CLINICAL STUDY

Polymorphisms in the interleukin-6 receptor gene are associated with bone mineral density and body mass index in Spanish postmenopausal women

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Abstract

Objective: Osteoporosis and obesity are complex diseases with a strong genetic component. Bone mineral density (BMD) and body mass index (BMI) linkage studies identified a locus at 1q21-23, where the interleukin-6 receptor (*IL6R*) gene is located. The *IL6R* and the gp130 receptors are the mediators of *IL6* action. Serum levels of *IL6* and s*IL6R* (the soluble form of *IL6R*) are higher in several diseases such as osteoporosis or obesity. Variants at *IL6R* have been associated with BMI and obesity. However, *IL6R* is an as-yet-unexplored osteoporosis candidate gene.

Design: In the present study we analysed two polymorphisms in the *IL6R* promoter, –1435 C/T (rs3887104) and –208 G/A (rs4845617), and the Asp358Ala polymorphism (rs8192284), in relation to both BMD and BMI in a cohort of 559 postmenopausal Spanish women.

Results: The promoter polymorphisms, –1435 C/T and –208 G/A were associated with femoral neck (FN) BMD ($P=0.011$ and $P=0.025$ respectively). The C-A and T-G promoter haplotypes were also associated with FN BMD. Additionally, the Asp358Ala variant was associated with lumbar spine BMD ($P=0.038$). Finally, the –208 G/A polymorphism and the C-G and C-A haplotypes were associated with BMI and obesity, where GG was the risk genotype ($P=0.033$ for BMI; $P=0.010$ for obesity).

Conclusion: These data suggest that variants in the *IL6R* gene are not only involved in the determination of BMI but also relevant for the determination of BMD. The *IL6R* gene may belong to the growing list of genes known to be involved in both phenotypes.

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Introduction

Osteoporosis and obesity are complex diseases with a strong genetic component. The heritability of bone mineral density (BMD) has been estimated to be between 0.6 and 0.9 depending on body site and sex (1, 2), whereas that of body mass index (BMI), a measurement of obesity, has been calculated to be between 0.5 and 0.6 (2). Genome-wide non-parametric linkage analysis is a good method for defining quantitative trait loci (QTLs). One of these loci, identified in relation to lumbar spine (LS) BMD, BMI and type 2 diabetes mellitus (T2DM), is located at 1q21-23 (3–5).

The *IL6R* gene maps to 1q21.3 and encodes the interleukin-6 (*IL6*) receptor- α . The action of *IL6* is mediated through two kinds of receptors: *IL6R* (also known as gp80, CD126, *IL-6R-1*, *IL-6R-A* or *IL-6R- α*) and gp130 (CD130, *IL-6R- β* or oncostatin M receptor). In contrast to gp130, whose expression pattern is wide and whose ligands include several interleukins, *IL6R* is

expressed in a more restricted cell pattern, including the osteoblast and osteoclast progenitors (6, 7), and only binds its natural ligand *IL6*.

The *IL6*–*IL6R* complex induces the homodimerization of gp130, which mediates signalling through the JAK-STAT and ras/MAP-kinase pathways (8). *IL6R* may be found both as a transmembrane and as a soluble molecule (s*IL6R*). The latter is produced by cleavage of the former, although it may also be generated by alternative splicing (9–12). The binding capacities of *IL6R* and s*IL6R* are similar, and the *IL6*–s*IL6R* complex acts as an agonist when it binds to gp130, allowing for *IL6* signalling in cells that do not express *IL6R* in their membranes. This process is referred to as trans-signalling (8).

IL6 is a pleiotropic cytokine that has multiple effects on different cell types (8). In bone, *IL6* is produced by osteoblasts, monocytes and T cells, and has an antiapoptotic and differentiating effect on osteoblasts (13–15). Also, and more importantly, *IL6* induces

osteoclastogenesis and osteoclast activity (16, 17) through RANKL and OPG produced by osteoblasts (6, 18). In human osteoblasts, IL6R cannot transmit IL6 signalling until it is shed into its soluble form (19).

It is generally accepted that serum levels of IL6 and sIL6R increase with age and postmenopausal status and are higher in several pathological processes such as osteoporosis, obesity or diabetes (20–26). IL6 is known to downregulate food intake and energy expenditure through the CNS (27). IL6-deficient mice develop mature-onset obesity (28) and are protected against osteoporosis after ovariectomy (29).

Several polymorphisms have been identified in the *IL6R* gene, some of which have been associated with obesity, diabetes and periodontitis in different studies (29–34). However, as yet, no polymorphisms of the *IL6R* gene have been studied in relation to osteoporosis. The aim of the present study is to evaluate two polymorphisms in the *IL6R* promoter, –1435 C/T and –208 G/A, and the Asp358Ala polymorphism in exon 9 of the gene in relation to LS BMD, femoral neck (FN) BMD, BMI and obesity status. These variants were selected based on their putative functionality. The Asp358Ala is the only non-synonymous SNP in the gene with a known MAF, and the promoter polymorphisms presented putative allele-specific binding capacities for different transcription factors, according to *in silico* analyses. In a cohort of postmenopausal Spanish women, these polymorphisms and/or their haplotypes were found to have effects on both BMD and BMI.

Materials and methods

Subjects

All participants were consecutive, unselected postmenopausal Spanish women attending the baseline visit at the outpatient clinic of the Menopausal Unit of the Hospital del Mar because of menopause. Patients were prospectively recruited and included regardless of their bone density values. Criteria for exclusion from the study were a previous diagnosis of Paget's disease of bone or osteomalacia, metabolic or endocrine disorder or if they were already undergoing treatment such as hormone replacement treatment. Women who had the menopause before the age of 35 were also excluded. The data recorded were age, the number of years since menopause (ysm) for the LS BMD and FN BMD measurements, height and weight. BMI was calculated as weight/height², and obesity was defined as BMI ≥ 30 kg/m²; 17.8% (*n* = 99) of the individuals in the cohort were obese. According to the WHO definition of osteoporosis and based on LS BMD, 30.9% of the participants were osteoporotic, 42.6% were osteopenic and 26.5% were normal. For FN BMD, 19% of the participants were osteoporotic, 54.1% were osteopenic and 26.9% were normal. Regarding osteoporotic

fractures, these were present in the cohort in the following percentages: 4.1% Colles, 7.7% vertebral, 0.5% FN and 4.7% other types. Given the small numbers, association with fracture was not performed. Blood samples and written informed consent were obtained according to the regulations of the Hospital del Mar Human Research Review Committee for Genetic Procedures. The main characteristics of the participants are listed in Table 1.

BMD analysis

LS BMD (L2–L4) was measured in all participants (*n* = 559) as part of the standard protocol of the Menopause Unit. FN BMD was also measured in 315 of the women. A dual-energy X-ray densitometer (QDR 4500 SL; Hologic) was used for the measurements. BMD was expressed in grams per square centimetre and in our centre the technique had an *in vivo* coefficient of variation of 1.0% for LS and 1.65% for FN measurements.

DNA extraction

Genomic DNA of the participants was isolated from peripheral leukocytes according to the BloodPrep chemistry protocol (Applied Biosystems). Purification procedures were conducted on an ABI PRISM 6700 Nucleic Acid Workstation (Applied Biosystems).

Genotyping

Genotyping of –1435 C/T and –208 G/A polymorphisms Typing of –1435 C/T (rs3887104) and –208 G/A (rs4845617) polymorphisms was performed using the SNaPshot method (Applied Biosystems). A PCR fragment was generated in a 25 µl reaction containing 20 ng genomic DNA, the indicated buffer, 5% DMSO, 4 mM MgCl₂, 0.2 mM of each d-NTP, 0.4 µM of each sense primer (5'-GGGGGTCCTTGAGAGAGTTC-3') and antisense primer (5'-CCGGCTCTTACACACTG-3') and 1.25 U Taq DNA polymerase

Table 1 Characteristics of the cohort.

Variable	N	Mean (S.D.)
Weight (kg)	558	64.7 (9.8)
Height (cm)	556	156.2 (6.2)
BMI (kg/m ²)	556	26.5 (3.9)
LS_age (years)	559	55.0 (8.4)
LS_ysm (years)	559	7.3 (8.2)
LS_BMD (g/cm ²)	559	0.847 (0.150)
FN_age (years)	315	58.6 (8.7)
FN_ysm (years)	315	10.5 (8.9)
FN_BMD (g/cm ²)	316	0.670 (0.114)

BMD, bone mineral density; BMI, body mass index; FN, femoral neck; LS, lumbar spine; ysm, years since menopause.

(GeneCraft). The PCR involved an initial denaturation step at 94 °C for 5 min, followed by 38 cycles of denaturation at 94 °C for 40 s, annealing at 59 °C for 30 s and polymerization at 72 °C for 40 s, with a final elongation step at 72 °C for 5 min. The SNaPshot reaction was performed in 10 µl volume, using 3 µl clean PCR product, 3 µl SNaPshot Multiplex Reaction Mix (Applied Biosystems) and 0.2 µM of each SNaPshot primer. Primer sequences were: –1435 C/T=5'-TTTTTTTTTTTCCAGGTGCTTGTCTTTCTTTT-3' and –208 G/A=5'-TTTTTTTTTTTTCGCACTGACCTGAGCCGG-3'. The reaction was run using a cycling protocol of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 30 s for 25 cycles. Clean SNaPshot products were mixed with HiDi formamide and GeneScan-120 LIZ size standard (Applied Biosystems) were denatured at 95 °C for 5 min and then loaded into an ABIPRISM 310 Genetic Analyzer (Applied Biosystems). GeneMapper software (Applied Biosystems) was used to analyse the data.

Genotyping of the Asp358Ala polymorphism A fragment of 286 bp containing the Asp358Ala (A/C) (rs8192284) polymorphic site was amplified by PCR using the following pair of primers: sense (5'-AAGCTTGTCAAATGGCCTGT-3') and anti-sense (5'-GGACCCATCTCACCTCAGAA-3') in a final volume of 25 µl containing 20 ng genomic DNA, the indicated buffer, 4 mM MgCl₂, 0.2 mM of each d-NTP, 0.4 µM of each primer and 1.25 U Taq DNA polymerase (Gene-Craft). The PCR consisted of an initial denaturing step at 94 °C for 5 min, followed by 35 cycles of denaturing at 94 °C for 40 s, annealing at 63 °C for 30 s and polymerization at 72 °C for 40 s, with a final elongation step at 72 °C for 5 min. The PCR product was digested with the restriction enzyme *Hinf*I (Takara) overnight at 37 °C. The homozygote CC samples remained uncut, while the AA homozygotes gave two DNA fragments of 101 and 185 bp.

Linkage disequilibrium calculations and haplotype reconstruction

The D' and r^2 linkage disequilibrium parameters were calculated using the Haploview software (35). Haplotype frequencies were obtained using Haploview and Haplo.score and showed no detectable differences (36). The Haplo.score software was also used to assign individual haplotypes and their related probabilities.

Statistical analyses

The statistical power was calculated using the Quanto v.1.1 software (37). For LS BMD (mean=0.847 and

s.d.=0.150), at a significance level of 0.05, MAFs of 0.2–0.4 and 460 individuals, there would be an 80% power to detect differences in BMD of 0.04 g/cm² (MAF=0.2) and 0.03 g/cm² (MAF=0.4) under an additive genetic model. For FN BMD (mean=0.670 and s.d.=0.114) and 250 individuals, there would be power to detect differences in BMD of 0.04 g/cm² (MAF=0.2) and 0.03 g/cm² (MAF=0.4) under an additive genetic model. For the categorical variable obesity/non-obesity, the sample size of this study ($n=83/385$), with MAFs of 0.2–0.4 and an OR=2, would give a statistical power of 80% for both the dominant and additive models.

All the analyses were performed using the SPSS v.11.5 statistical software (SPSS Inc). ANOVA or covariance (ANCOVA) was used to assess the effect of each polymorphism or haplotype on LS BMD, FN BMD and BMI. The covariables used were those with the greatest clinical relevance (BMI and ysm for BMD; ysm for BMI). General, dominant and recessive models were analysed. The χ^2 and Fisher's exact tests were used to assess Hardy–Weinberg equilibrium (HWE) and the obesity phenotype among different genotypes and haplotypes. A value of $P<0.05$ was considered statistically significant.

Results

Genotypes and haplotypes of IL6R

The IL6R promoter polymorphisms rs3887104 and rs4845617, located –1435 and –208 nucleotides from the translation start site respectively, were genotyped in the cohort. They are separated by 1227 bp and were found to be in strong linkage disequilibrium ($D'=1$ and $r^2=0.11$). Accordingly, only three of the four possible haplotypes were found in the sample. In contrast, the promoter polymorphisms were not in linkage disequilibrium with the exon 9 polymorphism, Asp358Ala (rs8192284), located ~50 kb downstream ($D'=0.50$ and $r^2=0.03$ for –1435 C/T; $D'=0.17$ and $r^2=0.03$ for –208 G/A). Genotypic and allelic frequencies are shown in Table 2. We estimated the short-range haplotypes, which encompass the polymorphisms in the promoter region. The population frequencies and the number of individuals bearing two, one or no copies of each haplotype are listed in Table 3.

Association between genotypes and LS BMD, FN BMD, BMI and obesity status

Results of the ANCOVA tests between the genotypes and the adjusted phenotypes are shown in Table 4. The two promoter polymorphisms were associated with adjusted FN BMD. The –1435 C/T polymorphism was associated under a dominant model ($P=0.011$) so that carriers of the CC genotype had higher FN BMD values

Table 2 Genotypic and allelic frequencies and Hardy–Weinberg equilibrium (HWE) *P* values.

Polymorphism	Genotype	N (%)	Allele	Frequency	HWE <i>P</i> value
–1435 C/T (rs3887104)	CC	349 (74.4)	C	0.86	0.48
	CT	109 (23.2)	T	0.14	
	TT	11 (2.3)			
	Total	469			
–208 G/A (rs4845617)	GG	179 (38.6)	G	0.62	0.59
	GA	214 (46.1)	A	0.38	
	AA	71 (15.3)			
	Total	464			
Asp358Ala (rs8192284)	AA	162 (33.4)	A (Asp)	0.57	0.62
	AC	232 (47.8)	C (Ala)	0.43	
	CC	91 (18.8)			
	Total	485			

than the rest of the cohort. The downstream polymorphism, –208 G/A, was associated with adjusted FN BMD under a recessive model ($P=0.025$). The AA genotype group had a significantly higher mean FN BMD value than the other two groups. Neither of the two promoter polymorphisms was associated with LS BMD. In contrast, the Asp358Ala polymorphism was found to be associated with LS BMD in a recessive manner ($P=0.038$). Subjects who were homozygotes for the C allele (Ala) had higher LS BMD than the remaining individuals.

In terms of body mass parameters, only the –208 G/A polymorphism was found to be associated with both BMI ($P=0.033$) and obesity ($P=0.010$), under a dominant model. The obesity OR for individuals bearing the GG genotype when compared with those bearing at least one copy of the A allele was 1.89 (95% CI: 1.18–3.04).

Association between haplotypes and LS BMD, FN BMD, BMI and obesity status

We analysed the relationship between the number of copies of each short-range haplotype and LS BMD, FN BMD, BMI or obesity status (Table 5). It was observed that carrying two copies of the C-A haplotype was associated with high FN BMD values ($P=0.041$) while carrying at least one copy of the T-G haplotype was associated with low FN BMD ($P=0.05$). Figure 1 summarizes these haplotype results for FN BMD. Individuals with C-A/C-A haplogenotype show

significantly higher FN BMD ($P=0.011$) than those bearing T-G/other haplogenotype. None of the short-range haplotypes was associated with LS BMD.

On the other hand, bearing two copies of C-G haplotype was associated with higher BMI values ($P=0.035$). Either two copies of the C-G haplotype or no copies of the C-A haplotype were also associated with obesity status ($P=0.021$ and $P=0.039$ respectively). The obesity OR for individuals bearing two copies of the C-G haplotype when compared with those bearing one or no copies was 1.95 (1.12–3.37). An OR of 0.59 (0.36–0.96) was obtained for individuals bearing one or two copies of the C-A haplotype when compared with those bearing no copy.

Discussion

This study is the first association analysis performed between polymorphisms in the *IL6R* gene and osteoporosis. We studied two polymorphisms in the promoter of this gene and a non-synonymous polymorphism in exon 9. The two promoter polymorphisms were significantly associated with FN BMD, and the missense change was associated with LS BMD, which suggests that *IL6R* has a role in bone mass determination.

For the promoter polymorphisms, we were able to identify two different genotypic or haplogenotypic groups that show lower or higher than average FN BMD levels (Fig. 1). The low FN BMD group was defined by the individuals bearing at least one copy of the –1435 T allele (the same group that carried at least one copy of the T-G haplotype, given that the T-A haplotype was absent from the cohort). The estimated mean FN BMD value of this group, which represented ~25% of the sample, was 0.037 g/cm² (0.009–0.066) lower than the corresponding value for the rest of the cohort. At the other end of the distribution, a high FN BMD group was defined by individuals who were homozygous for the A allele at –208 (all were homozygous for the C-A haplotype, given that the T-A haplotype was absent). This group included more than 15% of the sample and exhibited an estimated mean FN

Table 3 Short-range haplotype frequencies and number of individuals bearing 0, 1 or 2 copies of each haplotype.

Short-range haplotypes	N (%)	Number of copies		
		0	1	2
C-G	402 (46.2)	122 (28.0)	224 (51.5)	89 (20.5)
C-A	345 (39.7)	159 (36.6)	207 (47.6)	69 (15.9)
T-G	123 (14.1)	323 (74.3)	101 (23.2)	11 (2.5)
Total	870			

Table 4 Association between interleukin-6 receptor (*IL6R*) polymorphisms and lumbar spine (LS) bone mineral density (BMD), femoral neck (FN) BMD, body mass index (BMI) and obesity status.

	BMD and BMI (mean (S.E.M)) ^a			Obesity status N (frequency) ^b	
	adj_LS_BMD ^c	adj_FN_BMD ^c	adj_BMI ^d	Non obese	Obese
–1455C/T					
N	468	262	468	385	83
CC	0.847 (0.007)	0.680 (0.007)	26.5 (0.2)	287 (82.2%)	62 (17.8%)
CT	0.840 (0.007)	0.642 (0.013)	26.3 (0.4)	89 (81.7%)	20 (18.3%)
TT	0.808 (0.043)	0.655 (0.100)	25.9 (1.2)	9 (90.0%)	1 (10.0%)
P value ^e	0.627	0.040	0.675	0.803	
P value ^f		0.011 (D)			
–208 G/A					
N	462	251	462	376	86
GG	0.847 (0.010)	0.668 (0.011)	27.0 (0.3)	134 (75.3%)	44 (24.7%)
GA	0.852 (0.009)	0.669 (0.009)	26.1 (0.3)	183 (85.9%)	30 (14.1%)
AA	0.847 (0.016)	0.707 (0.015)	26.8 (0.4)	59 (83.1%)	12 (16.9%)
P value ^e	0.917	0.081	0.038	0.025	
P value ^f		0.025 (R)	0.033 (D)	0.010 (D) ^g	
Asp358Ala					
N	484	256	484	402	82
AA (AspAsp)	0.847 (0.011)	0.664 (0.011)	26.6 (0.3)	130 (80.2%)	32 (19.8%)
CA (AspAla)	0.850 (0.009)	0.675 (0.009)	26.3 (0.3)	196 (84.5%)	36 (15.5%)
CC (AlaAla)	0.882 (0.014)	0.693 (0.015)	26.2 (0.4)	76 (84.4%)	14 (15.6%)
P value ^e	0.115	0.276	0.695	0.505	
P value ^f	0.038 (R)				

^aANCOVA test.^b χ^2 test.^cAdjusted by BMI and years since menopause.^dAdjusted by years since menopause.^eGeneral P value.^fAlternative P value. D, Dominant model; R, recessive model.^gFisher's exact.

BMD that was 0.038 g/cm² (0.005–0.071) higher than the corresponding value for the rest of the cohort. Therefore, it seems that both polymorphisms are important in defining FN BMD.

Although there is no evidence of the functionality of the –1435 C/T and –208 G/A polymorphisms, these promoter SNPs showed allele-specific consensus sequences for factors such as Cdx2 or NF-1, according to analyses performed with the Genomatix and Signal Scan software packages (38, 39). It is also possible that these polymorphisms could be tagging other functional ones. According to HapMap data, the polymorphisms lie in a haplotypic block of more than 20 kb at the 5' flank of the *IL6R* gene, which includes 'synonymous' polymorphisms (according to an $r^2 \geq 0.8$) for the –208 G/A SNP. No data for the –1435 C/T polymorphism are available in HapMap.

In contrast to the promoter SNPs, the Asp358Ala polymorphism was found to be associated with LS BMD and not with FN BMD. This difference may be explained by the fact that LS and FN have different proportions of trabecular and cortical bone and are partially determined by different genetic variants (2).

Homozygotes for the Ala allele showed higher LS BMD values. The Asp358 is located in the site that is more frequently cleaved to produce the soluble sIL6R (40). Functional analyses have shown that point

mutations changing it to Gly, Phe, Leu or Arg reduce the shedding to 22–54% of the wild-type sIL6R and the double point mutation Gln357-Asp358 to Ala357-Ala358 reduces the shedding to 34%. Consequently, the Ala358 allele could be related to lower shedding and therefore to lower sIL6R levels. This, in turn, could lead to reduced osteoclastogenesis, which would be consistent with the higher BMD observed. However, we cannot rule out the possibility that this SNP is in high linkage disequilibrium with other functional polymorphisms. The Asp358Ala polymorphism lies in an LD block of 32 kb, which contains seven confirmed synonymous intronic polymorphisms.

We also investigated the relationship of the *IL6R* polymorphisms with obesity and found the –208 G/A SNP to be associated with both BMI and obesity status in the cohort. In particular, the GG genotype conferred an increased risk of obesity (OR = 1.89 (1.18–3.04)) and a similar effect was observed when looking at haplotypes: individuals who were homozygous for the C-G haplotype or those carrying no copies of the C-A haplotype (which are all homozygotes –208 GG, given the strong linkage disequilibrium observed) had higher BMI. Previous studies have associated polymorphisms of the *IL6R* gene with BMI. In particular, the Asp358 allele was related to higher BMI in a group of Pima Indians (30) and in a Spanish population (33). In contrast,

Table 5 Association between interleukin-6 receptor (*IL6R*) short-range haplotypes and lumbar spine (LS) bone mineral density (BMD), femoral neck (FN) BMD, body mass index (BMI) and obesity status.

	BMD and BMI Variables (mean (S.E.M.)) ^a			Obesity status N (frequency) ^b	
	adj_LS_BMD ^c	adj_FN_BMD ^c	adj_BMI ^d	Non obese	Obese
CG Haplotype					
N	434	234	434	355	79
0 copies of C-G	0.843 (0.012)	0.686 (0.012)	26.4 (0.3)	100 (82.6%)	21 (17.4%)
1 copy of C-G	0.854 (0.009)	0.666 (0.009)	26.2 (0.2)	190 (84.8%)	34 (15.2%)
2 copies of C-G	0.850 (0.014)	0.682 (0.015)	27.2 (0.4)	65 (73.0%)	24 (27.0%)
P value ^e	0.790	0.398	0.099	0.049	
P value ^f			0.035 (R)	0.021(R)	
CA Haplotype					
N	434	234	434	355	79
0 copies of C-A	0.848 (0.011)	0.668 (0.012)	26.9 (0.3)	121 (76.6%)	3 (23.4%)
1 copy of C-A	0.854 (0.009)	0.669 (0.009)	26.1 (0.3)	177 (85.5%)	30 (14.5%)
2 copies of C-A	0.843 (0.016)	0.704 (0.016)	26.7 (0.4)	57 (82.6%)	12 (17.4%)
P value ^e	0.810	0.124	0.112	0.089	
P value ^f		0.041 (R)		0.039 (D) ^g	
TG Haplotype					
N	434	234	434	355	79
0 copies of T-G	0.851 (0.008)	0.682 (0.007)	26.6 (0.2)	265 (82.0%)	58 (18.0%)
1 copy of T-G	0.851 (0.014)	0.651 (0.014)	26.3 (0.4)	81 (80.2%)	20 (19.8%)
2 copies of T-G	0.810 (0.043)	0.655 (0.100)	25.9 (1.2)	9 (90.0%)	1 (10.0%)
P value ^e	0.646	0.148	0.744	0.727	
P value ^f		0.050 (D)			

^aANCOVA test.^b χ^2 test.^cAdjusted by BMI and years since menopause.^dAdjusted by years since menopause.^eGeneral P value.^fAlternative P value. D, dominant model; R, recessive model.^gFisher's exact.

Hamid *et al.* (31), Wang *et al.* (32) and the present study failed to detect this association in a Danish, an American and another Spanish population respectively. Differences in study design and in the age, sex and ethnicity of the patients make it difficult to draw comparisons between these studies. A comprehensive analysis of the complete *IL6R* genomic region in a high-powered study might help to clarify the role of *IL6R* variants in obesity. Additionally, functional analyses of particular SNPs might help to understand the molecular basis of the positive association results.

In this study the -208 G/A polymorphism at the *IL6R* gene was found to be associated with FN BMD and BMI simultaneously. While women who were homozygous for the major allele (G) were at higher risk of obesity, those who were homozygous for the minor allele (A) had higher average FN BMD. This is not the first time that polymorphisms in a gene are related simultaneously to osteoporosis and obesity. For example, genes such as *IL6* and *RANK*, both participating in bone resorption, were associated with BMD and with BMI (41–43). Although it is generally considered that obese women are at lower risk of osteoporosis, there are some controversial results that question this assumption (44). It has recently been proposed that while lean mass is positively correlated with BMD, fat mass is inversely correlated with it (45). A positive

correlation between BMI and FN BMD was observed in our cohort but it disappeared when the sample was stratified according to the -208 G/A genotype. Obesity and osteoporosis are diseases that share several

Haplogenotype	T-G/_	C-G/C-G or C-G/C-A	C-A/C-A
-1435 C/T	T_	CC	CC
-208 G/A	G_	G_	AA
N (%)	54 (23%)	140 (60%)	40 (17%)

Figure 1 Mean adjusted FN BMD and standard error for three haplogenotype categories. Individuals bearing C-A/C-A (17%) had higher FN BMD and individuals carrying at least one copy of the T-G haplotype (23%) had lower FN BMD than the rest of the cohort. Comparison of the two extreme groups showed a significant difference (ANCOVA, $P=0.011$).

features, including a genetic basis. They both can be traced to dysregulation of a common precursor cell. Both diseases are under CNS control and regulated by multiple shared signal molecules such as oestrogens, leptin or IL6 (44). In particular, Deng *et al.* (2) observed that BMI and BMD shared ~10–20% genetic variation and suggested that this could be caused by shared genes with pleiotropic effects on both BMI and BMD. It was also observed that the magnitude of shared genetic and environmental components was higher between FN BMD and BMI than between LS BMD and BMI. This is in agreement with the results of this study in which the –208 G/A polymorphism of *IL6R* is related to BMI and FN BMD, but not to LS BMD.

In summary, we reported a significant association between polymorphisms of the *IL6R* gene and BMD, a surrogate marker for osteoporosis. We also observed an association with obesity. Although our sample size is intermediate and the statistical power is therefore limited, the results of this study suggest that *IL6R* influences these two phenotypes. It is necessary to replicate these results in other populations and it would be very interesting to perform complementary functional analyses.

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