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**SHORT REPORT**

# *VAV3* Gene Polymorphism Is Associated with Paget's Disease of Bone

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*Background and Aims:* Paget's disease of bone (PDB) is a focal bone disorder affecting the skeleton segmentally. The disease affects osteoclasts which increase in size, number, and activity. One of the etiopathogenic hypotheses is that the disease is genetic. It has been reported that Rho GEF Vav3 is an essential factor in the regulation of osteoclast function, and alteration of the *VAV3* gene could influence the development of the disease. The aim of our study was to perform an association study between variants of the *VAV3* gene and the risk of developing Paget's disease of bone. *Patients and Methods:* The genotypic and allelic distribution of the *VAV3* c.892A>T/p.T298S (rs7528153) polymorphism was compared between a cohort of 238 Spanish subjects with PDB and a cohort of 253 healthy subjects. *Results:* Our results indicated that individuals carrying the *VAV3* rs7528153 TT genotype were at a significantly increased risk of developing PDB ($p < 0.001$, odds ratio [OR] = 3.15, 95% confidence interval [95% CI] = 1.77–5.61). *Conclusions:* These results suggest that inheriting the *VAV3* rs7528153 polymorphism is a likely susceptibility factor for developing Paget's disease of bone.

## Introduction

Paget disease of bone (PDB) (OMIM: 167250) is a common disease characterized by focal areas of increased and disorganized bone turnover. Some patients are asymptomatic, whereas others develop complications such as pain, osteoarthritis, fracture, deformity, deafness, and nerve compression syndromes. A change occurs in the bone remodelling consisting in an increase in bone resorption followed by an excessive bone formation that results in a variegated and anarchic bone structure that alters the mechanical properties. The main alteration resides in osteoclasts, which increase in number and size and contain many more nuclei than normal (Ralston and Layfield, 2012; Singer *et al.*, 2006). The PDB is the most common metabolic bone disease after osteoporosis. In Spain, the PDB prevalence is 1.2% within the population above 55 years (Corral-Gudino *et al.*, 2013). PDB is primarily caused by dysregulation of osteoclast differentiation and function. There is increasing evidence that this is due, in part, to genetic factors (Ralston, 2008; Albagha *et al.*, 2011, 2013).

Cell behavior is partly controlled by Rho GTPases that regulate specific filamentous actin structures involved in cell migration, adhesion, and morphogenesis by acting as binary switches cycling between inactive (GDP-bound) and active (GTP-bound) conformational states (Schmidt and Hall, 2002). Rho GTPases activation is mediated by GEFs (guanine nucleotide exchange factors) stimulating the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) (Bustelo, 2001; Turner and Billadeau, 2002). Vav proteins are the best characterized family of mammalian Rho GEFs. There are three mammalian Vav proteins: Vav1, Vav2, and Vav3 (Turner and Billadeau, 2002). Several reports indicate that Vav proteins are activated by integrins in multiple hematopoietic cell types and might regulate Rho GTPases and the actin cytoskeleton in response to cell adhesion and extension (Gakidis *et al.*, 2004).

Osteoclasts are very mobile cells involved in bone resorption. They differentiate from monocyte/macrophage lineage precursors in response to the ligation of RANKL (receptor activation of NF-κB ligand) to RANK receptor and macrophage colony-stimulating factor receptor (c-FMS) (Teitelbaum and Ross, 2003). Mature osteoclasts are characterized by the presence of many podosomes, dynamic adhesive structures consisting of an F-actin nucleus surrounded by avβ3 integrin, and other cytoskeletal proteins (Lakkakorpi and Väänänen, 1991; Teitelbaum and Ross, 2003). In the start of bone resorption, the osteoclasts polarize and are subjected to large morphological changes that involve the formation of actin rings surrounding the area of resorption. One osteoclast

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performs several cycles of bone resorption, so their ability to remodel skeleton is crucial for the correct function (Lakkakorpi and Väänänen, 1991; Destaing *et al.*, 2003). It has been reported that the Rho GEF Vav3 (OMIM: 605541) (NM_006113.4) is an essential factor in the regulation of osteoclast function, being crucial in osteoclast polarization required for the initiation of the resorptive activity (Faccio *et al.*, 2005). There are several evidences that indicate that alterations in Vav3 cause alterations in bone metabolism. $Vav3^{-/-}$ mice display increased bone mass and are protected against bone loss (Faccio *et al.*, 2005).

Thus, the aim of our study was to characterize whether variations in *VAV3* gene could modify the risk of developing PDB. We have studied *VAV3* c.892A>T/p.T298S (rs7528153) polymorphism to evaluate its putative role in the susceptibility of suffering from PDB. We have selected the *VAV3* rs7528153 polymorphism because it is localized in the SH domain of the Vav3 protein and the SH domain is crucial in the Vav3 function because it allows the exchange of GDP for GTP. In addition, *VAV3* rs7528153 polymorphism is a nonsynonym polymorphism with a population frequency of the minor allele higher than 10% in the European population (29.62%) and it is located in a sequence highly conserved throughout the evolution.

## Materials and Methods

### Subjects

We have studied 238 patients who were suffering from PDB. In cases of familial PDB, we have included only one affected patient of each family. According to the prevalence of PDB, we calculated that a series of 152 patients is representative of our PDB population (www.surveysoftware.net/sscalce.htm). Patients were recruited in the Metabolic Bone Unit at the University Hospital of Salamanca (Province of Salamanca, Spain) between January 1990 and February 2014. As a control group, 253 sex-matched healthy subjects above 40 years old without history of PDB were recruited to the same hospital during the same period of time. From each patient, we collected clinical variables such as gender, age of diagnosis, family history, number of affected bones, presence of fractures, skull involvement, and cranial nerve involvement. All patients and control subjects gave informed consent to participate and the local ethics committee approved the study.

### DNA isolation and genotyping

Genomic DNA was extracted from peripheral blood of patients and control subjects by the standard phenol/chloroform procedure. Genotyping of *VAV3* rs7528153 polymorphism was performed using TaqMan 5′-exonuclease allelic discrimination assays (Applied Biosystems) that contain sequence-specific forward and reverse primers to amplify a fragment containing the polymorphic sequence and two probes labeled with VIC and FAM dyes to detect both alleles (c_447698_10) (Schleinitz *et al.*, 2011). Polymerase chain reactions (PCRs) were carried out using TaqMan universal PCR Master Mix (Applied Biosystems) following manufacturer's instructions in a Step-One Plus Real-time PCR system (Applied Biosystems). To assess reproducibility, a randomly selected 5% of the samples were regenotyped and all of these genotypes matched with genotypes initially designated.

Table 1. Clinical Characteristics of Paget Disease of Bone Patients

| | *PDB patients* |
|---|---|
| Male sex, *n* (%) | 132 (55.4) |
| Diagnostic age above 60 years, *n* (%) | 188 (78.9) |
| Family history of PDB, *n* (%) | 25 (10.6) |
| Less than three affected bones, *n* (%) | 177 (74.3) |
| Presence of fractures, *n* (%) | 17 (7.2) |
| Involvement of the skull, *n* (%) | 90 (37.8) |
| Cranial nerve involvement, *n* (%) | 33 (13.8) |

PDB, Paget disease of bone.

### Statistical analyses

Goodness of fit to Hardy–Weinberg equilibrium expectations was assessed by chi-squared test for *VAV3* rs7528153 polymorphism in control group. Odds ratios and 95% confidence intervals were estimated using unconditional logistic regression models to evaluate the association with PDB risk. Homozygous for the allele with greater frequency among controls was used as the reference genotype. These statistical analyses were performed using SPSS 21 software. For the analysis, differences with a $p$ value <0.05 were considered as statistically significant.

## Results

A total of 238 PDB patients and 253 healthy subjects were analyzed in this study. The clinical characteristics for each patient are summarized in Table 1. The genotype distributions of *VAV3* c.892A>T/p. T298S (rs7528153) polymorphism in control samples are in Hardy–Weinberg equilibrium. We found statistically significant differences in genotype distribution between PDB patients and controls. Homozygous TT genotype of *VAV3* c.892A>T/p. T298S (rs7528153) polymorphism was associated with an increased risk of developing PDB. The study of recessive and dominant codominance confirmed these results (Table 2). We also found significant differences in the allelic distribution, thus being a carrier of allele T confers an increased risk of developing PDB (Table 2).

Table 2. Distribution of the Genotypic and Allelic Frequencies of the *VAV3* c.892A>T (rs7528153) Polymorphism in Cases and Controls

| | *PDB patients,* n *(%)* | *Controls,* n *(%)* | *OR (95% CI)* | p |
|---|---|---|---|---|
| Genotype | | | | |
| AA | 95 (39.9) | 131 (51.8) | 1.00 | |
| AT | 95 (39.9) | 101 (39.9) | 1.29 (0.88–1.90) | 0.186 |
| TT | 48 (20.2) | 21 (8.3) | 3.15 (1.77–5.61) | **<0.001** |
| AA+AT | 190 (79.8) | 232 (91.7) | 1.00 | |
| TT | 48 (20.2) | 21 (8.3) | 2.79 (1.61–4.82) | **<0.001** |
| AA | 95 (39.9) | 131 (51.8) | 1.00 | |
| AT+TT | 143 (60.1) | 122 (48.2) | 1.61 (1.13–2.31) | **0.009** |
| Allele | | | | |
| A | 285 (59.9) | 363 (71.7) | 1.00 | |
| T | 191 (40.1) | 143 (28.3) | 1.70 (1.30–2.22) | **<0.001** |

Significant $p$-values are represented in bold.
CI, confidence interval; OR, odds ratio.

## Discussion

PDB occurs as a consequence of an increase of bone resorption followed by an excessive bone formation. The main alteration resides in osteoclasts that increase in size, number, and activity (Singer *et al.*, 2006; Ralston and Layfield, 2012). It has been reported that the Rho GEF Vav3 is an essential factor in the regulation of osteoclast function (Faccio *et al.*, 2005). Vav3 is expressed in mature osteoblasts and osteoclasts. It has been reported that $Vav3^{-/-}$ mice showed an increase in density and thickness of bone trabeculae (Faccio *et al.*, 2005), which coincides with the histology of pagetic bone (Ralston and Layfield, 2012). Increased bone density in $Vav3^{-/-}$ mice is because of a low bone resorption: bone formation predominates versus bone resorption (Faccio *et al.*, 2005). Low bone resorption is due to defective maturation of osteoclasts, and furthermore, it has been reported that Vav3 influences both the maturation of osteoclasts and the development of the normal functions of mature osteoclasts (Faccio *et al.*, 2005).

Several genetic factors have been related with the PDB (Ralston, 2008; Ralston and Layfield, 2012), so we hypothesize that polymorphic mutations in VAV3 gene could influence the development of the disease. VAV3 c.892A>T (rs7528153) polymorphism leads to an amino acid change Thr-Ser at codon 298 localized in the SH domain of the Vav3 protein. Our results showed that carriers of the T allele as well as carriers of homozygous TT genotype (Ser/Ser) of *VAV3* c.892A>T (rs7528153) polymorphism are associated with an increased risk of developing PDB in a Spanish cohort of patients.

The pagetic bone is characterized by an increase in density and thickness of the bone trabeculae. The balance resorption–bone formation is lost and it occurs as an excess bone resorption followed by an excessive bone formation that results in increased bone density (Ralston, 2008; Ralston and Layfield, 2012). Vav3 is an essential factor in the osteoclast polarization required for the initiation of the resorptive activity (Faccio *et al.*, 2005). Vav3 shuttles Rac-GDP to its activated GTP-associated state. Rac-GTP prompts association of lysosome-derived secretory vesicles with microtubules and, therefore, the resorptive activity of osteoclasts (Faccio *et al.*, 2005). The exchange of GDP for GTP is mediated by SH domain of Vav3 protein (Bustelo, 2001). The VAV3 c.892A>T (rs7528153) polymorphism is localized in the SH domain; therefore, our hypothesis is that carrying a serine at position 298 could increase the activation of Rac-GTP and bone resorption. Further analyses are necessary to confirm this hypothesis.

In conclusion, this study highlights for the first time that *VAV3* rs7528153 polymorphism could be a genetic biomarker of high risk of suffering from PDB, although additional studies would be necessary to validate our findings.

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## Author Disclosure Statement

No competing financial interests exist.

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