***WNT3A* gene polymorphisms are associated with bone mineral density variation in postmenopausal mestizo women of an urban Mexican population: findings of a pathway-based high-density single nucleotide screening**

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Abstract Osteoporosis (OP) is a common skeletal disorder characterized by low bone mineral density (BMD) and is a common health problem in Mexico. To date, few genes affecting BMD variation in the Mexican population have been identified. The aim of this study was to investigate the association of single nucleotide polymorphisms (SNPs) located in genes of the Wnt pathway with BMD variation at various skeletal sites in a cohort of postmenopausal Mexican women. A total of 121 SNPs in or near 15 Wnt signaling pathway genes

and 96 ancestry informative markers were genotyped in 425 postmenopausal women using the Illumina GoldenGate microarray SNP genotyping method. BMD was measured by dual-energy X-ray absorptiometry in total hip, femoral neck, Ward's triangle, and lumbar spine. Associations were tested by linear regression for quantitative traits adjusting for possible confounding factors. SNP rs752107 in *WNT3A* was strongly associated with decreased total hip BMD showing the highest significance under the recessive model

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($P=0.00012$). This SNP is predicted to disrupt a binding site for microRNA-149. In addition, a polymorphism of the Wnt antagonist *DKK2* was associated with BMD in femoral neck under a recessive model ($P=0.009$). Several *LRP4*, *LRP5*, and *LRP6* gene variants showed site-specific associations with BMD. In conclusion, this is the first report associating Wnt pathway gene variants with BMD in the Mexican population.

Keywords Bone mineral density · Polymorphisms · Wnt pathway · Postmenopausal Mexican women

Introduction

Osteoporosis (OP) is a common skeletal disorder with high morbidity and mortality characterized by low bone mineral density (BMD), microarchitectural deterioration of bone and increased susceptibility to fractures, especially of the hip, spine, and wrist (NIH Consensus Development Panel on Osteoporosis 2001). This disease is clinically defined by the measurement of bone mineral density (BMD), a widely used predictor for diagnosis of osteoporosis and fracture risk (Duncan and Brown 2010). In the Mexican population, OP is a serious public health problem causing up to 30,000 osteoporotic fractures of the femur or hip every year, with an annual expenditure of about 97 million dollars in health services in 2006. Increased life expectancy in the Mexican population has contributed to a significant increase in the incidence and prevalence of this entity, and it has been estimated that 16 % of women aged above 50 years (1.3 million) are affected with this disease (Clark et al. 2010). Both genetic and environmental factors are known to influence BMD variation, and heritability for this trait has been estimated between 50–80 % (Ralston and de Crombrughe 2006).

Although genome-wide linkage and candidate gene association studies have revealed multiple genetic *loci* related to BMD variation, conflicting results have been observed and associations with several loci have not been replicated in other populations (Wu et al. 2013). Several Wnt pathway genes have been associated with BMD phenotypes, ranging from osteoporosis (Butler et al. 2011) to the high bone mass (HBM) (Boyden et al. 2002). Among these, *LRP5* is recognized as one of the key bone regulatory genes that might influence BMD in the general population, and *LRP5* polymorphisms have been consistently associated with BMD

in different populations (Richards et al. 2008; van Meurs et al. 2008; Rivadeneira et al. 2009). More recently *WNT3A*, known to affect osteoblast differentiation (Monroe et al. 2012), was found to be associated with BMD in Australian postmenopausal women (Sims et al. 2008); a meta-analysis combining five GWAS identified a large linkage disequilibrium (LD) block encompassing *LRP4* associated with femoral neck and lumbar spine BMD (Styrkarsdottir et al. 2008). It has also been suggested that *LRP6* polymorphisms influence BMD (Sims et al. 2008; van Meurs et al. 2008); however, the results have been conflicting (Mencej-Bedrac et al. 2009; Yerges et al. 2010). Recently, Estrada et al. (2012) performed a meta-analysis including data from 17 genome-wide association studies of European and East Asian ancestry, highlighting the critical role of several genes (*WNT16*, *LRP5*, *DKK1*, *LRP4*, *WNT4*, *WNT5*, *SFRP4*, and *MEF2C*) and biological pathways involved in BMD variation.

The majority of studies examining the relationship of genetic factors with BMD variation and OP have been performed in populations of European and Asian ancestry (Liu et al. 2013; Wu et al. 2013). In the Mexican population, specific genetic factors of BMD variation remain largely unknown as only a few candidate gene studies have been reported in reduced samples and/or analyzing few SNPs (Lisker et al. 2003; Gómez et al. 2007; Magaña et al. 2008; Falcón-Ramírez et al. 2011; Rojano-Mejía et al. 2013; Falcón-Ramírez et al. 2013). The aim of the present study was to seek associations of Wnt pathway gene variants with BMD at various skeletal sites in Mexican mestizo postmenopausal women.

Methods and subjects

Sample population

Only women born in Mexico whose parents and grandparents identified themselves as Mexican mestizos were included in the study. A total of 425 postmenopausal women over 45 years of age, without spontaneous menses for at least 1 year, attending in the Instituto Mexicano del Seguro Social (IMSS) located in Cuernavaca, Morelos State, were recruited. All were participating in the third stage of an ongoing, long-term cohort study focusing on lifestyle and chronic

disease. Details of the study cohort, methodology, and participants' baseline characteristics have been reported elsewhere (Lazcano-Ponce et al. 2009; Denova-Gutiérrez et al. 2011). The ethics committees of all participating institutions approved the study protocol and informed consent forms. This is a cross-sectional analysis of the cohort study database.

Bone mass density measurement (assessment)

BMD was measured in total hip (TH), Ward's triangle (WT), femoral neck (FN), and lumbar spine L2–L4 (LS) using a dual-energy X-ray absorptiometry (DXA) Lunar DPX NT instrument (Lunar Radiation Corp., Madison WI). Measurements are presented in grams per square centimeter. T-score was used to analyze BMD data, which is the number of standard deviations (SDs) by which an individual BMD is below that of an average peak BMD in a race and gender-matched population, in accordance with recommendations of the World Health Organization. Standard calibration of instruments was performed daily using the phantom provided by the manufacturer; technicians ensured the daily variation coefficient (VC) was within normal operational standards and that in vivo VC was lower than 1.5 % (Lazcano-Ponce et al. 2009).

Candidate genes and SNP selection

Candidate genes were chosen from genetic pathways related to osteoblast or osteoclast proliferation, differentiation, ossification, communication, and activation in bone-related hormone metabolism or in bone matrix formation, based on previous reports identifying associations with BMD variation and/or OP from candidate gene and GWA studies. SNP selection was based on public information available in dbSNP (Genome Build version 36.2; <http://www.ncbi.nlm.nih.gov>) using the following criteria: minor allele frequency (MAF) >5 % in Caucasians, SNPs with functional relevance, and SNPs previously identified in association studies. To complete the design, TagSNPs were selected with the Tagger program <http://www.broad.mit.edu/mpg/tagger/>; de Bakker et al. 2005), using pairwise tagging with a r^2 threshold of 0.8. A total of 288 SNPs in or near 45 genes involved in the Wnt signaling or *RANK-RANKL-OPG* pathways or located within the *C6orf97/ESR1*, *ESR2*, *CKAP5-LRP4-C11orf49* regions were selected. Finally, 96 ancestry informative markers (AIMs) were included

in the design to rule out false associations due to stratification. These AIMs distinguish mainly Amerindian and European ancestry ($\delta > 0.44$) and have been validated in other study of the Mexican population (Kosoy et al. 2009). The selected SNP design was sent to Illumina (San Diego, CA, USA) and screened through a proprietary algorithm that predicts performance on the Illumina platform.

Genotyping

Genomic DNA was extracted from peripheral blood of all participants, using a commercial isolation kit (QIAGEN systems Inc., Valencia, CA). Genotyping was performed on Illumina BeadStation using the GoldenGate assay. Duplicate control samples were genotyped on each chip, which also served as internal controls for quality of clustering and reproducibility. The primary analysis of the genotyping data with the Illumina GenomeStudio software v.2011.1 was followed by visual inspection and assessment of data quality and clustering. Genotyping accuracy was also assessed by genotype clustering using the Illumina GeneTrain score, which is a measure of the clustering confidence of individual SNP alleles.

Quality control

After genotype calling, the following quality control (QC) criteria were applied using the PLINK software (Purcell et al. 2007), subject and SNP genotyping success rate ≥ 95 %; minor allele frequency ≥ 0.05 % and departure from Hardy–Weinberg equilibrium (HWE) at P value ≥ 0.001 . A total of 374 SNPs and 411 Mexican postmenopausal women meet QC criteria and were further analyzed. One hundred twenty-one of these SNPs were located within or near Wnt pathway genes. The full list of genes and SNPs is described in Supplementary Table S1.

Statistical analysis

Data are described as mean $\pm$ standard deviation for quantitative variables and as absolute and relative frequencies for qualitative variables. Linkage disequilibrium between loci was analyzed with Haploview (Barrett et al. 2005) and statistical analyses were performed using PLINK software (Purcell et al. 2007) and SPSS v20.0 (SPSS, Chicago, IL, USA). P value <0.05 was

accepted as statistically significant. Initially, the 121 SNPs from the Wnt signaling pathway were tested for single-marker allelic association, adjusting for age, body mass index (BMI), blood glucose levels, and ancestry. Alternative inheritance models were evaluated when the single-marker allelic association showed a trend of significance. Logistic regression models were used to evaluate the effect of having one or more risk alleles with BMD, adjusting by the above mentioned confounding factors. Statistical power was calculated with Quanto 1.1 software (Gauderman and Morrison 2006) for a significance level of 0.05 and MAF of 5 % in 425 postmenopausal women with a minimal power of 80 % to detect differences in BMD of 0.020 g/cm² under an additive model. The presence of population stratification was investigated by the principal components analysis (PCA) using the smartpca program in the Eigensoft 3.0 package (Price et al. 2006; Patterson et al. 2006). The significance threshold after multiple test correction for each gene was estimated by considering the effective number of independent marker loci, using the single nucleotide spectral decomposition software (SNPSpD), (Nyholt 2004). This approach can be applied to obtain an effective number of independent marker loci (Meff). The application of SNPSpD to our 121 SNPs sample set gave a Meff of 102 SNPs, which leads to a significance threshold of 0.05/102=0.0005.

Results

Data from 14 individuals whose DNA did not genotype successfully were excluded. In addition, 10 SNPs were removed from further analysis for any of the following motives: monomorphism, call rate below 95 %, minor allele frequency (MAF) less than 5 %, and deviation from Hardy–Weinberg equilibrium. The mean call rate for SNPs after data cleaning exceeded 97.0 %. Data from 11 more participants were excluded because their African ancestry exceeded the mean reported for the Mexican mestizo population (Supplementary Fig. 1). Overall, the gene pool of the study sample showed 57 % Native Amerindian, 35 % European, and 7 % African component, which is similar to that previously reported in mestizos from the state of Guerrero (Silva-Zolezzi et al. 2009).

General characteristics of the study population are described in Table 1. In the initial analysis, BMD was significantly associated with 12 of the 121 SNPs located

Table 1 General characteristics of the study population

Variable	Mean (SD)
<i>N</i>	399
Age (year)	62.23 (9.09)
Weight (kg)	66.13 (11.81)
Height (cm)	153.19 (5.83)
BMI (kg/m ²)	28.17 (4.78)
BMD (g/cm ²)	
Total hip	0.927 (0.133)
Ward triangle	0.708 (0.142)
Femoral neck	0.878 (0.125)
Lumbar spine L2–L4	0.997 (0.151)
Blood glucose (mg/dl)	104.87 (29.21)
Hormone replacement therapy ^a	26.31 % (105)
Tobacco use ^a	33.08 % (132)
Contraceptive therapy ^a	39.34 % (157)
Calcium dietary supplementation ^a	32.51 % (126)

BMI body mass index, BMD bone mineral density

^a Percent (*n*)

in the Wnt pathway (Supplementary Table S1). Four of these SNPs (rs3121310, 4653533, 752107, and 1745420) were located within the *WNT3A* gene (Supplementary Table S2). Linear regression analyses adjusting by age, BMI, blood glucose levels, and ancestry showed that the rs752107 T allele was associated with decreased BMD at all sites tested, with the highest significance under the recessive model. The remainder three *WNT3A* SNPs showed site-specific BMD associations: The T allele of rs4653533 and the C allele of rs1745420 were significantly associated with higher BMD-TH ($P_{\text{dom}}=0.009$ and $P_{\text{add}}=0.004$, respectively) and with higher BMD-FN ($P_{\text{add}}=0.015$ and $P_{\text{add}}=0.016$, respectively) but were not significantly associated with BMD-WT and BMD-LS. Moreover, the AA genotype of rs3121310 was significantly associated with decreased BMD-WT ($P_{\text{rec}}=0.024$) and BMD-LS ($P_{\text{rec}}=0.045$) (Table 2).

Table 3 describes the association of rs419764 within the *DKK2* gene with BMD parameters. The T allele was significantly associated with increased BMD-TH ($P_{\text{add}}=0.030$), BMD-FN ($P_{\text{rec}}=0.009$), and BMD-WT ($P_{\text{rec}}=0.033$) but not with BMD-LS. Moreover, only rs11039024 of the *LRP4* gene showed site-specific BMD associations, associated with decreased BMD-LS under a dominant model ($P=0.048$). The SNPs

Table 2 Association of the *WNT3A* gene with bone mineral density in postmenopausal Mexican women

				Model	β (95 % CI)	<i>P</i>
rs3121310	GG	GA	AA			
	Mean (SD)	Mean (SD)	Mean (SD)			
BMD-TH (g/cm ²)	0.931 (0.134)	0.937 (0.126)	0.914 (0.142)	–	–	NS
BMD-FN (g/cm ²)	0.888 (0.132)	0.881 (0.119)	0.872 (0.122)	–	–	NS
BMD-WT (g/cm ²)	0.730 (0.159)	0.718 (0.138)	0.697 (0.140)	Recessive	–0.36 (–0.068; –0.005)	0.024
				Additive	–0.022 (–0.039; –0.005)	0.012
BMD-LS (g/cm ²)	1.012 (0.153)	1.005 (0.143)	0.973 (0.168)	Recessive	–0.038 (–0.076; –0.001)	0.045
				Additive	–0.020 (–0.040; –0.001)	0.057
rs4653533	CC	CT	TT			
BMD-TH (g/cm ²)	0.923 (0.132)	0.945 (0.127)	1.040 (0.107)	Dominant	0.033 (0.008; 0.057)	0.009
				Recessive	0.076 (0.011; 0.141)	0.022
				Additive	0.032 (0.011; 0.053)	0.003
BMD-FN (g/cm ²)	0.875 (0.122)	0.889 (0.127)	0.987 (0.108)	Dominant	0.025 (0.002; 0.048)	0.035
				Recessive	0.061 (0.00016; 0.123)	0.051
				Additive	0.024 (0.005; 0.044)	0.015
BMD-WT (g/cm ²)	0.715 (0.145)	0.721 (0.147)	0.810 (0.132)	–	–	NS
BMD-LS (g/cm ²)	1.002 (0.155)	0.991 (0.142)	1.084 (0.120)	–	–	NS
rs752107	CC	CT	TT			
BMD-TH (g/cm ²)	0.937 (0.132)	0.936 (0.126)	0.890 (0.143)	Recessive	–0.063 (–0.095; –0.031)	0.00012
				Additive	–0.020 (–0.036; –0.005)	0.010
BMD-FN (g/cm ²)	0.887 (0.130)	0.885 (0.117)	0.849 (0.121)	Recessive	–0.056 (–0.087; –0.026)	0.00028
				Additive	–0.019 (–0.033; –0.004)	0.012
BMD-WT (g/cm ²)	0.727 (0.152)	0.720 (0.138)	0.683 (0.145)	Recessive	–0.063 (–0.100; –0.026)	0.001
				Additive	–0.023 (–0.041; –0.006)	0.010
BMD-LS (g/cm ²)	1.011 (0.152)	1.009 (0.149)	0.943 (0.150)	Recessive	–0.077 (–0.121; –0.034)	0.001
				Additive	–0.027 (–0.048; –0.006)	0.011
rs1745420	GG	GC	CC			
BMD-TH (g/cm ²)	0.923 (0.132)	0.940 (0.127)	1.040 (0.107)	Dominant	0.030 (0.007; 0.053)	0.012
				Recessive	0.076 (0.011; 0.141)	0.022
				Additive	0.030 (0.010; 0.050)	0.004
BMD-FN (g/cm ²)	0.876 (0.123)	0.886 (0.125)	0.987 (0.108)	Dominant	0.023 (0.001; 0.045)	0.040
				Recessive	0.061 (0.0001; 0.123)	0.051
				Additive	0.023 (0.004; 0.043)	0.016
BMD-WT (g/cm ²)	0.715 (0.146)	0.719 (0.146)	0.810 (0.132)	–	–	NS
BMD-LS (g/cm ²)	1.002 (0.153)	0.992 (0.149)	1.084 (0.120)	–	–	NS

Values are mean and standard deviation (*SD*) for bone mineral densities (g/cm²). *P* values were adjusted by age, BMI, blood glucose levels, and ancestry

BMD-TH total hip bone mineral density, *BMD-FN* femoral neck bone mineral density, *BMD-WT* Ward's triangle bone mineral density, *BMD-LS* lumbar spine bone mineral density

(*LRP5*) rs638051 was associated with increased BMD-TH under a recessive model ($P=0.048$), rs599083 was associated with increased BMD-FN ($P_{\text{dom}}=0.012$) and

BMD-WT ($P_{\text{dom}}=0.045$), and rs7125942 (*LRP5*) was associated with increased BMD-FN ($P_{\text{dom}}=0.021$). Finally, rs1181334 (*LRP6*) was associated with

Table 3 Association of the *DKK2* gene with bone mineral density in postmenopausal Mexican women

rs419764	CC	CT	TT	Model	β (95 % CI)	<i>P</i>
	Mean (SD)	Mean (SD)	Mean (SD)			
BMD-TH (g/cm ²)	0.928 (0.128)	0.942 (0.140)	0.926 (0.159)	Dominant	0.024 (0.00048; 0.047)	0.045
				Additive	0.022 (0.002; 0.042)	0.030
BMD-FN (g/cm ²)	0.880 (0.122)	0.884 (0.130)	0.917 (0.152)	Recessive	0.077 (0.019; 0.136)	0.009
				Additive	0.024 (0.005; 0.042)	0.014
BMD-WT (g/cm ²)	0.718 (0.142)	0.717 (0.153)	0.752 (0.190)	Recessive	0.077 (0.006; 0.148)	0.033
BMD-LS (g/cm ²)	1.002 (0.152)	1.000 (0.155)	1.022 (0.132)	–	–	NS

Values are mean and standard deviation (SD) for bone mineral densities (g/cm²). *P* values were adjusted by age, BMI, blood glucose levels and ancestry

BMD-TH total hip bone mineral density, *BMD-FN* femoral neck bone mineral density, *BMD-WT* Ward's triangle bone mineral density, *BMD-LS* lumbar spine bone mineral density

decreased BMD-TH under an additive model ($P=0.014$) (Table 4). After correction for multiple testing, only the association of BMD with the rs752107 polymorphism remained significant ($P=0.0005$).

Discussion

The Wnt signaling pathway is crucial in growth and maintenance of several organs and tissues including bone (Monroe et al. 2012). Mutations in *WNT3A* (c.152A>G, p.K51R) and *DKK1* (c.359G>T, p.R120L) have been shown to cause childhood-onset primary osteoporosis, manifested as reduced bone mineral density, peripheral fractures, and/or vertebral compression fractures (Korvala et al. 2012). Moreover, common polymorphisms in genes belonging to this pathway have been nominally associated with BMD in Australian postmenopausal women using an extreme phenotype approach (Sims et al. 2008). In the current study, we replicated the association of *WNT3A* and *DKK2* polymorphisms with BMD in the Mexican population, providing further evidence of the role of this pathway in BMD variation. In agreement with the observation of Sims et al. (2008), the T allele of rs752107 in *WNT3A* was consistently associated with BMD at all skeletal sites tested in Mexican postmenopausal women. The T allele frequency in Mexican mestizo women was 33 %, similar to that reported in Caucasians (30 %) and Mexican Americans (29 %) (www.hapmap.org) but higher than that observed in Australian women.

Recently, association analyses identified three polymorphisms within 3'-UTRs of mRNAs that were

significantly associated with femoral neck BMD. Further functional studies, showed that these polymorphisms may contribute to susceptibility to BMD variation, most likely through their effects on altered binding affinity of microRNAs (miRNAs) (Lei et al. 2011). Mirsnpscore (<http://www.bigr.medisin.ntnu.no/mirsnpscore>) and MirSNP databases (<http://cmbi.bjmu.edu.cn/mirsnp>) predicted that rs752107 disrupt a binding site for miR-149. This miRNA has been reported to be highly expressed in osteoarthritis samples, a disease with a high BMD phenotype (Díaz-Prado et al. 2012). It can be speculated that miR-149 may bind tightly to WNT3A mRNA transcripts containing the C allele, and thus negatively regulate WNT3A protein translation. Conversely, the presence of the T allele may break the miRNA binding site leading to increased WNT3A protein translation. Higher WNT3A protein levels would be expected to negatively regulate osteoblast proliferation and differentiation (Qiu et al. 2011; Hu et al. 2013), decreasing bone formation and BMD. Thus, women with the TT genotype would be expected to have lower BMD than women with the CC genotype, consistent with the association observed in the present study (Table 2). Further studies are required to elucidate the molecular mechanism by which this SNP and miR-149 regulate BMD variation in the Mexican postmenopausal women and to determine whether rs752107 is in linkage disequilibrium with other functional polymorphisms.

Interestingly, rs4643533, rs3121310, and rs1745420 in *WNT3A* showed site-specific effects on BMD. Although rs4653533 was previously associated with

Table 4 Bone mineral density in postmenopausal Mexican women according to genotype for *LRP4*, *LRP5*, and *LRP6* polymorphisms

				Model	β (95 % CI)	<i>P</i>
<i>LRP4</i> gene						
rs10838635	AA	AG	GG			
	Mean (SD)	Mean (SD)	Mean (SD)			
BMD-TH (g/cm ²)	0.924 (0.132)	0.934 (0.134)	0.937 (0.129)	–	–	NS
BMD-FN (g/cm ²)	0.885 (0.119)	0.880 (0.128)	0.883 (0.125)	–	–	NS
BMD-WT (g/cm ²)	0.718 (0.149)	0.719 (0.145)	0.719 (0.147)	–	–	NS
BMD-LS (g/cm ²)	1.011 (0.149)	0.996 (0.158)	1.005 (0.143)	–	–	NS
rs11039024	CC	CT	TT			
BMD-TH (g/cm ²)	0.923 (0.134)	0.932 (0.130)	0.945 (0.133)	–	–	NS
BMD-FN (g/cm ²)	0.884 (0.121)	0.877 (0.128)	0.891 (0.123)	–	–	NS
BMD-WT (g/cm ²)	0.716 (0.147)	0.718 (0.148)	0.727 (0.143)	–	–	NS
BMD-LS (g/cm ²)	1.015 (0.153)	0.991 (0.155)	1.005 (0.142)	Dominant	–0.031 (–0.061; –0.00024)	0.048
rs6485702	TT	TC	CC			
BMD-TH (g/cm ²)	0.937 (0.128)	0.930 (0.135)	0.930 (0.131)	–	–	NS
BMD-FN (g/cm ²)	0.878 (0.131)	0.879 (0.124)	0.893 (0.120)	–	–	NS
BMD-WT (g/cm ²)	0.720 (0.147)	0.716 (0.145)	0.725 (0.149)	–	–	NS
BMD-LS (g/cm ²)	0.999 (0.149)	0.998 (0.155)	1.015 (0.151)	–	–	NS
<i>LRP5</i> gene						
rs638051	GG	GA	AA			
BMD-TH (g/cm ²)	0.926 (0.124)	0.928 (0.142)	0.953 (0.127)	Recessive	0.030 (0.00019; 0.059)	0.048
BMD-FN (g/cm ²)	0.887 (0.120)	0.872 (0.130)	0.895 (0.121)	–	–	NS
BMD-WT (g/cm ²)	0.723 (0.146)	0.709 (0.151)	0.736 (0.140)	–	–	NS
BMD-LS (g/cm ²)	1.017 (0.153)	0.981 (0.146)	1.021 (0.159)	–	–	NS
rs599083	TT	TG	GG			
BMD-TH (g/cm ²)	0.929 (0.129)	0.938 (0.135)	0.924 (0.138)	–	–	NS
BMD-FN (g/cm ²)	0.872 (0.123)	0.897 (0.125)	0.889 (0.125)	Dominant	0.026 (0.006; 0.046)	0.012
BMD-WT (g/cm ²)	0.709 (0.141)	0.736 (0.149)	0.714 (0.165)	Dominant	0.025 (0.001; 0.049)	0.045
BMD-LS (g/cm ²)	0.996 (0.157)	1.022 (0.140)	0.974 (0.149)	–	–	NS
rs7125942	CC	CG	GG			
BMD-TH (g/cm ²)	0.930 (0.132)	0.942 (0.141)	0.885 (0.057)	–	–	NS
BMD-FN (g/cm ²)	0.879 (0.125)	0.906 (0.123)	0.865 (0.056)	Dominant	0.035 (0.005; 0.065)	0.021
				Additive	0.028 (0.001; –0.055)	0.039
BMD-WT (g/cm ²)	0.716 (0.145)	0.741 (0.163)	0.705 (0.047)			NS
BMD-LS (g/cm ²)	1.000 (0.153)	1.016 (0.149)	1.005 (0.157)	–	–	NS
<i>LRP6</i> gene						
rs1181334	GG	GT	TT			
BMD-TH (g/cm ²)	0.940 (0.131)	0.886 (0.133)	0.880 (0.042)	Dominant	–0.037 (–0.066; –0.007)	0.014
				Additive	–0.035 (–0.063; –0.007)	0.014
BMD-FN (g/cm ²)	0.887 (0.126)	0.857 (0.116)	0.815 (0.021)	–	–	NS
BMD-WT (g/cm ²)	0.725 (0.147)	0.686 (0.143)	0.700 (0.014)	–	–	NS
BMD-LS (g/cm ²)	1.006 (0.154)	0.981 (0.145)	0.950 (0.014)	–	–	NS

Values are mean and standard deviation (*SD*) for bone mineral densities (g/cm²). *P* values were adjusted by age, BMI, blood glucose levels, and ancestry

BMD-TH total hip bone mineral density, *BMD-FN* femoral neck bone mineral density, *BMD-WT* Ward's triangle bone mineral density, *BMD-LS* lumbar spine bone mineral density

BMD (Sims et al. 2008), to our knowledge, the two latter SNPs have not been previously associated with BMD variation or osteoporosis. Overall, the magnitude of the effects of *WNT3A* polymorphisms on BMD is relatively small, as expected for the role of common polymorphisms in complex traits.

The present study also confirmed the association of *LRP4*, *LRP5*, and *LRP6* gene variations with BMD as previously observed in GWAS and meta-analyses in individuals of European ancestry (Styrkarsdottir, et al. 2008; Richards et al. 2008). However, although the nonsynonymous rs3736228 in *LRP5* and rs2302685 in *LRP6* are the most frequently studied SNPs in candidate gene studies (van Meurs et al. 2008; Tran et al. 2008; Lee et al. 2009), they were not associated with BMD in Mexican women. This lack of association is in accordance with the report of Falcón-Ramírez et al. (2013), who analyzed rs3736228, rs4988321, rs627174, and rs901824 in the *LRP5* gene in a small case–control study of unrelated Mexican women.

The Mexican population is admixed with a complex genetic structure including genes of Native American (51 %), European (45.4 %), and African origin (3.7 %) (Price et al. 2007; Silva-Zolezzi et al. 2009). Because population stratification may be a source of spurious associations (Cardon and Palmer 2003), we used two different approaches to detect population stratification. Principal component analyses (Patterson et al. 2006) showed that the vast majority of women (98 %) were clustered together; the inflation factor as estimated by Genomic Control (Devlin and Roeder 1999) was $\lambda = 1.005$. This combined evidence suggests that population stratification was most likely not a confounding factor in this study.

It is noteworthy that most SNPs previously associated with BMD in European populations were not replicated in this study or in previous studies in the Mexican population. Potential reasons for this lack of association include differences in study methods and phenotype data, different genotyping methods and SNP genotype quality control, racial/ethnic differences in BMD, and insufficient sample size (Delezé et al. 2000; Nam et al. 2013; Wu et al. 2013). Among other limitations, this study was based on a cross-sectional design, and the SNPs were selected from previous reports mainly in European populations. Moreover, it must be pointed out that only the association of rs752107 with BMD remained significant after correction for multiple testing, probably because of the reduced sample size and the

elevated number of SNPs tested. Further fine mapping and confirmation studies are needed in Mexican population to determine the main associated variants.

In conclusion, this is the first report associating Wnt pathway gene variants with BMD in the Mexican population. Further cross-sectional and longitudinal studies are required to further analyze complex genomic architecture of BMD variation and osteoporosis in the Mexican population.

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