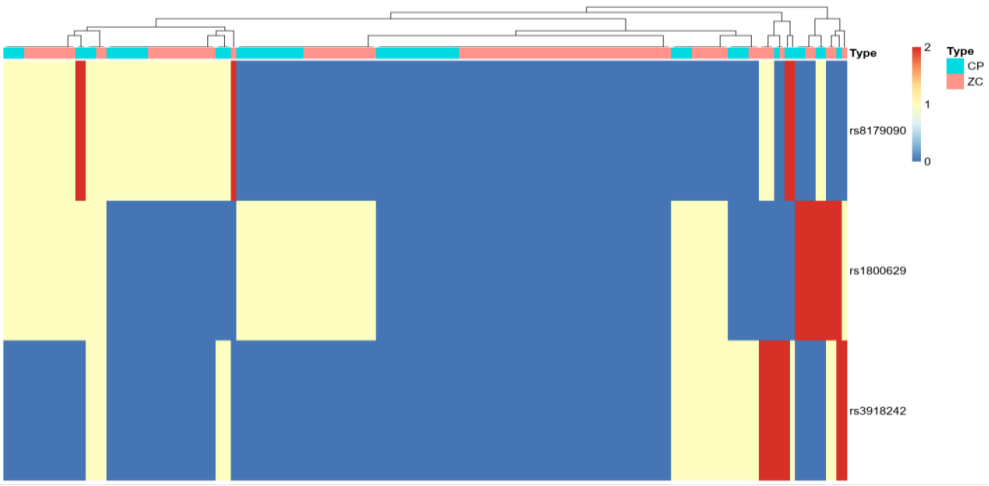


**Figure 6:** Genotype heat map of the Li Nationality based on rs8179090, rs1800629 and rs3918242. 0 is for homozygous major allele, 1 is for heterozygous genotype, and 2 is for homozygous minor allele.

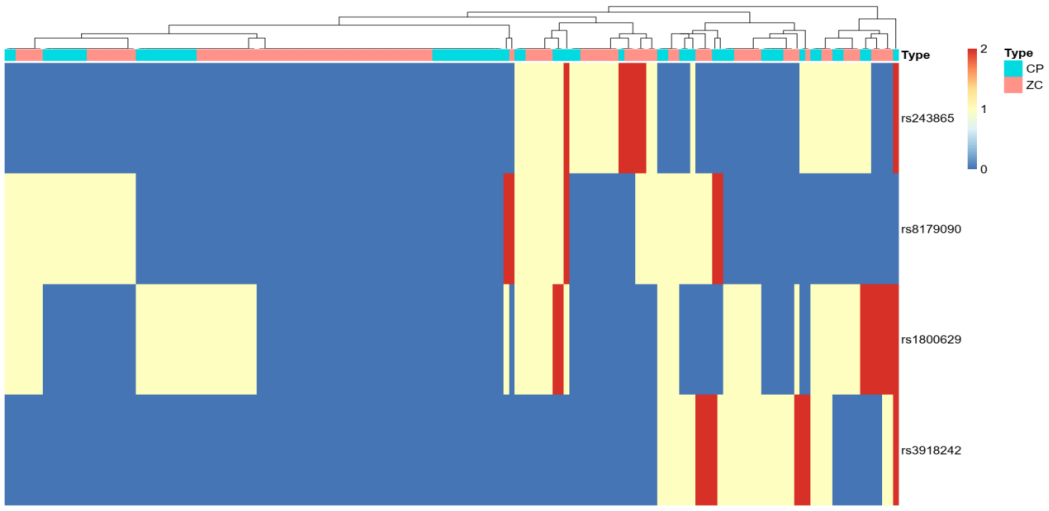


**Figure 7:** Genotype heat map of the Li Nationality based on rs243865, rs8179090, rs1800629 and rs3918242. 0 is for homozygous major allele, 1 is for heterozygous genotype, and 2 is for homozygous minor allele.

## 4. Discussion

The presence of periodontopathic bacteria induces the increased production of pro-inflammatory cytokines from the host, resulting in periodontal disease progression. The genotypic and allelic distributions of SNPs TNF-A-308A/G, MMP-2-1306C/T, MMP-9-1562C/T, TIMP-2-418G/C and IL-13-1112C/T were examined both in the Li nationality and Han nationality, living in Hainan Province with and without CP.

In this study, no statistically significant differences were found in genotypic or allelic distribution between CP and control groups for these five SNPs. The allele frequency difference and genotype frequency difference of rs1800629, rs243865, rs8179090 and rs1143634 in the healthy controls and

the CP patients of Han Nationality and of Li Nationality were less than 0.1. Only the genotype frequency difference of rs3918242 was greater than 0.1 in the healthy controls and the CP patients of Han Nationality. This may due to the significant deviation in age sampling in the Li Nationality. The age of the CP patients was significantly higher than that of the healthy controls, thus, the genetic background of the healthy controls was not exactly the healthy controls, or the potentially CP individuals in the healthy controls sampled have not yet reached the age of onset. TNF-A has been shown to participate in bone metabolism and is elevated in the patients with periodontitis. Hence, we selected the TNF-A encoded gene as a candidate gene that might be involved in periodontitis susceptibility.

TNF-A gene polymorphisms have been studied in periodontitis in various populations, but the results are inconsistent. This case-control study examined one SNP in TNF-A promotor area: -308G/A, along with the relationship with periodontitis. The results showed no significant genotype frequency difference or allele frequency difference in the Han Nationality or Li Nationality with or without CP. This was consistent with several studies. Menezes and Colombo (Menezes & Colombo, 2008) as well as Moreira et al. (Moreira, Costa, Gomez, Gollob, & Dutra, 2009) demonstrated that the TNF-A (-308) polymorphism was not linked to periodontal disease among Brazilians. In addition, Donati et al. (Donati, Berglundh, & Hyto¨nen, 2005) did not find any link between chronic periodontitis and the TNF- A -308 polymorphism in Swedish Caucasian subjects of a northern European origin. MMP and TIMP play a role in the development of inflammatory periapical lesions, and TIMP prohibitted the expression of MMP. MMP-2-1306C/T, MMP-9-1562C/T and TIMP-2-418G/C were analysed and proved to have no impact on genetic predisposition to periodontal disease.

Data derived from the present study should be interpreted with caution. First, this study included data only from the Li nationality and Han nationality in Hainan Province; thus, the results can only be extrapolated to these two ethnic groups. Second, the sample size in the present study is relatively small, though larger than those in many previous studies. It is estimated that thousands of participants are needed to obtain meaningful results for genetic case-control study, since small odds ratios (ranging from 1.1 to 1.50) were obtained in most associations (Yamamoto et al., 1997). For further study in the future, in vitro and in vivo functional studies are needed to determine whether the SNP or haplotypes demonstrating significant associations with CP susceptibility in the present work could influence the mRNA or protein levels in the context of CP.

In addition, gene-gene and gene-environment interactions and family linkage analysis in different ethnical groups consisting of large-scale samples might be meaningful for obtaining a better insight into the pathogenesis of CP.

## 5. Conclusions

In conclusion, our study meticulously explored the association between polymorphisms in TNF-α, MMP-2, MMP-9, TIMP-2, and IL-13 genes and the predisposition to chronic periodontitis among individuals of the Li ethnic group in Hainan Province. Despite the established roles of these genes in the immune response to periodontal disease, our findings indicate no significant correlation between the selected single nucleotide polymorphisms and chronic periodontitis in the studied populations. This outcome underscores the complexity of genetic factors influencing chronic periodontitis and suggests that other genetic, environmental, or lifestyle factors may play more critical roles in its development among the Li and Han populations. The absence of a direct genetic association also points to the potential for broader, multifactorial strategies in managing oral health and its implications for physical fitness and mental well-being, especially in athletes. Future research should, therefore, expand to include a wider array of genetic markers, as well as consider the multifaceted interactions between genetic predispositions, oral health practices, and systemic health outcomes. This holistic approach may yield more insightful correlations and strategies to enhance both oral and overall health, particularly for those in physically demanding professions or activities.

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