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# Osteoprotegerin Gene (*OPG*) Polymorphisms Associated with Peri-Implantitis Susceptibility in a Chinese Han Population

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Data Interpretation D  
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Funds Collection G

AG 1,2 **Jian Zhou**  
BCDEF 1 **Yimin Zhao**

1 State Key Laboratory of Military Stomatology, Department of Prosthetic Dentistry, School of Stomatology, The Fourth Military Medical University, Xi'an, Shaanxi, P.R. China  
2 Department of Stomatology, 253<sup>th</sup> Hospital of Chinese PLA, Huhhot, Inner Mongolia, P.R. China

**Corresponding Author:** Yimin Zhao, e-mail: zhaoyiminger@yeah.net  
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**Background:** The aim of this study was to investigate the association between T950C (rs2073617) and G1181C (rs2073618) polymorphisms of the osteoprotegerin gene (*OPG*) and the susceptibility of peri-implantitis in the Chinese Han population.

**Material/Methods:** 110 patients with peri-implantitis and 116 healthy persons from the Chinese Han population were included in this study using a case-control design; rs2073617 and rs2073618 in *OPG* were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The linkage disequilibrium (LD) and haplotype analysis were performed with Haploview software. Hardy-Weinberg equilibrium (HWE) was assessed in the control group based on the genotype distributions of *OPG* polymorphisms. The genotype, allele, and haplotype distribution differences between the case and control groups were analyzed by chi-square test, and the relative risk of PD was expressed by odds ratio (OR) and 95% confidence interval (CI).

**Results:** The study results showed that people carrying the CC genotype of rs2073618 were more likely to have peri-implantitis than GG genotype carriers (OR=2.18, 95% CI=1.03-4.62,  $p=0.04$ ). In addition, patients with the C allele had 1.47 times the risk of suffering from peri-implantitis (OR=1.47, 95% CI=1.01-2.13,  $p=0.04$ ), but not rs2073617 polymorphism. The G-C haplotype frequency of rs2073618-rs2073617 in *OPG* was significantly correlated to the increased susceptibility of peri-implantitis (OR=2.27, 95% CI=1.20-4.30).

**Conclusions:** *OPG* rs2073618 polymorphism may be related to the risk of peri-implantitis, but not rs2073617. Moreover, haplotype is also a non-ignorable risk factor.

**MeSH Keywords:** Haplotypes • Peri-Implantitis • Polymorphism, Genetic

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## Background

With the development in social economy and the growing maturity of dental implant technology, dental implants have become the preferred solution for people to repair some or all of their missing teeth. Peri-implantitis is an inflammation occurring in osseous tissue surrounding the dental implant and can develop as a chronic progressive marginal inflammation, which can cause loss of bone tissue and function around the implant [1]. There are similarities in aspects of microbiology and clinical and pathological manifestations between peri-implantitis and periodontitis, but there are still differences in the rate and extent of inflammation, as well as in cell composition [2,3]. Studies have shown chronic periodontitis was significantly associated with cytokine gene polymorphisms and environmental factors [4,5]; therefore, when studying the cause of peri-implantitis, the role of molecular genetic susceptibility and environmental factors should be considered.

Osteoprotegerin (*OPG*) is a cytokine receptor, as well as a new member of the tumor necrosis factor (TNF) receptor superfamily, also known as osteoclastogenesis inhibitory factor (OCIF), or tumor necrosis factor receptor superfamily member 11B (TNFRSF11B) and belongs to a protein that is encoded by the TNFRSF11B gene in humans, which was discovered in 1997 by Simonet [6]. It is also a secretory glycoprotein that regulates the function of bone resorption, comprising 401 amino acid residues arranged into seven structural domains. It is found as either a 60-kDa monomer or 120-kDa dimer linked by disulfide bonds [7]. Its main function is to inhibit osteoclast formation. *OPG* is expressed in various tissues and organs, including the heart and blood vessels [8].

Animal tests have shown that *OPG* gene knockout mice manifest severe bone loss and artery calcification [9,10]. *OPG* gene knockout mice studies suggest that the *OPG* gene can prevent the occurrence of arterial calcification [11,12]. Current studies indicate that *OPG* gene promoter and intron polymorphism *loci* include T950C, T149C, T245G, C889T, G1181C, A163G, G209A, A6890C, and C1217T [13]. Reports found that *OPG* gene polymorphism was related to the decrease in bone mineral density and bone fracture in postmenopausal or elderly patients with osteoporosis. However, there are few research studies about *OPG* gene polymorphism and peri-implantitis. Therefore, in this study we investigated the association of *OPG* rs2073617 and rs2073618 polymorphisms with genetic susceptibility to peri-implantitis in the Chinese Han population, in order to investigate the theoretical foundation for the mechanism of peri-implantitis.

## Material and Methods

### The case and control groups

This study aimed to test the correlation between *OPG* polymorphisms and peri-implantitis. All study participants were informed of the research process and signed informed consent forms. The study was authorized by the Ethics Committee of State Key Laboratory of Military Stomatology, Department of Prosthetic Dentistry, School of Stomatology, The Fourth Military Medical University. Trained epidemiological investigators collected blood samples and the process of sample collection was conducted according to the national ethics criteria of human genome research.

The case group included 110 patients with peri-implantitis who were selected from the clinical inpatients in the Department of Oral Implantology of the State Key Laboratory of Military Stomatology, Department of Prosthetic Dentistry, School of Stomatology, The Fourth Military Medical University, and who were diagnosed by pathobiology between December 2014 to January 2015, including 89 males and 21 females. The age range was 18–67 years, with an average age of  $42.85 \pm 11.21$ . Inclusion criteria were as follows: IMZ or Frialit-2 (German Friadent Corp) implants for the repair of dental implants within the oral cavity; no loosening of implants; swelling of the mucosa around the implant, bleeding on probing, x-ray examination of implant crown has bone resorption through shadow; and the absorption of alveolar bone around implants was greater than 3 mm. The exclusion criterion was peri-implantitis caused by mechanical overload.

The control group included 116 persons who had a successful implant, also at the State Key Laboratory of Military Stomatology, Department of Prosthetic Dentistry, School of Stomatology, The Fourth Military Medical University, during the same period, and included 94 men and 22 women. The age range was 17–69 years, with an average age of  $43.02 \pm 10.94$ . The patients were all from the Chinese Han population, but they had no blood relationship to one another.

### DNA extraction

For all participants, 3 mL peripheral venous blood was collected after a 12-hour fast; the blood sample was placed in a tube containing disodium ethylene diamine tetraacetic acid (EDTA-2Na) as an anticoagulation, and then stored at  $-80^{\circ}\text{C}$ . DNA was extracted according to the manufacturer's instructions; peripheral blood leucocyte genome DNA of all samples was extracted using Beijing TIANGEN biochemical blood genome DNA extraction kit, and then stored at  $-20^{\circ}\text{C}$ .

**Table 1.** Primer sequences of *OPG* rs2073617, rs2073618.

SNP		Primer sequence	Tm (°C)
rs2073618	For.	5'-CCAAGCCCCTGAGGTTT-3'	68°C
	Rev.	5'-GGAGACCAGGTGGCAGC-3'	
rs2073617	For.	5'-CCTGGGGGATCCTTCC-3'	54°C
	Rev.	5'-AAGTATCGCCTTGA-3'	

### The determination of genotypes in *OPG* polymorphisms

The genotypes of *OPG* were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primer design (Genebank database of NCBI) was adopted to find the complete sequence of *OPG* and the sequences of rs2073618 and rs2073617 polymorphisms. Complying with general primer design principles, we designed the PCR primers using Primer Premier 5.0 software, and synthesized in Shanghai Sangon Biotech Co., Ltd. The primer sequences are listed in Table 1. The PCR reaction mixture was 25 µL, including 1.0 µL DNA template, 1.0 µL each of forward and reverse primers, 1.0 µL Taq DNA enzyme, 2.0 µL dNTPs, 2.5 µL 10× buffer solution, and 16.5 µL sterilized double distilled water. The amplification conditions of the PCR were 95°C pre-denaturation for 5 minutes, followed by 45 cycles of 94°C degeneration for 10 seconds, 68°C/54°C annealing for 30 seconds, 72°C extension for 60 seconds, and finally 72°C extension for 5 minutes. The PCR products were checked using 1% agarose gel electrophoresis (AGE). The enzyme reaction mixture was 20 µL, including 3.0 µL restriction enzyme (ExoI for rs2073618 and HindIII for rs2073617), 2.0 µL 10 x buffer solution, 7.0 µL PCR products, and 8.0 µL double distilled water. The mixture was digested for 16 hours in a water bath at 37°C. The enzyme-digested products were separated using 3% agarose gel electrophoresis (AGE); the final outcome was observed in an imaging system.

### Statistical analysis

Hardy-Weinberg equilibrium (HWE) was used to detect group differences. The frequencies of genotype, allele, and haplotype in *OPG* polymorphisms were determined by the direct counting method. Linkage disequilibrium (LD) and its correlation coefficient ( $D'$  value) were calculated using Haploview software. The comparison of genotypes, alleles, and haplotypes between the two groups was tested by chi-square test. Measurement data was expressed by  $\bar{x} \pm s$  and%. The effects of different genotypes, alleles, and haplotypes on peri-implantitis were estimated through odds ratio (OR) and 95% confidence interval (CI). All statistical analysis was accomplished by PASW Statistics 18.0 software;  $p < 0.05$  was considered statistically significant.

## Results

### General characteristics of study objects

This study included 110 patients with peri-implantitis (case group) and 116 controls (control group). In the case group, women accounted for 19.09%, and the sex (male/female) ratio was 4.24: 1; the sex ratio for the control group was similar, 4.27: 1. Hence, there was no significant difference between the two groups by sex ( $p > 0.05$ ). The case group mean age was  $42.85 \pm 11.21$ , and the control group mean age was  $43.02 \pm 10.94$ ; there was no significant different between the two groups by age ( $p > 0.05$ ).

### The genotype distributions of *OPG* polymorphisms in case and control groups

The genotype distribution of *OPG* rs2073618 and rs2073617 polymorphisms in the control group conformed to HWE ( $p = 0.93$ ,  $p = 0.31$ , respectively). This result suggests that our population had a similar genetic background to the Mendelian population. The genotype and allele distributions of two SNPs in *OPG* are shown in Table 2. GG, GC, and CC genotype frequencies in rs2073618 were 28.18%, 45.46%, and 26.36% respectively in the case group and 36.20%, 48.28%, and 15.52% respectively in the control group. The GC allele frequency was 50.91% and 49.09% in the case group and 60.34% and 39.66% in the control group. Thus, the CC genotype and C allele distributions showed statistically significant differences between the two groups ( $p = 0.04$ ,  $p = 0.04$ , respectively), and their carriers were more likely to develop infection compared with those carrying the TT genotype and T allele (CC vs. GG: OR=2.18, 95% CI=1.03–4.62; C vs. G: OR=1.47, 95% CI=1.01–2.13).

The GG, GC, and CC genotypes frequencies of rs2073617 were 32.14%, 45.24%, and 22.62% respectively in the case control group and 39.66%, 50.00%, and 10.34% respectively in the control group. There were no significant differences between the two groups based on genotypes and alleles ( $P > 0.05$ ), which demonstrated that this polymorphism was not correlated with the susceptibility to peri-implantitis.

**Table 2.** Frequency comparisons of genotypes and alleles in *OPG* gene polymorphisms.

Genotype/allele	Case, n=110 (%)	Control, n=116 (%)	$\chi^2$	P	OR (95% CI)	$P_{HWE}$
rs2073618						0.93
GG	31 (28.18)	42 (36.20)	–	–	1.00	
GC	50 (45.46)	56 (48.28)	0.39	0.53	1.21 (0.66–2.21)	
CC	29 (26.36)	18 (15.52)	4.23	0.04	2.18 (1.03–4.62)	
G	112 (50.91)	140 (60.34)	–	–	1.00	
C	108 (49.09)	92 (39.66)	4.08	0.04	1.47 (1.01–2.13)	
rs2073617						0.31
TT	40 (32.14)	46 (39.66)	–	–	1.00	
CT	51 (45.24)	58 (50.00)	0.01	0.97	1.01 (0.57–1.78)	
CC	19 (22.62)	12 (10.34)	1.99	0.16	1.82 (0.79–4.21)	
T	131 (54.76)	150 (64.66)	–	–	1.00	
C	89 (45.24)	82 (35.34)	1.25	0.26	1.24 (0.85–1.82)	

HWE – Hardy-Weinberg equilibrium.

**Table 3.** Analyses of LD and haplotypes in *OPG* rs2073618, rs2073617 polymorphisms.

Haplotype SNP1-SNP2	Case 2n=220 (%)	Control 2n=232 (%)	$\chi^2$	P	OR (95% CI)
G-T	100 (45.45)	132 (56.90)	–	–	1.00
C-C	77 (35.00)	74 (31.90)	2.29	0.13	1.37 (0.91–2.07)
G-C	31 (14.09)	18 (7.76)	6.61	0.01	2.27 (1.20–4.30)
C-T	12 (5.46)	8 (3.44)	2.13	0.15	1.98 (0.78–5.03)

SNP1 – rs2073617; SNP2 – rs2073618.

**Haplotype analysis of *OPG* rs2073618, rs2073617 polymorphisms**

The LD and haplotype analysis of *OPG* rs2073618 and rs2073617 was performed by Haploview software. Strong LD was discovered between them ( $D' = 0.93$ ), and four haplotypes were found: G-T, C-C, G-C, C-T (rs2073618-rs2073617) (Table 3). The data show distributions of G-C haplotype, with obvious differences between the two groups ( $p = 0.01$ ) compared with G-T haplotype, which indicates this haplotype could increase the risk of peri-implantitis (OR=2.27, 95% CI=1.20–4.30).

**Discussion**

Peri-implantitis is the most common complication after implantation of support dentures; moreover, it is the main reason for failure of dental implant restoration. Peri-implantitis is the inflammation of the hard and soft tissue around the implant. It can result in supporting bone loss, which leads to

failure of the bone union. It is similar to chronic adult periodontitis. The incidence rate is 5% to 8% [3]. General symptoms are bleeding around the implant on probing, festering around implant, probing depth increase, sinus formation, gingival recession, gingival swelling, and on radiological examination appearance around the implant of bone resorption. Peri-implantitis is most often found during a return follow-up visit, and requires timely, correct diagnosis and treatment to avoid complete loss of synostosis, which can lead to failure of the implant [14]. In addition, persistent inflammation can lead to the destruction of the periodontal and peri-implant tissues [15]. At present, genetic differences are not a common factor known to affect the immune response of periodontopathogens [16]. The etiology and pathogenesis is not yet fully understood. Therefore, in this study we investigated the relationship between genetic susceptibility and peri-implantitis.

*OPG* is a secreted glycoprotein that has the function of protecting bone and preventing bone resorption [6]. The human *OPG* gene (TNFRSF11B) is located on chromosome 8q24,

which is a single-copy gene. It contains five exons, and the total length of the sequence is 29kb. It consists of 401 amino acid residues, including a signal peptide composed of 21 amino acids and seven structural domains (D1-D7). *OPG* mRNA is expressed in all tissues of the body, including lung, heart, kidney, liver, gastrointestinal tract, spinal cord, thyroid, and bone tissue; it is also expressed in fibroblasts, aortic smooth muscle cells, B lymphocytes, and other cells. Cardiovascular studies have shown that plasma osteoprotegerin could be used for prognosis of patients with cardiovascular disease [17–19]. However, little research has investigated the correlation between it and peri-implantitis.

The eleventh member of the *OPG* ligand family is a receptor activator of nuclear factor  $\kappa$  ligand (RANKL), also known as TNFSF11, a protein related to the TNF ligand super-family and which is the stimulating factor for osteoclasts. The RANK/RANKL/*OPG* pathway plays an important role in osteoclast formation, activation, and regulation of bone resorption [20,21]. The RANKL gene is closely related to the occurrence of bone resorption in other diseases, such as periodontitis, so it is appropriate to speculate that it is one of the major determining genes in peri-implantitis [22]. One study showed that RANKL gene polymorphism was significantly associated with bone resorption, and for the person carrying RANKL gene polymorphic locus, metabolism increased and bone mineral density decreased [23]. By analysis of allele linkage through human venous blood, Kadkhodazadeh et al. confirmed the correlation between RANKL gene polymorphism and peri-implantitis [24]. In addition, Kadkhodazadeh et al. [25] found that another major *OPG* gene in the RANK/*OPG*/RANKL pathway was also associated with the occurrence of peri-implantitis. The single nucleotide polymorphism (SNP) of the gene had T950C and G1181C, but only the genetic polymorphism of G1181C was related to the occurrence of peri-implantitis. This result is in agreement with the results of our study.

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## Conclusions

Our study supported the correlation between *OPG* polymorphisms and peri-implantitis in patients from a Chinese Han population. There are many limitations to our study, such as small sample size and environmental factors. To confirm our study results and achieve the goals of early diagnosis and timely treatment of peri-implantitis, further research should be conducted using well designed larger studies that include various populations.

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