Association of Interleukin 10 Haplotype with Low Bone Mineral Density in Korean Postmenopausal Women

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Osteoporosis is a disease characterized by exaggerated loss of bone mass, with as much as 50 to 85% of the variation in bone mineral density (BMD) commonly accepted as being genetically determined. Although intensive studies have attempted to elucidate the genetic effects of polymorphisms on BMD and/or osteoporosis in several genes, the genes involved are still largely unknown. The possible associations of genetic variants in five-candidate genes (*IL10*, *CCR3*, *MCPI*, *MCP2* and *GC*) with spinal BMD were investigated in Korean postmenopausal women (n = 370). Fourteen SNPs in five candidate genes were genotyped, and the haplotypes of each gene constructed. The associations of adjusted spinal BMD by age, year since menopause (YSM) and body mass index (BMI), with genetic polymorphisms, were analyzed using multiple regression models. Genetic association analysis of Korean postmenopausal women revealed that *IL10* -592A > C and/or *IL10* *ht2* were associated with decreased bone mass, whereas no significant associations were observed with all polymorphisms in other genes. The levels of spinal BMD in individuals bearing the *IL10* -592CC genotype were lower (0.78 ± 0.16) than those in others (0.85 ± 0.17) ($P = 0.02$), and the BMD of *IL10* *ht2* bearing individuals were also lower (0.82 ± 0.15) than those in others (0.85 ± 0.17) ($P = 0.04$). Our results suggest that variants of

IL10 might play a role in the decreased BMD, although additional study might need to be followed-up in a more powerful cohort.

Keywords: Bone mineral density, Haplotype, Interleukin 10, Osteoporosis, Single nucleotide polymorphism

Introduction

Osteoporosis is a common systemic skeletal disease, defined as both a decrease in bone mass and strength, which increases the fracture risk. Recent studies have shown that osteoporosis is strongly associated with genetic components and genetic factors, which play major roles in the development of peak bone mass, with heritability accounting for about 50-85% of the variance in bone mass. This was supported by twin studies showing a much higher concordance of bone mineral density (BMD) in monozygotic than dizygotic twins (Slemenda *et al.*, 1991; Kanis *et al.*, 1994; Young *et al.*, 1995; Arden and Spector 1997; Eisman 1999; Stewart and Ralston 2000). Numerous candidate genes and their genetic variants, including estrogen receptor (*ER*), transforming growth factor beta1 (*TGFB1*), Interleukin-1 receptor antagonist (*IL1RA*), Interleukin6 (*IL6*), collagen 1A1 (*COL1A1*) and osteoprotegerin (*OPG*), (Kobayashi *et al.*, 1996; Murray *et al.*, 1997; Keen *et al.*, 1998; Yamada *et al.*, 2001; Arko *et al.*, 2002; Garcia-

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Giralt *et al.*, 2002), have been proposed and implicated as genetic markers for BMD, since Morrison and his colleagues reported that vitamin D receptor (*VDR*) gene polymorphism was involved in bone metabolism (Morrison *et al.*, 1992; Morrison *et al.*, 1994). Although these intensive studies attempted to elucidate these genetic effects of polymorphisms for BMD and/or osteoporosis, the genes involved are still largely unknown.

Interleukin 10 (IL10) is a potent anti-inflammatory and a powerful T_H2 cell cytokine. It is produced by various cells, such as T_H2 cells, eosinophils and lymphoid cells, and its roles involved inhibiting both replication of macrophage/monocyte and T-cell lymphocyte and the secretion of inflammatory cytokines (IL1, TNFA, IL6, IL8 and IL12) (de Waal Malefyt *et al.*, 1992; Wanidworanun and Strober 1993; Roth *et al.*, 1996; Groux *et al.*, 1997; Asseman and Powrie, 1998). IL10 down-regulates the expressions of MHC-I and -II molecules and the production of T_H1 cytokines, as well as promotes B cell activation, regulates immunoglobulin class switching and maintains B cell viability by inhibiting apoptosis (Moore *et al.*, 1993). IL10 thus regulates both cellular and hormonal immune responses, so IL10 significantly regulates various diseases, like autoimmune disease, AIDS and chronic hepatitis C infection. *IL10* polymorphisms in the promoter regions (-1082 G > A, -819 C > T and -592 A > C) and their haplotype combination (Rees *et al.*, 2002) may also affect IL10 levels, and have been demonstrated to play an important role in association with various diseases (Eskdale *et al.*, 1998; Shin *et al.*, 2000; Vidigal *et al.*, 2002). It was recently found that IL10 suppresses osteoblastic differentiation in mouse bone marrow cultures (Van Vlasselaer *et al.*, 1993; Van Vlasselaer *et al.*, 1994), and exerts a reciprocal action on the differentiation of osteoclasts and macrophages from their shared precursor. This potent inhibition of osteoclast formation by IL10 suggests that IL10 might play a role in the modulation of bone loss in inflammatory disorders (Owens *et al.*, 1996).

CCR3, a chemokine receptor, and MCP1 and MCP2, chemotactic cytokines are involved in immune responses. Abnormal T-cell expression of several chemokine receptors, including CCR3, and the levels of soluble chemokines in the plasma/serum were observed in patients with SLE (Eriksson *et al.*, 2003). In recent studies, several polymorphisms of *CCR3*, *MCP1* and *MCP2* were identified and their association with several diseases, such as asthma, fibrosis and HCV examined (Fukunaga *et al.*, 2001; Szalai *et al.*, 2001; Hellier *et al.*, 2003). However, the association of the polymorphisms of these three genes with the BMD and/or osteoporosis has not been established. Vitamin D plays a major role in calcium homeostasis and bone turnover, and vitamin D binding protein (group specific component (*GC*), alternatively *DBP*) may play an important role as both a major protein for the vitamin D metabolites and osteoclast differentiation, as well as implicate an association between the polymorphism of vitamin-D binding protein and osteoporosis by bone loss or resorption

(Adebanjo *et al.*, 1998). The polymorphisms in Vitamin D binding protein were reported to be associated with lumbar spine BMD, BMI, plasma DBP, IGF-1 and femoral neck BMD (Rapado *et al.*, 1999).

In this study, five genes (*IL10*, *CCR3*, *MCP1*, *MCP2* and *GC*) were selected as candidates for BMD levels, and whether genetic polymorphisms in the *IL10* gene are associated with spinal BMD in Korean postmenopausal women investigated.

Materials and Methods

Subjects Blood samples were obtained, with informed consent, from 370 Korean postmenopausal women who visited the Samsung Cheil hospital for routine check-ups. The DNA was collected from blood samples, as described in Kim *et al.* (2003). The mean ($\pm$ SD) age, height, weight and spinal BMD of subjects in the study were 54.86 ($\pm$ 6.54), 155.24 ($\pm$ 4.70), 55.30 ($\pm$ 6.82) and 0.84 ($\pm$ 0.17), respectively. The BMI (body mass index) was then calculated using the following formula: BMI = weight (kg)/[height (m)]². The spinal BMD of each participant was measured by dual energy X-ray absorptiometry (DEXA, Lunar Pixi, USA). The device was calibrated twice daily using a standard BMD phantom provided by the manufacturer. The precision error of the device was 1.2%.

Genotyping by single base extension (SBE) PCR was performed under touch down conditions (Schunck *et al.*, 1995). For genotyping of polymorphic sites, the amplification and extension primers were designed for single-base extension (SBE) (Vreeland *et al.*, 2002; Zhang and Li, 2003). Primer extension reactions were performed with the SNaPshot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, USA). Information regarding the primers is summarized in Table 1. To clean up the primer extension reaction, one unit of SAP (shrimp alkaline phosphatase) was added to the reaction mixture and incubated at 37°C for 1 h, followed by 15 min at 72°C for enzyme inactivation. The DNA samples, containing extension products, and Genescan 120 Liz size standard solutions were added to Hi-Di formamide (Applied Biosystems), according to the recommendation of the manufacturer. The mixture was incubated at 95°C for 5 min, followed by 5 min on ice, and then electrophoresis was performed using the ABI Prism 3100 Genetic Analyzer. The results were analyzed using the ABI Prism GeneScan and Genotyper program (Applied Biosystems).

Statistics The Lewontin's D' ($|D'|$) between all pairs of biallelic loci were examined (Hedrick 1987; Hedrick and Kumar 2001). Haplotypes of each individual were inferred using the algorithm developed by Stephens *et al.* (2001) (PHASE), which uses a Bayesian approach incorporating *a priori* expectations of a haplotypic structure from population genetics and coalescent theory. The genetic effects of inferred haplotypes were analyzed in the same way as the polymorphisms. Means and standard deviations (SD) of the phenotypes, as well as the *P* values were calculated by multiple regression analyses controlling for age (continuous value) YSM, and BMI as co-variables.

Table 1. Sequences of amplifying and extension primers for genotyping of the polymorphisms of the *IL10*, *CCR3*, *MCP1*, *MCP2* and *GC* genes by the single-base extension method

Gene	Locus*	Sequences	
<i>IL10</i>	<i>-1082A>G</i>	Forward	5'-CCAACTGGCTCCCCTTACCTTCTAC-3'
		Reverse	5'-CAGGATTCCATGGAGGCTGG-3'
		Extension	5'-ACTTTCCTCTTACCTATCCCTACTTCCCC-3'
	<i>-819T>C</i>	Forward	5'-GGGTGAGGAAACCAATTCTCAG-3'
		Reverse	5'-GGTAGTGCTACCATGACCCC-3'
		Extension	5'-GTACCCTTGTACAGGTGATGTAA-3'
	<i>-592A>C</i>	Forward	5'-AGGTGGAACATGTGCCTGAG-3'
		Reverse	5'-CTCAAAGTTCCCAAGCAGCC-3'
		Extension	5'-TTCCATTTTACTTTCCAGAGACTGGCTTCCTACAG-3'
	<i>+3930T>C</i>	Forward	5'-ATAGCTGACCCAGCCCCCT-3'
		Reverse	5'-AAATCGTTCACAGAGAAGCTCAGT-3'
		Extension	5'-GCTCAGTAAATAAATAGAAATGGGGGTTGAGGTATCAGAGGTAATAAATATTCTAT-3'
<i>CCR3</i>	<i>-22557G>A</i>	Forward	5'-GTAGGTGTCGCTTTTCCAG-3'
		Reverse	5'-TGGCCCCATACTACCTCTA-3'
		Extension	5'-AATCAATGATGATGGTCCAGGCAGGTGGAAGTA-3'
	<i>-520T>G</i>	Forward	5'-TGACAGGAGAAATGGACATGG-3'
		Reverse	5'-TACTGTCTCTTACGGCATTTTGC-3'
		Extension	5'-TATAATCAATGATGATCAATTTCCCCATTAATAATGAATG-3'
	<i>-174C>T</i>	Forward	5'-ACCCCAACAATTTCTGCT-3'
		Reverse	5'-CCTTCGTAAAAACACACCAGTT-3'
		Extension	5'-ATTATAATCAATGATGATGCATATCAGAGGTGCCAGGA-3'
	<i>+51T>C (Y17Y)</i>	Forward	5'-GGGAGAAGTGAAATGACAACCT-3'
		Reverse	5'-AAGAGGCCACAGTGAACAC-3'
		Extension	5'-TTGAGACCTTTGGTACCACATCCTACTA-3'
<i>MCP1</i>	<i>-2518G>A</i>	Forward	5'-TGGGAATTTCCACTCACTTC-3'
		Reverse	5'-CCAGGACTAGAGACTGTGCAA-3'
		Extension	5'-AGAAGTGGGAGGCAGACAGCT-3'
	<i>-2076A>T</i>	Forward	5'-TGGGAATTTCCACTCACTTC-3'
		Reverse	5'-CCAGGACTAGAGACTGTGCAA-3'
		Extension	5'-AAGTTCATGGTAAAGGATGCACTAAC-3'
<i>MCP2</i>	<i>+1311A>C (K46Q)</i>	Forward	5'-CTTCACCTAAGGACCAAGGGCT-3'
		Reverse	5'-TCTCAGTCCATGTATGAAGGCTCA-3'
		Extension	5'-TCCCCCACAGCTTCAAGACC-3'
<i>GC</i>	<i>+27279T>C (C283C)</i>	Forward	5'-CATTCGTTTTGTAGCTGCCTGA-3'
		Reverse	5'-ACATCTTTGTTTGTGGGCAACTC-3'
		Extension	5'-GATGATGACAATTTATCCACAAAGAATTCTAAGTTTGAAGACTG-3'
	<i>+31417T>G (D416E)</i>	Forward	5'-GACTGGCAGAGCGACTA-3'
		Reverse	5'-TCATTGCAAAGACAGCCAAG-3'
		Extension	5'-TCATGATTATAATCAATGATGATAGAGCGACTAAAAGCAAAATTGCCTGA-3'
	<i>+31428C>A (T420K)</i>	Forward	5'-GACTGGCAGAGCGACTA-3'
		Reverse	5'-TCATTGCAAAGACAGCCAAG-3'
		Extension	5'-ATAATCAATGATGATAGCTTGTTAACCAGCTTTGCCAGTTCC-3'

* Calculated from transcriptional start site in promoter region (*IL10* -1082A>G, *IL10* -819T>C, *IL10* -592A>C, *MCP1* -2518G>A, and *MCP1* -2076A>T), and from translational start site (*CCR3* -22557G>A, *CCR3* -520T>C, *CCR3* -174C>T, *CCR3* +51T>C (Y17Y), *MCP2* +1311A>C (K46Q), *GC* +27279T>C (C283C), *GC* +31417T>C (D416E), and *GC* +31428C>A (K420T)).

Results

The rare allele frequencies of each SNP were 0.083 (*IL10* -1082A > G), 0.309 (*IL10* -819T > C), 0.312 (*IL10* -592A >

C), 0.038 (*IL10* +3930T > C), 0.261 (*CCR3* -22557 G > A), 0.401 (*CCR3* -520 T > G), 0.360 (*CCR3* -174 C > T), 0.036 (*CCR3* +51T > C (Y17Y)), 0.340 (*MCP1* -2518 G > A), 0.058 (*MCP1* -2076A > T), 0.280 (*MCP2* +1311A > C (K46Q)),

Table 2. Frequencies of single nucleotide polymorphisms (SNPs) in the *IL10*, *CCR3*, *MCP1*, *MCP2* and *GC* genes in the Korean population (n=370)

Gene	Locus	Region	Genotype			Frequency*	Heterozygosity
<i>IL10</i>	<i>-1082A>G</i>	Promoter	A	AG	G	0.089	0.162
			305	57	4		
	<i>-819T>C</i>	Promoter	T	CT	C	0.309	0.427
			177	148	38		
	<i>-592A>C</i>	Promoter	A	AC	C	0.309	0.427
			177	148	38		
<i>CCR3</i>	<i>+3930T>C</i>	3'UTR	T	CT	C	0.040	0.076
			339	27	1		
	<i>-22557G>A</i>	Intron1	G	AG	A	0.263	0.388
			201	139	27		
	<i>-520T>G</i>	Intron3	T	GT	G	0.405	0.482
			131	178	61		
<i>MCP1</i>	<i>-174C>T</i>	Intron3	C	CT	T	0.364	0.463
			146	179	45		
	<i>+51T>C (Y17Y)</i>	Exon4	T	CT	C	0.038	0.073
			341	28	0		
	<i>-2518G>A</i>	Promoter	G	AG	A	0.349	0.455
			139	102	51		
<i>MCP2</i>	<i>-2076A>T</i>	Promoter	A	AT	T	0.063	0.150
			312	41	2		
<i>GC</i>	<i>+1311A>C (K46Q)</i>	Exon3	A	AC	C	0.282	0.405
			192	139	33		
	<i>+27279T>C (C283C)</i>	Exon8	T	CT	C	0.109	0.194
			293	70	5		
	<i>+31417T>G (D416E)</i>	Exon11	T	GT	G	0.248	0.373
			185	122	20		
	<i>+31428C>A (T420K)</i>	Exon11	C	AC	A	0.221	0.345
			173	76	22		

*Frequencies of rare alleles.

0.107 (*GC +27279T>C (C283C)*), 0.262 (*GC +31417T>G (D416E)*) and 0.218 (*GC +31428C>A (T420K)*) in the Korean population (Table 2). Haplotypes were constructed in each gene, and common haplotypes (freq. > 0.03) were used in further analysis (Table 3). Four haplotypes in *IL10*, three in *MCP2* and four in *GC* were observed. In *CCR3*, four major haplotypes concerning over 99.6% of the distribution were also shown, but the minor haplotypes were excluded. Coefficients of linkage disequilibrium ($|D'|$) of all pair-wise SNPs in each gene were also calculated (Table 4). Haplotypes with either equivalence to single polymorphisms or frequencies less than 3% were excluded from the statistical analysis.

The associations of polymorphisms in the *IL10*, *CCR3*, *MCP1*, *MCP2* and *GC* genes with spinal BMD were analyzed in Korean postmenopausal women (n = 370). The summary of the statistical analysis is shown in Table 5. The genotype and haplotype distributions, means and standard deviations (SD) of spinal BMDs and *P* values of the three alternative analyzing models (co-dominant, dominant, and recessive) for regression analyses are also shown.

IL10 -592A>C and *IL10 ht2 (A-C-C-T)* showed an association with the decrease of spinal BMD ($P = 0.02-0.07$), whereas no significant associations were showed in the other polymorphisms and haplotypes of the *CCR3*, *MCP1*, *MCP2* and *GC* genes. The *IL10 -592A>C* showed an association with spinal BMD, i.e., the levels of spinal BMD in individuals bearing the *IL10 -592CC* genotype were lower (0.78 ± 0.16) than those in the others (0.85 ± 0.17) ($P = 0.03$ in recessive model). *IL10 ht2 (A-C-C-T)* also showed an association, i.e., the levels of BMD in *IL10 ht2* bearing individuals were lower (0.82 ± 0.15) than those in the others (0.85 ± 0.16) ($P = 0.03$ in dominant model).

Discussion

Osteoporosis is a multifactorial disease, with great impact on morbidity and mortality mainly in postmenopausal women. Although factors related to life-style and habits are recognized may influence bone mass formation, leading to greater or

Table 3. Frequencies of haplotypes constructed in the *IL10*, *CCR3*, *MCP1* and *GC* genes in Korean population (n=370)

Gene	Haplotype	Locus				Frequency
<i>IL10</i>		-1082A>G	-819T>C	-592A>C	+3930T>C	
	<i>ht1</i> (A-T-A-T)	A	T	A	T	0.689
	<i>ht2</i> (A-C-C-T)	A	C	C	T	0.221
	<i>ht3</i> (G-C-C-C)	G	C	C	C	0.040
	<i>ht4</i> (G-C-C-T)	G	C	C	T	0.050
<i>CCR3</i>		-22557G>A	-520T>G	-174C>T	+51T>C (Y17Y)	
	<i>ht1</i> (G-T-C-T)	G	T	C	T	0.600
	<i>ht2</i> (A-G-T-T)	A	G	T	T	0.224
	<i>ht3</i> (G-G-T-T)	G	G	T	T	0.136
	<i>ht4</i> (A-G-C-C)	A	G	C	C	0.036
<i>MCP1</i>		-2518G>A	-2076A>T			
	<i>ht1</i> (G-A)	G	A			0.662
	<i>ht2</i> (A-A)	A	A			0.278
	<i>ht3</i> (A-T)	A	T			0.060
		+27279T>C (C283C)	+31417T>G (D416E)	+31428C>A (T420K)		
<i>GC</i>	<i>ht1</i> (T-T-C)	T	T	C		0.373
	<i>ht2</i> (T-T-A)	T	T	A		0.232
	<i>ht3</i> (T-G-C)	T	G	C		0.273
	<i>ht4</i> (C-T-C)	C	T	C		0.122

There were no ambiguous haplotypes phasing due to absolute LD ($|D'| = 1$ and $d^2 = 1$) and/or complete LD ($|D'| = 1$ and $d^2 \neq 1$) between the SNPs in the *IL10*, *MCP1* and *GC* genes. *IL10* -819 and *IL10* -592 are in absolute LD ($d^2 = 1$). *IL10* -1082, (*IL10* -819: *IL10* -592) and *IL10* +3930 are in complete LD ($|D'| = 1$). *GC* +27279, +31417 and +31428 are in complete LD, and *MCP1* -2518 and -2076 are also in complete LD. Rare haplotypes of *CCR3* (<0.03 of frequency) were excluded from further analysis.

Haplotypes were constructed by an algorithm developed by Stephens *et al.* (Stephens *et al.* 2001). *IL10* *ht1* and *ht3* are equivalent to *IL10* -592 and *IL10* +3930, respectively. *GC* *ht2*, *ht3* and *ht4* are equivalent to *GC* +31428, *GC* +31417 and *GC* +27279, respectively, and *MCP1* *ht1* and *ht3* are equivalent to *MCP1* -2518 and *MCP1* -2076, respectively. Haplotypes with either equivalence with a single polymorphism or with frequencies less than 3% were excluded from the statistical analysis.

lower bone mass, the majority of the variation in the bone mineral density (BMD) might be genetically controlled (Kanis *et al.*, 1994; Eisman, 1999). BMD is the major factor determining bone strength and, consequently, osteoporotic fracture risk, and can be considered a quantitative polygenic trait. It has been shown that several genes are associated with BMD, although conflicting results also exist (Kanis *et al.*, 1994; Morrison *et al.*, 1994; Kobayashi *et al.*, 1996; Murray *et al.*, 1997; Keen *et al.*, 1998; Eisman, 1999; Yamada *et al.*, 2001; Arko *et al.*, 2002; Garcia-Giralt *et al.*, 2002).

IL10 is a powerful T_H2 -cell cytokine, and the MCP gene family is a beta-chemokine that binds primarily to C-C chemokine receptors, such as CCR2 or CCR3. CCR3 is a beta-chemokine receptor abundant on eosinophils, mast cells, basophils and a subset of T_H2 -like T-lymphocytes, and most of the action of beta-chemokines is mediated through the CCR3 receptor (Combadiere *et al.*, 1995; Garcia-Zepeda *et al.*, 1996; Heath *et al.*, 1997). *GC* was a potential candidate for association with BMD because of its function in vitamin D

binding. A plausible relation of these genes (*IL10*, *CCR3*, *GC*, *MCP1* and *MCP2*) and their polymorphisms with BMD has been proposed, although there was no direct evidence, so is still controversial. In the present study, by screening 14 SNPs in five candidate genes, including *IL10*, *CCR3*, *MCP1*, *MCP2* and *GC*, *IL10* -592A > C, and a common *IL10* haplotype, and *IL10* *ht2*, which is also common in Caucasians (0.29, n = 1,813) and African Americans (0.41, n = 858) (Shin *et al.*, 2000), were observed to be associated with decreased BMD in Korean postmenopausal women. *IL10* polymorphisms, which have been reported to be related with *IL10* expression and transcriptional activity, may be an important genetic factor in BMD. *IL10*, produced mainly by macrophages, work as a potent immunosuppressive cytokine by down-regulating the expressions of T_H1 cytokines and co-stimulatory molecules (Redpath *et al.*, 2001). The two SNPs, *IL10* -1082 T > C and -592A > C, contributing to *IL10* *ht2* were particularly interesting because several previous reports have demonstrated quantitative differences in *IL10* transcription

Table 4. Linkage disequilibrium coefficients ($|D'|$) between the SNP loci in the *IL10*, *CCR3*, *MCPI* and *GC* genes.

Gene	Polymorphisms	Linkage disequilibrium coefficients ($ D' $)			
<i>IL10</i>		<i>-1082A>G</i>	<i>-819T>C</i>	<i>-592A>C</i>	<i>+3930T>C</i>
	<i>-1082A>G</i>	-	1	1	1
	<i>-819T>C</i>		-	1	1
	<i>-592A>C</i>			-	1
	<i>+3930T>C</i>				-
<i>CCR3</i>		<i>-22557G>A</i>	<i>-520T>G</i>	<i>-174C>T</i>	<i>+51T>C (Y17Y)</i>
	<i>-22557G>A</i>	-	0.799	0.968	0.981
	<i>-520T>G</i>		-	1	0.997
	<i>-174C>T</i>			-	1
	<i>+51T>C (Y17Y)</i>				-
<i>MCPI</i>		<i>-2518G>A</i>	<i>-2076A>T</i>		
	<i>-2518G>A</i>	-	1		
	<i>-2076A>T</i>		-		
<i>GC</i>		<i>+27279T>C (C283C)</i>	<i>+31417T>G (D416E)</i>	<i>+31428C>A (T420K)</i>	
	<i>+27279T>C (C283C)</i>	-	1	1	
	<i>+31417T>G (D416E)</i>		-	1	
	<i>+31428C>A (T420K)</i>			-	

Linkage disequilibrium coefficients ($|D'|$) were calculated between all pairs of biallelic loci (Hedrick and Kumar 2001; Hedrick 1987). *IL10* 819 T>C and *IL10* -592 A>C are related by absolute LD ($r^2=1$)

and/or expression mediated by alternative alleles or haplotypes. It has been reported that the differences in nuclear-binding activity and IL10 production were mediated by the *IL10* -592A > C (Shin *et al.*, 2000) and *IL10* -1082A > G polymorphisms (Turner *et al.*, 1997; Rees *et al.*, 2002) and the *IL10* promoter haplotype (Crawley *et al.*, 1999; Edwards-Smith *et al.*, 1999; Zhang *et al.*, 2002). Among the haplotypes, *IL10* *ht2* was associated with an increase in IL10 production, and positive associations with several diseases have also been detected: AIDS progression (Shin *et al.*, 2000), HCV infection and resistance to antiviral therapy (Edwards-Smith *et al.*, 1999; Yee *et al.*, 2001; Vidigal *et al.*, 2002), advanced alcoholic liver disease (Grove *et al.*, 2000), EBV infection (Helminen *et al.*, 2001), gastric cancer (Wu *et al.*, 2002), cervical cancer (Stanczuk *et al.*, 2001), multiple myeloma (Zheng *et al.*, 2001) and cutaneous malignant melanoma (Howell *et al.*, 2001). Because the roles of IL10 in these diseases suggest that *IL10* polymorphisms play a critical role in immunity, inflammation progress and cancer development, by modulating IL10 production, it might also be proposed that the change in IL10 levels by two *IL10* polymorphisms was due to the decreased BMD in this study. However it is unclear which polymorphisms are due to the suggestive associations with BMD, as two *IL10* polymorphisms (*IL10* -592A > C & *IL10* *ht2*) share a 91% distribution (Table 3).

In summary, *IL10* -592A > C and/or *IL10* *ht2* among the polymorphisms tested in the five-candidate genes, including

IL10, *CCR3*, *MCPI*, *MCP2* and *GC*, showed an association with decreased BMD in Korean postmenopausal women. Additional study might need to be followed-up in a more powerful cohort. There is also a need to clarify whether the inhibition of innate immunity by increased IL10 production in *IL10* -592A > C and *IL10* *ht2* bearing individuals accelerates the loss of bone mineral density (BMD).

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Table 5. Regression analysis of spinal BMD of patients with SNPs and haplotypes in the *IL10*, *CCR3*, *MCPI*, *MCP2* and *GC* genes in Korean postmenopausal women

Gene	Locus	N (mean±SD)			P		
		C/C*	C/R*	R/R*	Co-dominant	Dominant	Recessive
<i>IL10</i>	-1082A>G	305(0.84 ± 0.17)	57(0.84 ± 0.17)	4(0.76 ± 0.21)	0.84	0.97	0.50
	-592A>C	177(0.85 ± 0.18)	148(0.84 ± 0.15)	38(0.78 ± 0.16)	0.02	0.06	0.03
	+3930 T>C	339(0.84 ± 0.16)	27(0.85 ± 0.17)	1(0.57 ± 0.00)	0.92	0.98	0.49
	ht2	221(0.85 ± 0.17)	116(0.83 ± 0.16)	20(0.79 ± 0.12)	0.03	0.04	0.20
	ht4	321(0.84 ± 0.16)	36(0.84 ± 0.17)	.	0.93	0.93	.
<i>CCR3</i>	-22557G>A	201(0.83 ± 0.17)	139(0.84 ± 0.15)	27(0.85 ± 0.17)	0.87	0.84	0.99
	-520T>G	131(0.83 ± 0.16)	178(0.85 ± 0.17)	61(0.83 ± 0.17)	0.99	0.51	0.38
	-174C>T	146(0.83 ± 0.16)	179(0.85 ± 0.17)	45(0.82 ± 0.18)	0.58	0.21	0.45
	+51T>C (Y17Y)	341(0.84 ± 0.17)	28(0.81 ± 0.14)	.	0.13	0.13	.
	ht1	58(0.82 ± 0.17)	174(0.85 ± 0.16)	129(0.83 ± 0.16)	0.88	0.29	0.56
	ht2	217(0.84 ± 0.17)	127(0.83 ± 0.15)	17(0.90 ± 0.18)	0.49	0.80	0.18
	ht3	270(0.84 ± 0.16)	84(0.85 ± 0.17)	7(0.69 ± 0.17)	0.63	0.99	0.10
	ht4	334(0.84 ± 0.17)	27(0.81 ± 0.14)	.	0.16	0.16	.
<i>MCPI</i>	-2518G>A	139(0.82 ± 0.15)	102(0.84 ± 0.16)	51(0.87 ± 0.21)	0.09	0.13	0.19
	-2076A>T	312(0.83 ± 0.15)	41(0.88 ± 0.23)	2(0.68 ± 0.17)	0.14	0.07	0.26
	ht2	163(0.82 ± 0.17)	92(0.85 ± 0.16)	36(0.85 ± 0.19)	0.32	0.26	0.66
<i>MCP2</i>	+1311A>C (K46Q)	192(0.83 ± 0.17)	139(0.84 ± 0.16)	33(0.86 ± 0.15)	0.67	0.67	0.81
<i>GC</i>	+27279T>C (C283C)	293(0.84 ± 0.17)	70(0.84 ± 0.16)	5(0.81 ± 0.15)	1.00	0.89	0.62
	+31417T>G (D416E)	185(0.85 ± 0.18)	122(0.83 ± 0.15)	20(0.85 ± 0.13)	0.96	0.66	0.44
	+31428C>A (T420K)	173(0.83 ± 0.16)	76(0.85 ± 0.18)	22(0.86 ± 0.16)	0.55	0.64	0.55
	ht1	99(0.84 ± 0.15)	115(0.84 ± 0.16)	39(0.84 ± 0.18)	0.93	0.97	0.74

*C/C, C/R and R/R represent homozygotes for common allele (C/C), heterozygotes (C/R) and homozygotes for rare allele (R/R). Genotype and haplotype distributions, means and standard deviations (SD) of spinal BMDs and *P* values of three alternative analyzing models (co-dominant, dominant and recessive) for regression analyses are shown. Spinal BMD was adjusted for age, YSM and BMI as co-variables.

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