

## Polymorphisms of the farnesyl diphosphate synthase gene modulate bone changes in response to atorvastatin

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**Abstract** Although their primary therapeutic indications are different, aminobisphosphonates and statins target enzymes in the mevalonate pathway, which is critical for bone homeostasis. Previous studies have shown that some polymorphisms of the gene encoding farnesyl diphosphate synthase (FDPS), the main target of aminobisphosphonates, modulate the response to these drugs. In this study, we explored whether those single nucleotide polymorphisms (SNPs) also influence the changes in bone mineral density (BMD) following therapy with statins. Sixty-six patients with coronary heart disease were studied at baseline and after 1-year therapy with atorvastatin. BMD was measured by DXA. Three SNPs of the FDPS gene (rs2297480, rs11264359 and rs17367421) were analyzed

by using Taqman assays. The results showed that there was no association between the SNPs and basal BMD. However, rs2297480 and rs11264359 alleles, which are in linkage disequilibrium, were associated with changes in hip BMD following atorvastatin therapy. Thus, patients with AA genotype at the rs2297480 locus had a  $0.8 \pm 0.8$  % increase in BMD at the femoral neck, whereas in patients with AC/CC genotypes, BMD showed a  $2.3 \pm 0.8$  % decrease ( $p = 0.02$ ). Similar results were obtained regarding changes of BMD at the femoral trochanter and when alleles at the rs11264359 locus were analyzed. However, there was no association between BMD and rs17367421 alleles. In conclusion, these results suggest that polymorphisms of the FDPS gene may influence the bone response to various drugs targeting the mevalonate pathway, including not only aminobisphosphonates but also statins.

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### Introduction

Atherosclerosis and osteoporosis are prevalent chronic disorders. They are an important cause of morbidity and mortality in elderly people and represent a large economical burden for the public health systems. These disorders are characterized by a long asymptomatic period prior to the development of complications, cardiovascular events and fractures, respectively [1, 2].

Several studies have shown an association between cardiovascular risk and fractures. Besides an increasing prevalence with advancing age, they share some risk factors, such as smoking, sedentarism and estrogen deficiency.

Moreover, it has been suggested that they might share genetic risk factors and some common pathophysiological mechanisms, mediated by pro-inflammatory cytokines (IL-1, IL-6, TNF), which may favor the growth and rupture of atheroma plaques, as well as bone resorption and consequently bone loss [3, 4].

Another link between osteoporosis and atherosclerosis is the mevalonate pathway, a common target for drugs used in both disorders. This pathway is essential for the synthesis of cholesterol. In fact, statins, major drugs in the prevention and therapy of atherosclerosis, inhibit 3-hydroxy-3-methylglutaryl-coenzyme-A reductase (HMGCR), an enzyme in the mevalonate pathway [5]. On the other hand, farnesyl diphosphate synthase (FDPS; also known as farnesyl pyrophosphate synthase or FPPS), another enzyme in the mevalonate pathway, is the main target for aminobisphosphonates, drugs that inhibit osteoclast-mediated bone resorption and are the first-line therapy for many patients with osteoporosis [6, 7]. Although the clinical relevance is unclear, statins have favorable effects on bone homeostasis in several experimental models [8–10].

Aminobisphosphonates and statins are highly effective drugs for increasing bone mass and lowering cholesterol levels, respectively. However, some patients do not show an optimal response. We have shown recently that polymorphisms of the FDPS gene are associated with differences in bone mineral density (BMD) following therapy with aminobisphosphonates [11]. In this study, we explored the hypothesis that those polymorphisms could also modulate the response to statins.

## Subjects and methods

### Subjects

We included 66 patients with acute coronary syndrome, diagnosed according to the criteria of the European Society of Cardiology. Patients with active cancer, alcoholism, chronic renal failure or disorders influencing bone metabolism were excluded. They were treated with various doses of atorvastatin according to their vascular risk. High-risk patients were given 40–80 mg/day, whereas low-risk patients received 10–20 mg/day. Blood samples were obtained after 8-h fasting. Cholesterol, triglycerides, HDL cholesterol and LDL cholesterol were measured using a Hitachi 917 autoanalyzer (Tokyo, Japan). Osteocalcin was measured by immunoassay (Immulite DPC, USA) with a 6.7 interassay coefficient of variation. BMD was measured by DXA (Lunar Corporation, Madison, WI, USA) at the lumbar spine and the hip, both at baseline and after 1 year of atorvastatin. The study was approved by

the institutional ethical committee, and all patients gave informed consent.

### Genotyping

We analyzed several single nucleotide polymorphisms (SNPs) that were previously selected among a set of tagging and functional polymorphisms of the FDPS gene, identified using Haploview and PupaSuite software [12, 13], as previously reported [11]. Thus, we studied 3 SNPs: rs2297480, located in the 5' region of FDPS gene, 778 bp upstream of the translation start site; rs11264359, an intronic SNP at +4,125 from the translation start site; and rs17367421, located in an intron at +8,543. DNA was isolated from peripheral blood with commercial methods (Qiagen or GE Healthcare). Alleles at each locus were genotyped by using specific Taqman assays (Applied Biosystems, Foster City, CA, USA).

### Data analysis

Hardy–Weinberg equilibrium was analyzed with an exact test implemented in HWSIM software (available at <http://krunch.med.yale.edu/hwsim/hwsim.txt>). The response of BMD to drug therapy was estimated as the percent change from baseline. Given the low frequency of homozygotes for the less common alleles, they were grouped with heterozygotes. Then, the differences between genotypes were tested by t tests. The influence of covariates was explored in multiple regression models.

## Results

The study group included 66 patients (27 women and 39 men), of these 90 % had acute myocardial infarction and 10 % had unstable angina. Mean age was  $61 \pm 10$  year; mean body mass index was  $28 \pm 4$  kg/m<sup>2</sup>. Twenty percent of the patients were hypertensive and 13 % diabetic. All women were postmenopausal. In addition to atorvastatin, 28 % received treatment with angiotensin converting enzyme inhibitor, 57 % with  $\beta$ -blockers, 20 % with nitrates, 2 % with angiotensin antagonists and 1 % with thiazides. The atorvastatin decreased the levels of cholesterol ( $180 \pm 47$  vs.  $154 \pm 36$  mg/dl,  $p = 0.0001$ ), LDL cholesterol ( $113 \pm 44$  vs.  $86 \pm 33$  mg/dl,  $p = 0.001$ ) and triglycerides ( $144 \pm 64$  vs.  $126 \pm 66$  mg/dl,  $p = 0.001$ ) and increased HDL cholesterol ( $39 \pm 12$  vs.  $46 \pm 11$  mg/dl,  $p = 0.001$ ). However, there were no relationship between changes in LDL cholesterol and changes in BMD. The atorvastatin decreased the levels of osteocalcin, bone marker of bone turnover ( $3.07 \pm 1.82$  vs.

**Table 1** Genotype frequencies (number and rounded percentages) and *p* values for departure of a Hardy–Weinberg equilibrium

SNP				<i>p</i> (HWE)
rs2297480	AA	AC	CC	
	43 (65.2)	21 (31.8)	2 (3.0)	>0.5
rs11264359	AA	AG	GG	
	41 (62.1)	22 (33.3)	3 (4.6)	>0.5
rs17367421	CC	CG	GG	
	61 (92.4)	5 (7.6)	0 (0)	>0.5

1.34 ± 1.33 ng/ml, *p* = 0.0001). We did not find differences in baseline and final osteocalcin across genotypes for any of the polymorphisms studied. Baseline BMD was 1.128 ± 0.230, 0.915 ± 0.150 and 0.776 ± 0.210 g/cm<sup>2</sup>, at the lumbar spine, femoral neck and femoral trochanter, respectively.

Genotype frequencies are shown in Table 1; there was no evidence for departure from Hardy–Weinberg equilibrium. Rs2297480 and rs11264359 alleles were in strong linkage disequilibrium (*p* < 0.0003).

We did not find differences in baseline BMD across genotypes for any of the polymorphisms studied (Table 2). However, alleles at loci rs11264359 and rs2297480 were associated with differences in the hip BMD changes following atorvastatin therapy. Whereas BMD tended to increase in patients homozygotes for the most common A allele at either the rs11264359 or the rs2297480 loci, it tended to decrease in patients with the less common alleles (G at rs11264359 and C at rs2297480) (Fig. 1). Changes in spine BMD were not significantly associated with the patients' genotypes. Very similar results were obtained when baseline BMD, age and sex were included in the analysis as covariates. There was no evidence for interaction between those variables and the genotypes. There was no association between the

genotypes and the dose of atorvastatin used. Likewise, there was no interaction between genotypes and the dose of atorvastatin on BMD response.

## Discussion

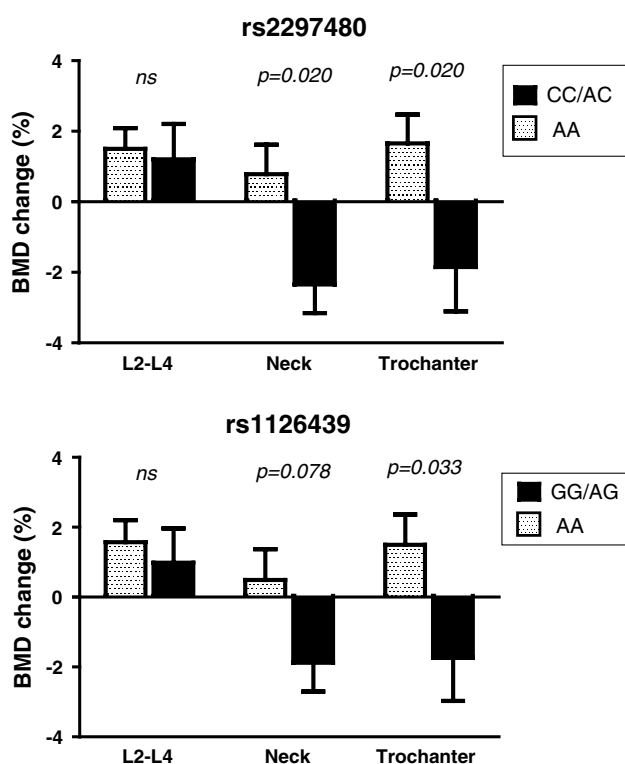
In the present study, we did not find an association of baseline BMD with common polymorphisms of the FDPS gene, thus confirming previous results by our own group in a different Spanish population [11]. However, we found an association of the rs2297480 and rs11264359 polymorphisms with BMD changes following atorvastatin therapy. Statins target the mevalonate pathway. This pathway is essential for cholesterol synthesis, but it also leads to the synthesis of a number of intermediate products, such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate that in turn contribute to the posttranslational modification of several regulatory proteins playing important roles in the activity and survival of a variety of cell types.

Following the seminal work by Mundy et al. [14], the effects of statins on bone metabolism have been widely studied. As reviewed recently by Ruan et al. [15], statins promote the differentiation of osteoblast precursors and inhibit osteoblast apoptosis, which tend to increase bone formation. On the other hand, these drugs have been shown to inhibit RANKL expression in several experimental models [15, 16]. RANKL is a critical factor required to induce the differentiation of hemopoietic precursors toward mature osteoclasts, the cells responsible for bone resorption [17]. Therefore, in experimental models, statins tend to stimulate bone formation and inhibit bone resorption, which results in a positive effect on bone mass.

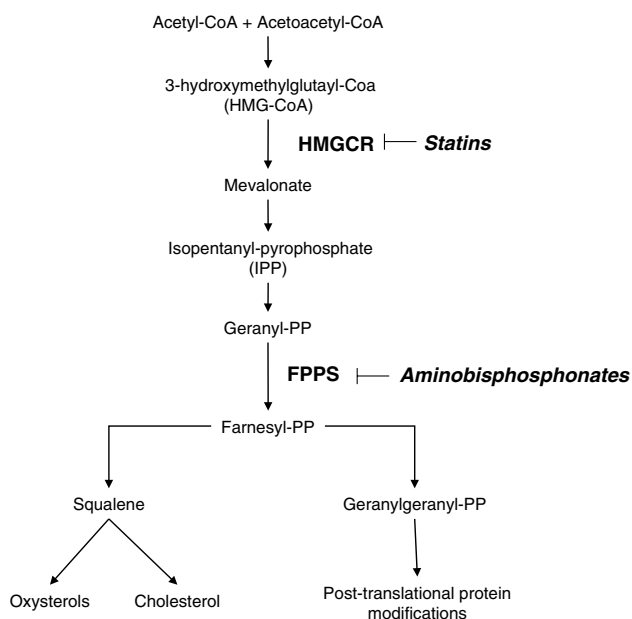
Statins inhibit HMGCR, an early enzyme in the mevalonate pathway, but they are not known to target FDPS (Fig. 2). Therefore, the mechanisms explaining the association between FDPS polymorphisms and statin-induced changes in BMD are unclear. Interestingly, a alleles at the

**Table 2** Baseline BMD at the spine (L2–L4) and femoral neck across genotypes

SNP				<i>p</i>	
rs2297480	AA	AC	CC		
	Spine	1.147 (0.241)	1.122 (0.210)	1.071	0.88
	Femoral neck	0.898 (0.157)	0.943 (0.147)	0.989 (0.098)	0.46
rs11264359	AA	AG	GG		
	Spine	1.150 (0.146)	1.222 (0.216)	1.071	0.96
	Femoral neck	0.914 (0.156)	0.916 (0.159)	0.989 (0.10)	0.86
rs17367421	CC	CG	GG		
	Spine	1.144 (0.280)	1.183 (0.214)	–	0.19
	Femoral neck	0.917 (0.155)	0.929 (0.135)	–	0.78



**Fig. 1** BMD changes (% over baseline) after 1 year of atorvastatin treatment in patients with different FDPS genotypes



**Fig. 2** Simplified view of the mevalonate pathway

rs2297480 and rs11264359 loci, associated with a better response of bone to statins in this study, were previously associated with a better response to bisphosphonates in a

different population [11]. This suggests that in some way, those alleles of the FDPS gene are associated with a higher susceptibility to drugs interfering the mevalonate pathway, either by inhibiting FDPS itself (bisphosphonates) or by decreasing substrate availability following the inhibition of an upstream enzyme, as statins do.

Statins may have additional indirect effects on the expression of FDPS, which could represent another mechanism linking FDPS genotype and statin-induced effects on bone. The mechanisms regulating FDPS expression have not been completely elucidated, but the promoter region has binding sites for several factors, including a sterol-response element and nuclear factor  $\gamma$  [18, 19]. Transcriptional activation induced by sterols is mediated by sterol-regulatory-element-binding protein 2 (SREBP-2) [20]. Expression and activity of the SREBP family depends on the cellular levels of cholesterol and other lipids [5, 21]. Therefore, statin therapy may modulate SEBP-2 and consequently FPPS expression, but it remains to be confirmed if this effect actually participates in the influence of statins on bone.

In summary, although these results should be considered preliminary until they are replicated in larger groups of patients, this study suggests that polymorphisms of the FDPS gene may influence the response of bone to different drugs targeting the mevalonate pathway, including not only aminobisphosphonates, but also statins.

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**Conflict of interest** Authors declare that they do not have conflicts of interest to declare.

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