OPG rs2073617 polymorphism is associated with upregulated OPG protein expression and an increased risk of intervertebral disc degeneration

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Abstract. The present study aimed to investigate the associations between three distinct osteoprotegerin (*OPG*) gene polymorphisms and the risk of intervertebral disc degeneration (IDD). A total of 200 IDD patients and 200 healthy controls were recruited from the Department of Spine Surgery at the First Affiliated Hospital of the University of South China (Hengyang, China) between January 2013 and May 2014. The allele, genotype and haplotype frequency distributions of three *OPG* polymorphisms in the study and control populations were analyzed by polymerase chain reaction prior to restriction fragment length polymorphism or high resolution melting assays. In addition, serum OPG levels were measured via an ELISA. The genotype and allele frequencies of the *OPG* rs2073617 polymorphisms were significantly higher in the IDD patients, as compared with the control group (P<0.05). Furthermore, carriers of the C allele exhibited a higher risk of IDD, as compared with carriers of the T allele (P<0.001). Conversely, the genotype and allele frequencies of the two other gene polymorphisms, rs2073618 and rs3102735, showed no significant differences between the patients and controls (P>0.05). The serum OPG levels were significantly higher in IDD patients with TT, TC and CC genotypes at the *OPG* rs2073617 polymorphism, as compared with the control group (P<0.05). Logistic-regression analysis suggested that high serum levels of OPG were positively correlated with IDD risk, whereas the T-C-A, T-G-A and T-G-G haplotypes were negatively correlated with IDD risk (P<0.05). Furthermore, the G-T-G haplotype was associated with protection against IDD (P=0.008), whereas the G-C-G haplotype was associated with an elevated susceptibility to IDD (P=0.007). The results of the present study suggested that *OPG* rs2073617 polymorphisms and upregulated serum levels of OPG were associated with an increased risk of IDD, whereas the T-C-A, T-G-A and T-G-G haplotypes were protective factors for IDD. The results of the present study suggested that the *OPG* gene polymorphism may have an important role in the progression of IDD, and its serum level may function as a valuable predictive indicator of the severity of degenerative disc diseases.

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Introduction

Intervertebral Disc Degeneration (IDD), which is also called degenerative disc disease, is the major cause of degenerative spinal disease and one of the most common ailments severely affecting quality of life in elderly populations (1). IDD is characterized by lower back pain, intervertebral disc herniation and spinal canal stenosis (2). Degeneration of the nucleus pulposus in spinal discs may reduce the production and secretion of extracellular matrix components, leading to changes in disc structure and resulting in the dysfunction of the discs (3-5). The incidence of IDD has been shown to be influenced by gender and age, with a prevalence in adolescents and young adults of <10%, which increases to 30-50% in middle adulthood (6). The exact pathophysiology underlying IDD is not well understood; however, genetic and environmental factors are thought to be involved (7-9). Previous studies have demonstrated that smoking, excessive biomechanical load, obesity, gender, age, decreased nutrition and other environmental factors are associated with a high risk of developing IDD (10-13). Furthermore, pro-inflammatory signaling pathways, tumor necrosis factor (TNF) and matrix degrading enzymes may have pivotal roles in the pathogenesis of IDD (2,14-16), and it has been suggested that osteoprotegerin (*OPG*), a member of the TNF receptor superfamily, has a crucial role in the etiology of IDD (17,18).

OPG, which is also called osteoclastogenesis inhibitory factor or tumor necrosis factor receptor superfamily member 11B, is a glycoprotein and cytokine receptor containing 401 amino acid residues (19,20). OPG is primarily

synthesized by osteoblasts; however, it is also expressed in various organs and tissues, including the heart, vessel walls, lungs, kidneys and bone (21,22). The main role of OPG is its function as a decoy receptor for receptor activator for nuclear factor-κB ligand (RANKL) (23-25); by competitively binding to RANKL, OPG prevents RANK/RANKL association and activation of RANK, thereby modulating bone resorption, decreasing the number of osteoclasts and acting as a regulator of osteoblast-osteoclast cross-talk in bone homeostasis (26,27). The RANK-RANKL-OPG system regulates the balance between osteoblasts and osteoclasts, and has a crucial role in bone formation and absorption (28). Previous studies have suggested that a deficiency of the *OPG* gene enhances osteoclastogenesis and secondary hyperactive osteoblasts in long bones and vertebral bones (17,29). Therefore, it has been hypothesized that factors affecting *OPG* gene regulation may be major genetic factors influencing bone mass and increasing the risk of fractures, osteoporosis and osteoarthritis (30,31). The human *OPG* gene is located on chromosome 8q23-24, has a length of ~29 kb and consists of 5 exons and 4 introns (32). Previous studies have demonstrated that changes in the OPG serum level are influenced by genetic polymorphisms, including 950T/C at the rs2073617 locus, 1181 G/C at the rs2073618 locus and 1181 G/C at the rs2073618 locus (33-35). The association between genetic polymorphisms in the *OPG* gene and bone mineral density has been the focus of previous investigations (36,37). Previous studies have implicated increased bone mineral density in the etiology of IDD (38,39); however, few studies have focused on a direct association between *OPG* genetic polymorphisms and the risk of IDD. Therefore, the present study aimed to investigate the associations between *OPG* genetic polymorphisms, serum OPG levels and IDD risk.

Materials and methods

Ethical approval and patient consent. The present study was approved by the Ethical Committee of the First Affiliated Hospital of the University of South China. Written informed consent was acquired from all study participants at the time of hospitalization. In addition, the present study was performed in accordance with the guidelines and principles of the Declaration of Helsinki (40).

Subjects. Between January 2013 and May 2014, a total of 200 patients with IDD, including 100 females and 100 males, at the Department of Spine Surgery of the First Affiliated Hospital of the University of South China (Hengyang, China) were enrolled in the present study. The average age of the patients was 52.6±6.6 years (age range, 40-62 years). All patients were diagnosed with IDD according to magnetic resonance imaging (MRI) results and IDD was confirmed by postoperative pathological analyses, according to previous studies (41,42). All patients presented with the typical clinical and physical symptoms of IDD, including: i) Chronic lower back pain with radiation to the lower limb; ii) spasticity and atrophy of the paravertebral and lower limb muscles; iii) limited activity; and iv) positive results in a nerve traction test. In addition, the patients showed obvious disc degeneration in the postoperative pathological analysis. Patients were excluded from the present study if they suffered from lumbar spinal stenosis. In addition, 200 age- and gender-matched healthy subjects from our hospital, including 100 men and 100 women aged between 42 and 62-years (average age, 52.1±5.4 years), were recruited. The MRI findings of the healthy controls showed normal intervertebral disc tissue (http://http://www.uscspine.com/conditions/back-degenerative-disc.cfm). No significant differences in age, gender, body mass index and smoking history were observed between the IDD patients and controls. The present study was approved by the Ethics Committee of the First Affiliated Hospital of the University of South China.

Specimen collection. Blood samples (10 ml) were drawn from the patient's elbow vein in the morning following an overnight fast, and 3 ml blood was added to ethylenediamine tetraacetic acid (EDTA) tubes (Sigma-Aldrich, St. Louis, MO, USA) for anticoagulation. Genomic DNA was extracted from the 3 ml blood samples using a Whole Blood Genomic DNA Extraction kit (cat. no. OSR-M102/M104; Tiangen Biotech, Co., Ltd., Beijing, China). The remaining blood (without EDTA) was allowed to clot for 1 h at room temperature, followed by centrifugation at 1006.2 x g for 10 min at 37˚C to obtain the serum, which was stored at -80˚C until further use.

High resolution melting (HRM) analysis. The primers used for HRM genotyping were designed by LightScanner® Primer Design software, version 1.0 (Idaho Technology, Inc., Salt Lake City, UT USA) and are presented in Table I. The polymerase chain reaction (PCR)-HRM analysis was performed using a final reaction volume of 11 μl, consisting of 1 μl genomic DNA (20 ng/l), 0.2 μl each of the forward and reverse primers (each 10 pmol), 5 μl 2X *Taq* PCR Master mix (containing *Taq* DNA polymerase, Mg^{2+} and dNTPs; Tiangen Biotech, Co., Ltd.), 3.6 μl sterilizing ultrapure water and 1 μl 10X LCGreen Plus dye (Idaho Technology, Inc.). The PCR reaction was conducted using the Type-it HRM PCR kit (cat. no. 206542; Qiagen GmbH, Hilden, Germany), according to the manufacturer's protocol. The PCR reaction conditions were as follows: Initial denaturation at 96˚C for 5 min, followed by 40 cycles of 96˚C for 20 sec, 57˚C for 20 sec and 72˚C for 20 sec. The HRM analysis was synchronized with the PCR reaction, as follows: 95˚C for 5 min, 40˚C for 2 min, 60˚C for 1 min and then heating from 72-95˚C at 0.1˚C/sec, during which time the melting curve data was collected. Each HRM analysis detected the standard three genotypes already known from each locus and the genomic DNA of study subjects. LightScanner software (Idaho Technology, Inc.) was used in the melting curve analysis to determine the genotypes.

PCR-restriction fragment length polymorphism (PCR-RFLP) assays. PCR primers were designed using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA, USA) and were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The sequences and lengths of the primers are shown in Table II. The PCR reaction system consisted of 100 ng genomic DNA, 125 ng each of the upstream and downstream primers, 12.5 μl 2X *Taq* PCR Master mix and 9.5 μl double-distilled H_2O, in a final volume of 25 μl. The cycling conditions were as follows: Initial denaturation at 95˚C for 5 min, followed by

Table I. Sequences of the primers used for polymerase chain reaction-high resolution melting genotyping of the osteoprotegerin gene.

Locus	Primer sequence	Product size (bp)
rs2073617	F: 5'-CTGGTAGGACAAATATTGG-3' R: 5'-ACTTACCATTTGCGATCACC-3'	88
rs2073618	F: 5'-CGTTGTCTTGAGAAGGTTGA-3' R: 5'-TCCTCAAAAACATGTCAGTGTG-3'	141
rs3102735	F: 5'- TCCCACTATCATGATTATTTCCC-3' R: 5'-ATTATAGGTTTTTAAGTA ATTTGT-3'	163

F, forward; R, reverse.

Table II. Sequences of the primers used for polymerase chain reaction-restriction fragment length polymorphism analysis of the three loci of the osteoprotegerin gene.

Locus	Primer sequence	Product size (bp)	Endonucleases
rs2073617	F: 5'-GAAGTGAAGGGGTCAGGCAGC-3' R: 5'-GTCTGTCTCTCTCTTGCTGTCTTCC-3'	342	*Hinc*II
rs2073618	F: 5'-GTCTGTCTCTCTCTTGCTGTCTTCC-3' R: 5'-GAGATGAAGACAGAAGGTTAATGAC-3'	340	*Xsp*I
rs3102735	F: 5'-TTCCTTCCCTTGAATCTGGTG-3' R: 5'-CTAAAGCCCGTGCTATTCTGC-3'	300	*Ase*I

F, forward; R, reverse.

30 cycles at 94˚C for 30 sec, 62˚C for 45 sec and 72˚C for 60 sec, and a final extension step at 72˚C for 10 min. *Hinc*II, *Xsp*I and *Ase*I restriction enzymes (Takara Biotechnology Co., Ltd., Dalian, China) were added to the PCR products and then 10% agarose gel electrophoresis was used to assess PCR fragment patterns. An automated DNA sequencer (model 370; Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to determine the genotypes of the rs2073617, rs2073618 and rs3102735 loci.

Measurement of OPG protein levels in serum. Serum levels of OPG were measured using an OPG ELISA kit (cat. no. K1011; Immundiagnostik AG, Bensheim, Germany), according to the manufacturer's protocol. Optical density (OD) values were measured at 450 nm using a microplate reader. The standard curve was drawn by setting standard concentrations as the vertical axis and OD values as the horizontal axis. All samples were measured twice and averaged, and the detection met laboratory quality control standards.

Construction of haplotypes. *OPG* rs2073617 and rs3102735 are located in the promoter region of the *OPG* gene, whereas rs2073618 is located in the exon 1 (43). Haplotype construction of these three genotypes of polymorphic loci was performed using the individual-driven Bayesian method implemented in PHASE 2.1 software (http://stephenslab.uchicago.edu/phase/download.html), as previously described (44).

Statistical analysis. SPSS software, version 18.0 (SPSS, Inc., Chicago, IL, USA) was used to perform data analyses. Hardy-Weinberg equilibrium was used to assess and confirm the representation of the selected samples in the population. Measurement data are presented as the mean ± standard deviation. One-way analysis of variance was used for comparisons between the three groups and Student's t-test was used to compare the measurement data between the groups. The non-parametric Wilcoxon signed-rank test was used to calculate the significance of comparisons when the data did not meet the normal distribution. The χ^2 test was applied to compare the distribution frequencies of the genotypes, alleles and haplotypes between the two groups. Logistic regression analysis was conducted to calculate the odds ratios (OR), and 95% confidence intervals (CI) represented the relative risk. All statistical tests were two-sided probability tests. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Genotyping of the OPG *gene by HRM analysis*. DNA fragments containing polymorphic loci were amplified by PCR and analyzed by HRM. The *OPG* gene has some common, functionally important genetic polymorphisms that have been associated with various human diseases. The most important polymorphisms of the *OPG* gene are rs2073617 (950T/C) and rs3102735 (163A/G), which are located in the promoter region, and rs2073618 (1181G/C), which is located in exon 1 (44). The

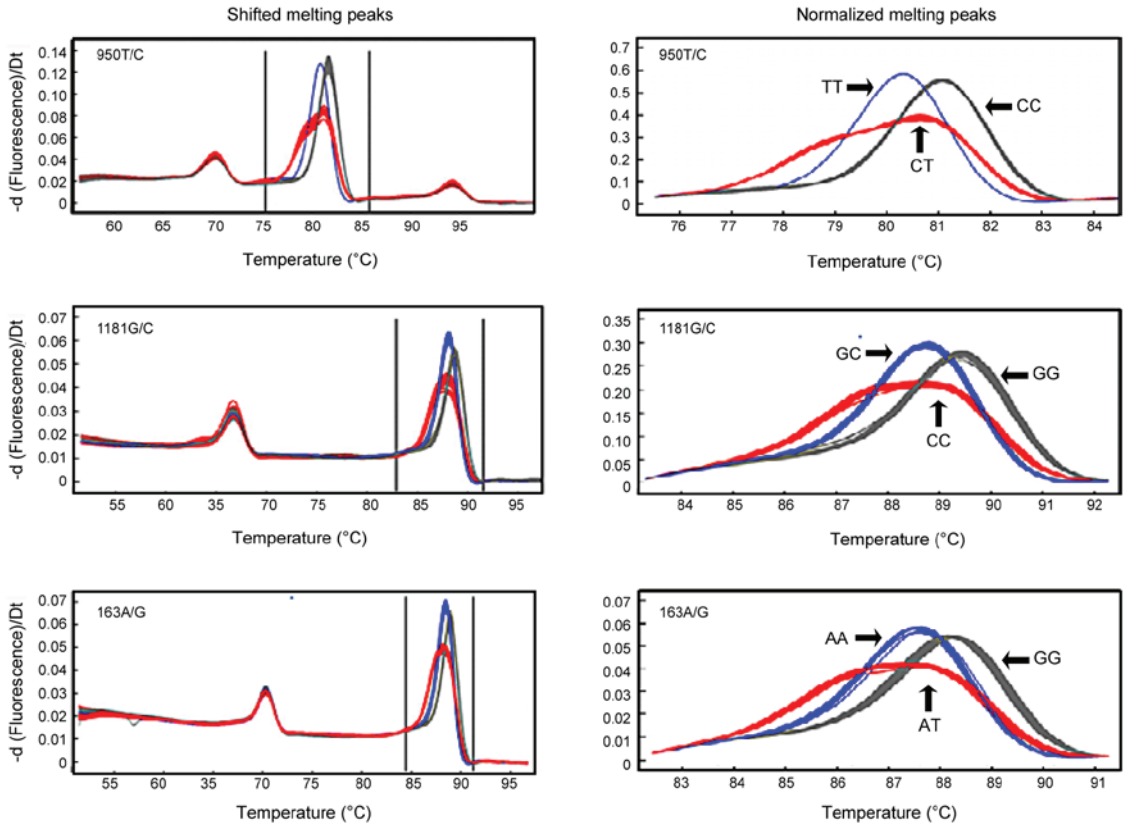

Figure 1. Melting curves of *OPG* genotyping by high resolution melting analysis. There were three different genotypes for the *OPG* rs2073617, rs2073618 and rs3102735 loci in the study population. *OPG*, osteoprotegerin.

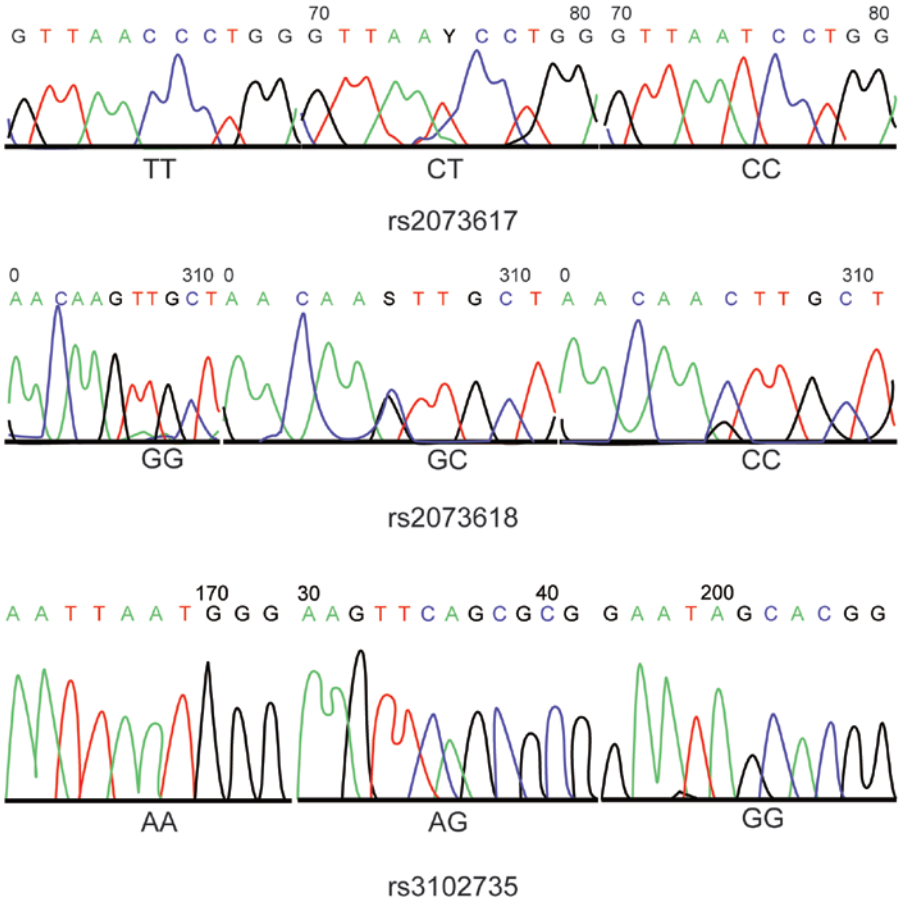

Figure 2. Results of the sequencing of 950T/C (rs2073617), 1181G/C (rs2073618) and 163A/G (rs3102735) polymorphisms of the osteoprotegerin gene.

Table III. Genotype and allele frequency distributions of osteoprotegerin genetic polymorphisms and their associations with intervertebral disc degeneration risk.

Locus	Genotype/Allele	Control Group [n (%)]	Case group [n (%)]	P1	OR (95% CI)	P2
rs2073617	TT	112 (56.0)	84 (41.8)	0.018	Ref.	
	TC	55 (32.8)	70 (35.2)		1.69 (1.07-2.67)	0.021
	CC	33 (11.2)	46 (23.0)		1.19 (1.10-3.16)	0.020
	TC+CC	88 (46.0)	116 (58.2)		1.76 (1.18-2.61)	0.005
	T	290 (72.4)	238 (59.4)	<0.001	Ref.	
	C	110 (27.6)	162 (40.6)		1.79 (1.33-2.41)	<0.001
rs2073618	GG	107 (53.5)	111 (55.2)	0.890	Ref.	
	CG	77 (38.5)	75 (37.8)		0.94 (0.62-1.42)	0.765
	CC	16 (8.0)	14 (7.0)		0.84 (0.39-1.81)	0.662
	CC+CG	93 (46.5)	89 (44.8)		1.04 (0.70-1.53)	0.845
	G	291 (72.8)	304 (74.1)	0.292	Ref.	
	C	109 (27.3)	96 (25.9)		1.07 (0.78-1.47)	0.682
rs3102735	AA	116 (58.4)	113 (56.6)	0.945	Ref.	
	AG	54 (26.4)	55 (27.0)		1.05 (0.87-1.17)	0.848
	GG	30 (15.2)	32 (16.4)		1.10 (0.62-1.92)	0.781
	AG+GG	84 (41.6)	87 (43.4)		1.06 (0.71-1.58)	0.761
	A	287 (71.6)	280 (70.1)	0.586	Ref.	
	G	113 (28.4)	120 (29.9)		1.09 (0.80-1.47)	0.586

P1, comparison of genotypes and allele frequencies between the case and control groups; P2, comparison with the control group in the risk assessment. OR, odds-ratio; CI, confidence interval; P, P-value.

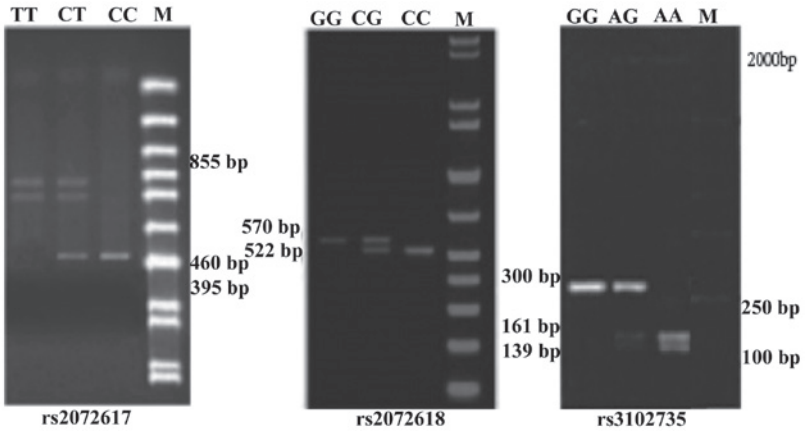

Figure 3. Agarose gel electrophoresis of the various genotypes of the osteoprotegerin rs2073617, rs2073618 and rs3102735 loci, following restriction enzyme digestion.

HRM analysis demonstrated that the 950T/C (rs2073617), 1181G/C (rs2073618) and 163A/G (rs3102735) *OPG* gene polymorphisms were associated with three genotypes (Fig. 1). The HRM genotyping results were consistent with the sequences of the polymorphisms, as determined by PCR and DNA sequencing (Fig. 2).

OPG genotyping. PCR-RFLP analysis of the *OPG* rs2073617 locus detected a single band of 855 bp corresponding to the homozygous wild-type genotype (TT), three fragments of 855 bp, 460 bp and 395 bp corresponding to the heterozygous mutated genotype (TC), and two fragments of 395 bp and 460 bp corresponding to the homozygous mutated genotype (CC). Similarly, for rs2073618, an undigested and single band of 570 bp corresponded to the homozygous wild-type GG genotype, three fragments of 570 bp, 522 bp and 48 bp corresponded to the heterozygous mutated GC genotype, and two bands of 522 bp and 48 bp corresponded to the homozygous mutant CC genotype, although the 48 bp fragment was too small to be visible. Furthermore, the *OPG* rs3102735 locus showed three genotypes: Homozygous wild-type (A/A) with 161 bp and 139 bp fragments; heterozygous mutated genotype (G/A) with 300 bp, 161 bp and 139 bp fragments, and homozygous mutated genotype (G/G) with a 300 bp fragment. The

Table IV. OPG serum levels in individuals with certain genetic polymorphisms of the *OPG* gene.

SNP	Genotype	Control group (ng/ml)	Case group (ng/ml)
rs2073617	TT	6.36±2.10	7.25±2.12[a]
	TC	6.56±2.45	7.86±2.70[a,b]
	CC	6.20±2.23	7.95±2.30[a,b]
rs2073618	CC	6.16±2.13	6.75±2.34
	CG	6.21±2.35	6.69±2.45
	GG	6.54±2.82	6.84±2.41
rs3102735	AA	6.56±2.13	6.86±2.30
	AG	6.21±2.16	6.76±2.42
	GG	6.14±2.52	6.69±2.74

Data are presented as the mean ± standard deviation. [a]P<0.05 vs. the control group; [b]P<0.05 vs. the homozygous wild-type genotypes. SNP, single nucleotide polymorphism; OPG, osteoprotegerin.

results of DNA sequencing confirmed the existence of these genotypes for the rs2073617, rs2073618 and rs3102735 loci (Figs. 2 and 3).

Allele and genotype frequency distributions of OPG polymorphisms. The genotype and allele frequencies of *OPG* rs2073617, rs2073618 and rs3102735 polymorphisms were in accordance with the Hardy-Weinberg equilibrium, thus suggesting that each polymorphism had reached equilibrium and that the selected sample was representative of the population (P>0.05). There were significant differences in the allele and genotype frequencies of the *OPG* rs2073617 polymorphisms between the case and control groups (P<0.05). In addition, patients carrying the C allele exhibited an increased risk of IDD, as compared with carriers of the other alleles (OR=1.79; 95% CI=1.33-2.41; P<0.001). However, no significant differences were observed in the allele and genotype frequencies of the rs2073618 and rs3102735 polymorphisms between the case and control groups (P>0.05; Table III).

Association between serum OPG levels and OPG genotype frequency distributions. Serum levels of OPG were significantly higher in IDD patients with TT, TC or CC genotypes at the *OPG* rs2073617 locus, as compared with the control group (all P<0.05). In addition, OPG serum levels were significantly higher in IDD patients harboring the TC or CC mutated genotypes, as compared with those carrying the wild-type homozygous (TT) genotype (P<0.05). No significant differences were observed between the patient and control groups in the OPG serum levels associated with the various genotypes of the rs3102735 and rs2073618 loci (P>0.05; Table IV and Fig. 4). These results suggest that TT, TC and CC genotypes may contribute to the elevation of serum OPG levels in IDD patients.

Statistical analysis of haplotype frequencies of three polymorphic loci. The T-G-A haplotype was shown to be a potential protective factor for IDD (OR=0.62; 95% CI=0.41-0.94; P=0.02), whereas the C-G-G haplotype was a potential risk factor for IDD (OR=2.24; 95% CI=1.09-4.60; P=0.02; Table V).

Logistic regression analysis. A multivariate stepwise logistic regression was performed to calculate the adjusted OR. The IDD was the dependent variable and the genotypes and haplotypes of *OPG* rs2073617, rs3102735 and rs2073618 loci, body mass index, smoking, OPG levels (>6.69 ng/ml as high levels and ≤6.69 ng/ml as low levels), age and gender, were the independent variables. The multivariate stepwise logistic regression results showed that upregulated OPG serum levels were positively correlated with IDD risk, whereas the T-C-A, T-G-A and T-G-G haplotypes were negatively correlated with IDD risk (P<0.05; Table VI).

Discussion

The pathogenesis of IDD is considered multifactorial, involving various genetic and environmental factors (7,9); however, the exact mechanism underlying IDD remains incompletely understood. The present study evaluated the associations between polymorphisms of the *OPG* gene and the risk of IDD, and demonstrated that the 950T/C variant at the rs2073617 locus was associated with an increased risk of IDD, as compared with the 950T/T wild-type genotype. Conversely, the 1181G/C (rs2073618) and 163A/G (rs3102735) SNPs were not associated with an increased susceptibility to IDD. These results suggested that the *OPG* gene, in particular the 950T/C (rs2073617) SNP, may serve as a potential indicator of IDD risk. Previous studies based on pathophysiological analyses have reported the involvement of various cytokines in IDD (45,46). In addition, the RANK/RANKL/OPG signaling pathway has been proposed to have a role in bone metabolism (26,47,48). Previous studies have demonstrated that disruption of the RANKL/OPG balance led to cartilage degradation due to mechanical loading and resulted in the progression of IDD or osteoarthritis (49,50). In addition, it has been reported that *OPG* genetic polymorphisms negatively influence bone resorption, and thereby regulate bone mineral density, resulting in the deterioration of patients with IDD (51,52). *OPG* gene expression has a crucial role in maintaining the integrity of endplate cartilage by preventing its resorption by osteoclasts (17).

In the present study, a haplotype analysis suggested that the G-T-G, T-C-A, T-G-A and T-G-G haplotypes were associated with protection against IDD, whereas the G-C-G haplotype was associated with an increased susceptibility to IDD. Furthermore, the serum levels of OPG were significantly higher in IDD patients with TT, TC and CC genotypes at the *OPG* rs2073617 locus, as compared with the control group (P<0.05), which suggested that TT, TC and CC genotypes may be significant risk factors for IDD development and that upregulated serum levels of OPG are correlated with IDD risk.

Insufficient nutrition has been shown to be involved in the degeneration of intervertebral discs (50,53,54). Metabolism and nutrient exchange at intervertebral discs is dependent on the interaction of endplate cartilage with intervertebral discs (55), and endplate cartilage has an important role in the biomechanical structure of intervertebral discs (55). *OPG* polymorphisms have been closely associated with the degeneration of endplate cartilage and intervertebral discs by

Table V. Frequency distributions of the haplotypes of osteoprotegerin genetic polymorphisms in the case and control groups.

Haplotypes	Case group	Control group	χ^2	P-value	OR (95% CI)
T-C-A	21 (10.5)	28 (14.0)	0.82	0.36	0.70 (0.40-1.32)
T-C-G	9 (4.5)	12 (6.0)	0.19	0.66	0.73 (0.30-1.79)
C-C-A	14 (7.0)	10 (5.0)	0.71	0.39	1.43 (0.61-3.30)
C-C-G	6 (3.0)	4 (2.0)	0.41	0.52	1.51 (0.42-5.45)
T-G-A	55 (27.5)	76 (38.0)	5.01	0.02	0.62 (0.41-0.94)
T-G-G	26 (13.0)	30 (15.0)	0.33	0.56	0.84 (0.48-1.50)
C-G-A	44 (22.0)	28 (14.0)	2.61	0.06	1.73 (1.03-2.91)
C-G-G	25 (12.5)	12 (6.0)	5.03	0.02	2.24 (1.091-4.60)

Data are presented as n (%). The haplotypes are presented as rs2073617-rs2073618-rs3102735. OR, odds-ratio; CI, confidence interval.

Table VI. Multivariate stepwise logistic regression analysis for the associated risk factors of patients with intervertebral disc degeneration.

Variable	B	S.E.	Wald	df	P-value	Exp (B)	95% CI
T-C-A	-1.022	0.455	5.051	1	0.025	0.360	0.148-0.878
T-G-A	-1.057	0.393	7.228	1	0.007	0.347	0.161-0.751
T-G-G	-0.877	0.442	3.942	1	0.047	0.416	0.175-0.989
High serum level of OPG	0.567	0. 141	7.791	1	0.005	1.758	1.183-2.611

The haplotypes are presented as rs2073617-rs2073618-rs3102735. High serum levels of OPG were defined as >6.69 ng/ml. B, partial regression coefficient; S.E., standard error of regression; Wald, Wald χ^2; df, degree of freedom; Exp (B), adjusted odds-ratio; 95% CI, 95% confidence interval; OPG, osteoprotegerin.

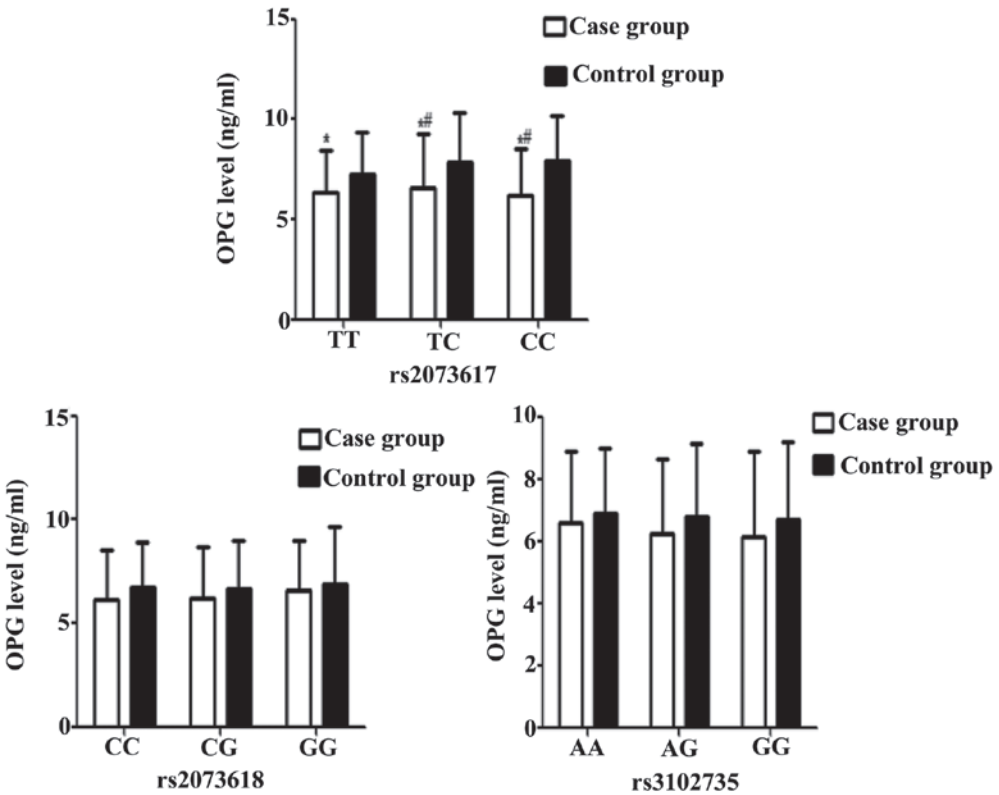

Figure 4. OPG serum levels associated with genetic polymorphisms at the rs2073617, rs2073618 and rs3102735 loci of the *OPG* gene in the case and control groups. Higher serum levels of OPG were detected in intervertebral disc degeneration patients harboring the TT, TC and CC genotypes of the *OPG* rs2073617 locus, as compared with the control group. *P<0.05 vs. the same genotype in the control group; #P<0.05 vs. the TT genotype. *OPG*, osteoprotegerin.

upregulating OPG serum levels; thus OPG has already been associated with an increased risk of IDD (53,56). Furthermore, a previous study demonstrated that the TT, TC and CC genotypes of the rs2073617 locus affected the protein expression levels of OPG and were associated with elevated OPG serum levels; thus suggesting that upregulated OPG expression is a high risk factor for IDD (57). In addition, it was reported that the CC genotype of the rs2073617 locus was associated with increased serum levels of OPG and increased production of OPG by osteoblasts in order to restore disrupted bone metabolism (58). Consistent with this, the present study demonstrated that IDD patients with the TT, TC or CC genotypes of the *OPG* rs2073617 polymorphism had significantly higher serum levels of OPG, as compared with the healthy controls. These results suggested that IDD patients with high OPG serum levels and the 950T/C SNP at the rs2073617 locus may be at a high risk of developing impaired bone metabolism and endplate cartilage degeneration, resulting in IDD.

In conclusion, the present study demonstrated that the *OPG* genetic polymorphism, 950T/C (rs2073617), was associated with an increased risk of IDD, and that the C allele may be a high risk factor for elevated IDD risk by promoting increased serum levels of OPG. In addition, the G-T-G haplotype was associated with protection against IDD, whereas the G-C-G haplotype was associated with an elevated risk of IDD. Furthermore, an elevated OPG serum level was positively correlated with IDD risk, whereas the T-C-A, T-G-A and T-G-G haplotypes were negatively correlated with IDD risk. These results suggested that genetic polymorphisms of the *OPG* gene may influence susceptibility to IDD by altering the protein serum levels of OPG. With continued efforts, the OPG rs2073617 polymorphism may emerge as a biomarker for the diagnosis of IDD, and targeting OPG may represent a novel and promising therapeutic strategy for biologically-induced disc repair in early stage disc degeneration.

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