

# Proangiogenic gene polymorphisms are associated with susceptibility to Paget's disease of bone and with its clinical features

Sirs,  
Paget's disease of bone (PDB) is a metabolic focal disease affecting one or more bones throughout the skeleton, being synergistic actions of environmental and genetic factors involved in its pathogenesis. One of the morphological characteristics of pagetic bone is hypervascularisation (1, 2). However, the process of angiogenesis in PDB has not been practically studied. We consider that this observation can reflect an alteration in bone angiogenesis accompanying osteoclastogenesis.

Vascular endothelial growth factor (VEGF) is a key regulator of physiological angiogenesis. The VEGF family consists of 6 homodimeric proteins and VEGFA is the most studied (3). VEGF receptors (VEGFR) and sensitivity to VEGF have been reported by both osteoblasts (OBL) and osteoclasts (OCL) (4), and a number of recent studies have shown evidence that VEGF and its receptors play vital roles coupling osteogenic and angiogenic processes (5-7).

The aim of our study was to characterise whether simple nucleotide polymorphisms (SNP) in proangiogenic genes could modify the occurrence and the clinical features of PDB. We have studied VEGFA rs699947, VEGFA rs833061 and VEGFR2 rs2071559 polymorphisms in a cohort of Spanish patients. These three SNPs are located in the promoter region of VEGFA and VEGFR2 genes.

A total of 264 PDB patients and 300 healthy subjects were analysed in the present study. Clinical and analytical variables such as gender, age at diagnosis, family history, number of affected bones, Renier's index, presence of complications and alkaline phosphatase levels were collected. All patients and healthy subjects gave informed consent to participate and the local ethics committee approved the study.

When we analysed VEGFR2 rs2071559 polymorphism, homozygous CC genotype and C allele were associated with increased risk of developing PDB (Table I). C allele was also associated with increased risk of developing complications of the disease ( $p=0.006$ ; OR=1.75 [IC=1.17-2.60]). VEGFR2 rs2071559 SNP could modify the susceptibility to develop PDB. Wang *et al.* showed that CC genotype of VEGFR2 rs2071559 SNP is associated with 68% decrease of transcriptional activity, resulting in reduced levels of VEGFR2 protein (8). Subsequently, Kariz *et al.* demonstrated that CC genotype of this SNP is associated with increased serum levels of VEGFA, suggesting that this increase could be the

**Table I.** Distribution of the genotypic and allelic frequencies of the VEGFR2 -604T>C (rs2071559) polymorphism in PDB patients and healthy subjects.

Genotype	Controls	PDB patients	p-value	OR (95% CI)
TT	77 (25.7%)	56 (21.2%)	—	1.00
TC	168 (56.0%)	127 (48.1%)	0.855	1.03 (0.68-1.57)
CC	55 (18.3%)	81 (30.7%)	<b>0.004</b>	<b>2.02 (1.24-3.29)</b>
TT+TC	245 (81.7%)	183 (69.3%)	—	1.00
CC	55 (18.3%)	81 (30.7%)	<b>0.001</b>	<b>1.97 (1.33-2.91)</b>
TT	77 (25.7%)	56 (21.2%)	—	1.00
TC+CC	223 (74.3%)	208 (78.8%)	0.214	1.28 (1.33-2.91)
Allele				
T	322 (53.7%)	239 (45.3%)	—	1.00
C	278 (46.3%)	289 (54.7%)	<b>0.005</b>	<b>1.40 (1.10-1.77)</b>

result of a compensation for a shortfall in VEGFA/VEGFR2 signalling (9). According to these studies, CC genotype of VEGFR2 rs2071559 SNP would induce the activation of different mechanisms to increase VEGF levels. Given that the degradation of extra cellular matrix (ECM) by OCLs is a major source of VEGFA (10), OCLs would boost their metabolic activity in order to increase ECM resorption and thus free the kidnapped VEGFA in the bone matrix. It is possible that this mechanism could favour the development of PDB and its complications.

In the analysis of VEGFA SNPs, we found that CC genotype of rs699947 was associated with a diagnosis at an older age ( $p=0.019$ ; average [ST]: AA=65.9 [13.0] vs. CC=70.7 [10.8]). PDB is usually diagnosed in patients older than 55 years (2). The age of presentation may have a genetic basis. This genotype may be associated with a later onset of the disease or a lower phenotypic expression of the disease that could delay its diagnosis. However, given the variability of the clinical manifestations and the frequency with which the diagnosis is made by radiological or laboratory incidental findings, we believe that this finding must be considered cautiously.

In conclusion, VEGFA and VEGFR2 SNPs are associated to development and phenotype of PDB in a Spanish cohort, highlighting the importance of angiogenesis in the pathogenesis of PDB. Further analysis in other series is needed to confirm these results.

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