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Promoter polymorphisms of the vascular endothelial growth factor gene is associated with an osteonecrosis of the femoral head in the Korean population

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Summary

Objective: Disruption of the vascular supply to the bone and subsequent hypoxia has been implicated in the pathogenesis of osteonecrosis of the femoral head (ONFH). Vascular endothelial growth factor (*VEGF*), a major inducer of angiogenesis, has been correlated with several pathological conditions, from inflammation to ischemic processes. A number of polymorphisms in the *VEGF* gene have been described as being associated with several diseases, such as diabetic retinopathy, prostate cancer and breast cancer. The aim of this study was to evaluate the association of *VEGF* gene polymorphisms with ONFH in a case–control study.

Methods: Three polymorphisms (−2578C>A, −634G>C and +936C>T) in *VEGF* were genotyped in 317 ONFH patients and 497 control subjects, using the TaqMan 5′ allelic discrimination assay. We performed the association analysis of genotyped single nucleotide polymorphisms (SNPs) and haplotypes with ONFH.

Results: The −634G>C genotype was significantly associated with an increased risk for ONFH in dominant model with odds ratio (OR) of 1.47, 95% confidence intervals (CIs) 1.08–2.01 with *P* value 0.015. Further analysis stratified by sex showed that the −634G>C genotype was also significantly associated with a high risk for male patients considering the dominant model with OR of 1.60, 95% CI 1.13–2.26 with *P* value 0.008. Haplotype association analysis did not provide a further delineation of the risk allele.

Conclusion: Our study is, to our knowledge, the first report that shows the −634G>C polymorphism in the *VEGF* promoter was associated with an increased susceptibility of ONFH in the Korean population.

Key words: Vascular endothelial growth factor (*VEGF*), Osteonecrosis of the femoral head (ONFH), Polymorphism, Angiogenesis, Haplotypes, Linkage disequilibrium (LD).

Introduction

Osteonecrosis (ON) is a pathologic process in which cell death in the components of bone occurs due to an interruption in the vascular supply of blood or a decreased blood flow. While ON can occur in many anatomical locations, the femoral head is most commonly affected. This is probably because of restricted perfusion[1,2]. As ON of the femoral head (ONFH) is a devastating disease that frequently leads to the progressive collapse of the femoral head, followed by degenerative arthritis of the hip joint, it mainly affects middle-aged men resulting in both a reduction in the quality of life and a tremendous amount of pain. The disease incurs substantial socioeconomic costs, as well as a burden for patients and their families[3,4].

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The precise pathophysiology of ONFH is not known, but it has been suggested that a common pathogenesis of ONFH involves an interruption of the circulation of blood to anterior–superior–lateral part of the femoral head, thus leading to ischemic insult and bone collapse[5]. The disease can be aggravated by several factors including steroid administration[6], alcohol abuse[7], arterial thrombosis or hypofibrinolysis[8], and systemic lupus erythematosus[9].

Angiogenesis is an important part of the bone repair process, and the close connection between blood vessels and bone has already been recognized[10]. Recently, the vascular endothelial growth factor (*VEGF*) gene transfer, using the adenoviral vector in a rabbit model of ON, resulted in the enhanced neovascularization of the necrotic bone[11,12]. Significantly, *VEGF* is highly expressed in the edematous area of the ON adjacent to the necrotic area[13], indicating that *VEGF* expression is correlated with bone tissue repair[14]. *VEGF* is important for bone formation, such as normal growth plate morphogenesis, which includes blood vessel invasion and cartilage remodeling[15]. *VEGF* has also been implicated in bone repair[16].

VEGF, a major inducer of angiogenesis during embryogenesis, has been implicated in neuronal survival, neuroprotection, regeneration, growth, differentiation, and migration[17–19]. It has also been correlated with several pathological conditions, from tumor proliferation to inflammatory and ischemic processes. The *VEGF* gene was reported to be polymorphic, especially in the promoter region (−2578, −1154, etc.), the 5′-untranslated region (UTR) (−634, −7) and in the 3′-UTR (+936). Several studies have shown that polymorphisms within the 5′-UTR have lead to differences in *VEGF* expression, and that they could influence the etiology of a variety of pathological conditions such as diabetic retinopathy[20,21], prostate cancer[22,23], and breast cancer[24].

Although there are various studies on the role of *VEGF* regarding bone repair for osteonecrotic bone[15,16], none of them have investigated the effects of the genetic polymorphisms of *VEGF* on ONFH. In previous study of *VEGF* with other diseases in Korea, *VEGF* gene was fully sequenced and linkage disequilibrium (LD) was estimated[25]. It has also been reported that the polymorphisms of *VEGF* promoter were associated with many diseases in Korea[25,26].

Therefore, in the present study, we have focused on the 5′-UTR, a promoter region, and the 3′-UTR of *VEGF* for genetic analysis of ONFH, as they have been shown to be highly polymorphic and reported to be associated with many diseases. We investigated the relationship between the incident risk for ONFH and polymorphisms in the genes for *VEGF*, which may be the major signal to couple angiogenesis and osteogenesis during bone repair.

Materials and methods

SUBJECTS

Blood samples and records were obtained from 317 unrelated patients with ONFH (255 men, 62 women; age: 49.7 ± 14) and 497 control subjects (396 men, 101 women; age: 41.6 ± 15) who visited Kyungpook National University Hospital (Daegu, Korea) for medical check up between 2002 and 2005. Diagnoses were established by the evidence of ON on magnetic resonance imaging (MRI) in Stage 1 of association research circulation osseons (ARCO)[27] classification system and plain radiographs in Stages 2, 3 and 4. The diagnosis of symptomatic ON was made using anteroposterior and lateral-pelvic radiographs and MRIs. Patients with a demonstrable history of direct trauma or with the possibility of a combination of causes were excluded. Control subjects were recruited from spouses of the patients and the general population. All individuals provided informed consent for their participation in the study and this project was approved by the Institutional Review Board.

GENOTYPING WITH FLUORESCENCE POLARIZATION DETECTION

For the genotyping of polymorphic sites, amplifying primers and probes were designed for the TaqMan assay[30]. Primer Express (Applied Biosystems) was used to design both the polymerase chain reaction (PCR) primers and the MGB TaqMan probes. One allelic probe was labeled with the 6-carboxyfluorescein (FAM)™ dye and the other was labeled with the fluorescent VIC® dye. PCRs were run in the TaqMan Universal Master mix without UNG (Applied Biosystems), with PCR primer concentrations of 900 nM and TaqMan MGB-probe concentrations of 200 nM. Reactions were performed in a 96-well plate format in a total reaction volume of 10 μl using 20 ng of genomic DNA. The plates then were placed in a thermal cycler (7500HT, Applied Biosystems) and heated at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and at 60°C for 1 min. The fluorescence intensity in each well of the plate was read. Fluorescence data files from each plate were analyzed using automated allele-calling software (SDS 2.1, Applied Biosystems). Genotyping quality control was performed in 10% of the samples by duplicate checking.

STATISTICS

We used χ^2 tests to determine whether individual variants were in Hardy–Weinberg equilibrium at each locus in the population. Logistical regression analyses were used to calculate the odds ratios (ORs) and their 95% confidence intervals (CIs) for SNP sites. The *P* values of the codominant, dominant and recessive models are shown. The haplotypes of each individual and their frequencies were constructed with an EM algorithm[31] and a permutation test was performed with genotyped SNPs. A Fisher's exact test or χ^2 test was applied in order to compare the frequency of discrete variables between the controls and patients. A *P* value of less than 0.05 was considered to be statistically significant.

Results

In order to investigate the association of *VEGF* gene polymorphism with ONFH, we analyzed the genotype of three SNPs (−2578C>A, −634G>C and +936C>T), one (−2578C>A) in promoter region, one (−634G>C) in the 5′-UTR and one (+936C>T) in the 3′-UTR, by a TaqMan assay. The genotype frequencies between ONFH patients and control groups were compared.

The genotype distributions of all loci were within the Hardy–Weinberg equilibrium ($P > 0.05$) (Table I). The *P* values of each polymorphism were analyzed with respect to a comparison between ONFH patients and the controls (Table II). The genotype and allele frequencies of −634G>C, in ONFH patients, were significantly different from those in the control group with *P* values 0.050, 0.037 (OR; 1.24, 95% CI; 1.01–1.52), respectively. In dominant genetic analysis, carriers of C allele in −634G>C (GC + CC) were significantly higher in the ONFH patients than in the controls (73.4% vs 65.3%, $P = 0.015$, OR; 1.47, 95% CI; 1.08–2.01). Next, since we observed a tendency showing a high incidence of ONFH in men and that the majority of patients in this study were male, the association of *VEGF* polymorphisms was further analyzed with ONFH subgroups stratified by sex. When compared to controls, the minor allele (C) of −634G>C showed a significantly higher frequency in ONFH patients than in controls ($P = 0.040$, OR; 1.27, 95% CI; 1.01–1.59) in male subgroup. The −634G>C genotype was also significantly associated with an increased risk for ONFH in codominant and dominant models ($P = 0.028$, OR; 1.31 (1.04–1.64) and $P = 0.008$, OR; 1.60 (1.13–2.26), respectively), suggesting that the C allele of −634G>C contributed to the susceptibility of ONFH. The allele frequency of the C allele of −634G>C in female patients was similar to the males, but the association between female patients and control was statistically not significant. This result is probably due to their small number of female patients (control 101, case 62) in our study (Table III), and a larger sample size may be required.

Table I
Frequencies of VEGF polymorphisms in ONFH subjects

Loci	Position	rs#	Genotype				Frequency	Heterozygosity	HWE*	
									Controls	Patients
−2578C>A	Promoter	rs699947	C 429	CA 328	A 51	N 808	0.266 (A)	0.390	0.744	0.266
−634G>C	5′-UTR	rs2010963	G 255	GC 414	C 138	N 807	0.428 (C)	0.490	0.776	0.087
+936C>T	3′-UTR	rs3025039	C 519	CT 254	T 29	N 802	0.194 (T)	0.313	0.903	0.977

**P* values of deviation from HWE among ONFH patients and normal subjects.

On the basis of the LD coefficient between polymorphisms, which indicated that −2578 and −634 polymorphisms are in tight LD, and that +936 polymorphism was only weakly associated with the other polymorphisms (Table IV). Based upon the LD coefficients, haplotypes were reconstructed and compared haplotype frequencies between normal controls and ONFH patients.

As shown in Table V, the frequency CC haplotype was significantly higher in patients with ONFH, conferring a risk for disease ($P = 0.032$, OR; 1.25, 95% CI; 1.02−1.53). Haploype, CG, was significantly lower in patients, suggesting that CG haplotype bearing −634G allele had protective effect against ONFH ($P = 0.047$, OR; 0.80, 95% CI; 0.64−1.00). However, since haplotype association analysis did not provide a higher OR or a more significant result, the effect may be brought about by the SNP-634G>C itself or by a mutation in complete LD with this SNP.

These results suggest that the −634G>C polymorphism of the *VEGF* promoter might be associated with an increased susceptibility of ONFH in the Korean population. In addition, this polymorphism may contribute to future studies regarding *VEGF* functions and ON.

Discussion

Although many pathophysiological models of bone necrosis have been proposed, the precise mechanism of ONFH has not been completely elucidated. Among several confounding pathogenic mechanisms for ONFH, a vascular hypothesis appears to be the most persuasive, presuming that a decrease in the local blood flow in the femoral head, due to vascular obstruction by any means, plays a pivotal role in the pathogenesis of ONFH[32,33]. All factors that decrease blood flow and lead to vascular insufficiency contribute to ON. Several studies, in human and animal models of ONFH, have shown that vascular abnormalities have resulted in thrombosis associated with abnormal thrombophilic coagulopathy and hypofibrinolysis and embolism, which contribute to the development of ONFH[8,34,35]. A reduction in shear stress, due to decreased blood flow, could also lead to the apoptosis of endothelial cells, which can ultimately contribute to osteogenesis as releasing osteogenic factors. The dysregulation of endothelial cell activating factors and stimulators of angiogenesis or repair processes could also affect the progression and outcome of ONFH[33]. A diminished blood flow, resulting from capillary

Table II
Genotypes and allelic frequencies of the VEGF gene polymorphisms between ONFH patients and controls

Position*	Genotype	Control, *n* (%)	ONFH, *n* (%)	OR† (95% CI)	*P*
−2578C>A	CC	263 (53.0)	166 (53.2)	1.00	0.647
	CA	199 (40.1)	129 (41.3)	1.03 (0.76−1.38)	
	AA	34 (6.85)	17 (5.45)	0.79 (0.43−1.46)	
	CA + AA‡	233 (47.0)	146 (46.8)	0.99 (0.75−1.32)	0.960
	C	725 (73.1)	461 (73.9)	1.00	0.725
	A	267 (26.9)	163 (26.1)	0.96 (0.77−1.21)	
−634G>C	GG	172 (34.8)	83 (26.6)	1.00	**0.050**
	GC	243 (49.1)	171 (54.8)	**1.46 (1.05−2.02)**	
	CC	80 (16.2)	58 (18.6)	**1.50 (0.98−2.30)**	
	GC+CC‡	323 (65.3)	229 (73.4)	**1.47 (1.08−2.01)**	**0.015**
	G	587 (59.3)	337 (54.0)	1	**0.037**
	C	403 (40.7)	287 (46.0)	**1.24 (1.01−1.52)**	
+936C>T	CC	317 (64.4)	202 (65.2)	1.00	0.990
	CT	157 (31.9)	97 (31.3)	0.98 (0.72−1.33)	
	TT	18 (3.66)	11 (3.55)	1.00 (0.46−2.16)	
	CT + TT‡	175 (35.6)	108 (34.8)	0.97 (0.72−1.30)	0.833
	C	791 (80.4)	501 (80.8)	1	0.836
	T	193 (19.6)	119 (19.2)	0.97 (0.75−1.26)	

*Calculated from the translation start site.

†Logistic regression analyses were used for calculating OR (95% CI).

‡Dominant analysis: homozygotes for the major allele vs heterozygotes + homozygotes for the minor allele.

Table III
Association of genotypes and alleles between the ONFH patients and controls stratified by sex

Subgroups	Loci	Normal controls	ONFH patients	Codominant		Dominant		Allele	
				OR*(95% CI)	*P*†	OR (95% CI)	*P*	OR (95% CI)	*P*
		n = 396	*n* = 255						
Male	−2578C>A	0.262	0.262	1.02 (0.79−1.32)	0.629	1.06 (0.77−1.46)	0.713	1.00 (0.78−1.29)	0.999
	−634G>C	0.402	0.460	**1.31 (1.04−1.64)**	**0.028**	**1.60 (1.13−2.26)**	**0.008**	**1.27 (1.01−1.59)**	**0.040**
	+936C>T	0.205	0.184	0.90 (0.68−1.20)	0.601	0.84 (0.60−1.18)	0.316	0.88 (0.66−1.17)	0.365
		n = 101	*n* = 62						
Female	−2578C>A	0.297	0.258	0.80 (0.47−1.37)	0.705	0.76 (0.41−1.44)	0.407	0.82 (0.50−1.36)	0.448
	−634G>C	0.426	0.459	1.19 (0.73−1.93)	0.613	1.04 (0.51−2.11)	0.908	1.14 (0.73−1.80)	0.559
	+936C>T	0.163	0.225	1.54 (0.86−2.75)	0.291	1.69 (0.87−3.30)	0.122	1.49 (0.84−2.62)	0.170

*Logistic regression analyses were used for calculating OR (95% CI).
†Values were analyzed by χ^2 test.

and venular microthrombosis, can lead to elevations in local intraosseous pressure and secondary arterial ischemia. Ischemia-induced necrosis can result in a collapse of the joint cartilage and subchondral bone[36,37]. During hypoxia, hypoxia-inducible factors bind to the hypoxia-responsive elements and the expression of *VEGF* can be induced. This leads to the stimulation of angiogenesis[38−40].

VEGF, a major inducer of angiogenesis, is important for bone formation processes, such as normal growth plate morphogenesis, including blood vessel invasion and cartilage remodeling[15] and it has also been implicated in bone repair[16,33]. These reports indicate that normal angiogenesis is central to tissue repair, and that *VEGF* may be the major signal in the coupling of angiogenesis and osteogenesis during bone repair[14,41].

The *VEGF* gene was reported to be polymorphic, especially in the promoter region, the 5′-UTR and in the 3′-UTR. Several transcription factor binding sites are found in the *VEGF* 5′-UTR and the transcriptional regulation of the gene is extremely complex[42]. Several studies have shown that polymorphisms within the 5′-UTR have lead to differences in *VEGF* expression between individuals and that they could influence the etiology of a variety of pathological conditions such as diabetic retinopathy[20] and Type 1 diabetes mellitus[43], with which *VEGF* has been associated.

In this study, we examined the potential association of *VEGF* polymorphisms with ONFH in Korean patients. First, we identified a significant association between the −634G>C polymorphism and the risk of ONFH. We also analyzed the −2578C>A and +936C>T polymorphisms of *VEGF*, but neither was associated with the risk of ONFH. Haplotype analysis also suggested the importance of −634G>C in susceptibility to ONFH. However, haplotype analysis did not provide a further delineation of the risk allele (as indicted by the similar effect sizes, *P* values and the fact that the haplotype bears the C allele alone), indicating that the effect may be driven by the −634G>C SNP itself or by a mutation in complete LD with this SNP.

Although many etiological factors contributed to pathogenesis of ONFH, the pathologic changes of bone marrow in ONFH were almost similar[2] and it is commonly accepted that the final common pathway for the development of ONFH involves an interruption of the circulation of blood. Although the allele frequency of the C allele of −634G>C in female patients was similar to the males, we were not able to detect an association between −634G>C polymorphisms and female patients of ONFH. Our present study has some limitations. The incidence of ONFH is relatively low and sex-biased. The number of cases in only 317, of which the mayority (80%) are males. And also, epidemiologic study has shown that alcohol-induced ONFH is most prevalent in Korea. The frequency of idiopathic and steroid-induced ON is relatively low. Therefore, association analysis strategy with subgrouped by sex or etiology also has some limitations such as sample number and sex-biased sample. In this study, female patients may not be large enough to assess a powerful stratified association analysis (control 101, case 62). Hence, the association might be false negative due to the small sample size and not statistically relevant (Table III). Nevertheless, it might be worthwhile to follow up the importance of the *VEGF* polymorphism with other subgroups (etiology, sex) of ONFH through larger cohort studies, considering its association with the risks of ONFH.

According to Watson *et al.*[44], the −634G>C polymorphism was significantly correlated with the *VEGF* expression of lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMC). The *VEGF* production was the highest for the GG homozygotes and the lowest for the CC homozygotes, and it also suggested that LPS may work through the myeloid zinc finger protein (MZF1) binding site, within which the −634G>C polymorphism

Table IV
Allele frequency and pairwise LD estimates among SNPs in VEGF gene

	SNPs	|D'|		
		−2578C>A	−634G>C (5′-UTR)	+936C>T (3′-UTR)
r^2	−2578C>A	−	1	0.238
	−634G>C (5′-UTR)	0.271	−	0.008
	+936C>T (3′-UTR)	0.038	0	−
	Minor allele (freq. %)	A (26.6)	C (42.8)	T (19.4)

Table V
Estimated haplotype frequencies of selected SNPs in ONFH patients and controls

Haplotype*		Overall			
−2578C>A	−634G>C	Control	Case	OR (95% CI)	*P*†
C	C	**0.407**	**0.461**	**1.25 (1.02−1.53)**	**0.032**
C	G	0.324	0.277	0.80 (0.64−1.00)	0.047
A	G	0.269	0.261	0.96 (0.77−1.21)	0.744

*Values were constructed by EM algorithm with genotyped SNPs.
†Values were analyzed by permutation test.

was predicted to be located. Therefore, we have suggested that decreased *VEGF* expression in the osteonecrotic bone area could affect angiogenesis, repair processes, the progression, and outcome of ONFH. Since the *VEGF* gene is highly polymorphic, there is the possibility that if the impact of a single locus is not overwhelming, the net effect of multiple polymorphisms may determine a *VEGF* production, thus conferring the clinical manifestations.

These results suggest that −634G>C polymorphism of the *VEGF* promoter is likely to be associated with susceptibility to ONFH. To our knowledge, our study is the first report that shows the *VEGF* polymorphisms and haplotypes are associated with ONFH in Korea. Further biological and/or functional evidences would be needed to confirm the suggestive correlations between the *VEGF* polymorphism and ONFH.

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References

1. Asano T, Takahashi KA, Fujioka M, Inoue S, Ueshima K, Hirata T, *et al*. Relationship between postrenal transplant osteonecrosis of the femoral head and gene polymorphisms related to the coagulation and fibrinolytic systems in Japanese subjects. Transplantation 2004;77(2): 220–5.
2. Mont MA, Hungerford DS. Non-traumatic avascular necrosis of the femoral head. J Bone Joint Surg Am 1995;77(3):459–74.
3. Assouline-Dayan Y, Chang C, Greenspan A, Shoenfeld Y, Gershwin ME. Pathogenesis and natural history of osteonecrosis. Semin Arthritis Rheum 2002;32(2):94–124.
4. Liu YF, Chen WM, Lin YF, Yang RC, Lin MW, Li LH, *et al*. Type II collagen gene variants and inherited osteonecrosis of the femoral head. N Engl J Med 2005;352(22):2294–301.
5. Atsumi T, Kuroki Y. Role of impairment of blood supply of the femoral head in the pathogenesis of idiopathic osteonecrosis. Clin Orthop Relat Res 1992;(277):22–30.
6. Fisher DE. The role of fat embolism in the etiology of corticosteroid-induced avascular necrosis: clinical and experimental results. Clin Orthop Relat Res 1978;(130):68–80.
7. Wang Y, Li Y, Mao K, Li J, Cui Q, Wang GJ. Alcohol-induced adipogenesis in bone and marrow: a possible mechanism for osteonecrosis. Clin Orthop Relat Res 2003;(410):213–24.
8. Jones LC, Mont MA, Le TB, Petri M, Hungerford DS, Wang P, *et al*. Procoagulants and osteonecrosis. J Rheumatol 2003;30(4):783–91.
9. Abu-Shakra M, Buskila D, Shoenfeld Y. Osteonecrosis in patients with SLE. Clin Rev Allergy Immunol 2003;25(1):13–24.
10. Trueta J, Buhr AJ. The vascular contribution to osteogenesis. V. The vasculature supplying the epiphysial cartilage in rachitic rats. J Bone Joint Surg Br 1963;45:572–81.
11. Yang C, Yang S, Du J, Li J, Xu W, Xiong Y. Vascular endothelial growth factor gene transfection to enhance the repair of avascular necrosis of the femoral head of rabbit. Chin Med J (Engl) 2003; 116(10):1544–8.
12. Katsube K, Bishop AT, Simari RD, Yla-Herttuala S, Friedrich PF. Vascular endothelial growth factor (VEGF) gene transfer enhances surgical revascularization of necrotic bone. J Orthop Res 2005;23(2):469–74.
13. Radke S, Battmann A, Jatzke S, Eulert J, Jakob F, Schutze N. Expression of the angiomatrix and angiogenic proteins CYR61, CTGF, and VEGF in osteonecrosis of the femoral head. J Orthop Res 2006; 24(5):945–52.
14. Street J, Bao M, deGuzman L, Bunting S, Peale FV Jr, Ferrara N, *et al*. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. Proc Natl Acad Sci U S A 2002;99(15):9656–61.
15. Schipani E, Ryan HE, Didrickson S, Kobayashi T, Knight M, Johnson RS. Hypoxia in cartilage: HIF-1alpha is essential for chondrocyte growth arrest and survival. Genes Dev 2001;15(21):2865–76.
16. Komatsu DE, Hadjiargyrou M. Activation of the transcription factor HIF-1 and its target genes, VEGF, HO-1, iNOS, during fracture repair. Bone 2004;34(4):680–8.
17. Greenberg DA, Jin K. VEGF and ALS: the luckiest growth factor? Trends Mol Med 2004;10(1):1–3.
18. Sun Y, Jin K, Xie L, Childs J, Mao XO, Logvinova A, *et al*. VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. J Clin Invest 2003;111(12):1843–51.
19. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. Endocr Rev 2004;25(4):581–611.
20. Awata T, Inoue K, Kurihara S, Ohkubo T, Watanabe M, Inukai K, *et al*. A common polymorphism in the 5′-untranslated region of the VEGF gene is associated with diabetic retinopathy in type 2 diabetes. Diabetes 2002;51(5):1635–9.
21. Ray D, Mishra M, Ralph S, Read I, Davies R, Brenchley P. Association of the VEGF gene with proliferative diabetic retinopathy but not proteinuria in diabetes. Diabetes 2004;53(3):861–4.
22. McCarron SL, Edwards S, Evans PR, Gibbs R, Dearnaley DP, Dowe A, *et al*. Influence of cytokine gene polymorphisms on the development of prostate cancer. Cancer Res 2002;62(12):3369–72.
23. Lin CC, Wu HC, Tsai FJ, Chen HY, Chen WC. Vascular endothelial growth factor gene-460 C/T polymorphism is a biomarker for prostate cancer. Urology 2003;62(2):374–7.
24. Krippl P, Langsenlehner U, Renner W, Yazdani-Biuki B, Wolf G, Wascher TC, *et al*. A common 936 C/T gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk. Int J Cancer 2003;106(4):468–71.
25. Kim Y, Nam YJ, Lee C. Haplotype analysis of single nucleotide polymorphisms in VEGF gene for vascular dementia. Am J Med Genet B Neuropsychiatr Genet 2006;141(4):332–5.
26. Seo JS, Lee SS, Kim SI, Ryu WH, Sa KH, Kim SU, *et al*. Influence of VEGF gene polymorphisms on the severity of ankylosing spondylitis. Rheumatology (Oxford) 2005;44(10):1299–302.
27. Gardeniers JWM. The ARCO perspective for reaching one uniform staging system of osteonecrosis. In: Scoutens A, Arlet J, Gardeniers JWM, Hughes SPF, Eds. Bone Circulation and Vascularization in Normal and Pathologic Conditions. New York: Plenum Press 1993:375–80.
30. Livak KJ. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal 1999;14(5–6):143–9.
31. Qin ZS, Niu T, Liu JS. Partition–ligation–expectation–maximization algorithm for haplotype inference with single-nucleotide polymorphisms. Am J Hum Genet 2002;71(5):1242–7.
32. de Camargo FP, de Godoy RM Jr, Tovo R. Angiography in Perthes' disease. Clin Orthop Relat Res 1984;(191):216–20.
33. Kerachian M, Harvey E, Cournoyer D, Chow T, Seguin C. Avascular necrosis of the femoral head: vascular hypotheses. Endothelium 2006; 13(4):237–44.
34. Irisa T, Yamamoto T, Miyanishi K, Yamashita A, Iwamoto Y, Sugioka Y, *et al*. Osteonecrosis induced by a single administration of low-dose lipopolysaccharide in rabbits. Bone 2001;28(6):641–9.
35. Miyanishi K, Yamamoto T, Irisa T, Yamashita A, Jingushi S, Noguchi Y, *et al*. Bone marrow fat cell enlargement and a rise in intraosseous pressure in steroid-treated rabbits with osteonecrosis. Bone 2002; 30(1):185–90.
36. Mirzai R, Chang C, Greenspan A, Gershwin ME. The pathogenesis of osteonecrosis and the relationships to corticosteroids. J Asthma 1999;36(1):77–95.
37. Inoue S, Horii M, Asano T, Fujioka M, Ogura T, Shibatani M, *et al*. Risk factors for nontraumatic osteonecrosis of the femoral head after renal transplantation. J Orthop Sci 2003;8(6):751–6.
38. Treins C, Giorgetti-Peraldi S, Murdaca J, Van Obberghen E. Regulation of vascular endothelial growth factor expression by advanced glycation end products. J Biol Chem 2001;276(47):43836–41.
39. Oosthuyse B, Moons L, Storkebaum E, Beck H, Nuyens D, Brusselmans K, *et al*. Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. Nat Genet 2001;28(2):131–8.
40. Stevens A, Soden J, Brenchley PE, Ralph S, Ray DW. Haplotype analysis of the polymorphic human vascular endothelial growth factor gene promoter. Cancer Res 2003;63(4):812–6.
41. Peng H, Wright V, Usas A, Gearhart B, Shen HC, Cummins J, *et al*. Synergistic enhancement of bone formation and healing by stem cell-expressed VEGF and bone morphogenetic protein-4. J Clin Invest 2002; 110(6):751–9.
42. Lukiw WJ, Ottlecz A, Lambrou G, Grueninger M, Finley J, Thompson HW, *et al*. Coordinate activation of HIF-1 and NF-kappaB DNA binding and COX-2 and VEGF expression in retinal cells by hypoxia. Invest Ophthalmol Vis Sci 2003;44(10):4163–70.
43. Yang B, Cross DF, Ollerenshaw M, Millward BA, Demaine AG. Polymorphisms of the vascular endothelial growth factor and susceptibility to diabetic microvascular complications in patients with type 1 diabetes mellitus. J Diabetes Complications 2003;17(1):1–6.
44. Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE. Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. Cytokine 2000;12(8):1232–5.