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Association of LRP5 genotypes with osteoporosis in Tunisian post-menopausal women

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Abstract

Background: Osteoporosis is a highly heritable trait. Among the genes associated with bone mineral density (BMD), the low-density lipoprotein receptor-related protein 5 gene (*LRP5*) has been consistently identified in Caucasians. However *LRP5* contribution to osteoporosis in populations of other ethnicities remains poorly known.

Methods: To determine whether *LRP5* polymorphisms Ala1330Val and Val667Met are associated with BMD in North Africans, these genotypes were analyzed in 566 post-menopausal Tunisian women with mean age of 59.5 ± 7.7 years, of which 59.1% have low bone mass (T-score < -1 at spine or hip).

Results: In post-menopausal Tunisian women, 1330Val was weakly associated with reduced BMD T-score at lumbar spine ($p = 0.047$) but not femur neck. Moreover, the TT/TC genotypes tended to be more frequent in women with osteopenia and osteoporosis than in women with normal BMD ($p = 0.066$). Adjusting for body size and other potential confounders, *LRP5* genotypes were no longer significantly associated with aBMD at any site.

Conclusions: The less common Val667Met polymorphism showed no association with osteoporosis. The Ala1330Val polymorphism is weakly associated with lower lumbar spine bone density and osteopenia/osteoporosis in postmenopausal Tunisian women. These observations expand our knowledge about the contribution of *LRP5* genetic variation to osteoporosis risk in populations of diverse ethnic origin.

Keywords: *LRP5*, Ala1330Val, Val667Met, Lumbar spine BMD, Fractures, Post-menopausal Tunisian population

Background

Osteoporosis is characterized by low bone mineral density (BMD) and micro architecture deterioration of bone tissue, leading to an increased risk of fractures that occur mostly at the hip, spine and wrist [1]. It is increasingly becoming a serious public-health issue because of the morbidity and mortality associated with the disease [2]. Hence, the identification and the management of women at highest risk of osteoporosis is a main concern.

Bone mineral density (BMD) is a primary predictor of osteoporotic fracture [3], and is used as a surrogate definition of osteoporosis [4]. BMD changes with age, peak levels being reached between the age of 20 and 30

and then decreasing during the later decades of life [5]. The bone mass acquired by adulthood varies from one population to another and is determined, essentially, genetically. In fact, genetic factors account for 50% to 80% of the interindividual variations in bone mineral density (BMD) [6-8].

To date, many genome-wide association studies (GWAS) were performed and multiple genes were identified to be associated with BMD and/or fragility fractures, including the low-density lipoprotein receptor-related protein 5 (*LRP5*) gene and other genes in the Wnt signalling pathway [9], like *CTNNA1*, and several genes in the RANKL/RANK signalling pathway, such as *TNFRSF11A* [10,11]. The Wnt signaling pathway has been shown to be related to bone mass and metabolism [12,13]. In osteoblasts, *LRP5* can transduce canonical signals to promote renewal of stem cells, stimulation of pre-osteoblast replication, induction of osteoblastogenesis, and inhibition of osteoblast and osteocyte apoptosis by increasing the levels of β -

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catenin and altering gene expression through the *Lef/Tcf* transcription factors [14]. It is known that *LRP5* is essential for normal morphology, development processes, mechano-sensation and formation of bone [15]. Mapped on chromosome 11q12–14, activating mutations in the *LRP5* gene are responsible for rare conditions with a “high bone mass phenotype” [13,16] as well as autosomal dominant osteopetrosis type 1 [17]. Conversely, inactivating mutations are responsible for the osteoporosis pseudoglioma syndrome [12]. In addition to these mutations, a number of polymorphisms were described in the *LRP5* gene (<http://www.ncbi.nlm.nih.gov/SNP>). One of these, the Ala1330Val (rs3736228, exon 18) polymorphism, was associated with attenuated bone gain in prepubertal boys [18], decreased BMD in Japanese [19], American [20], Australian [21], and Dutch populations [22]. It was also associated with an increased risk of fragility fractures in postmenopausal Australian women [21] and in elderly Dutch men [22]. Similarly, the Val667Met (rs4988321, exon 9) polymorphism was associated with attenuated bone gain in prepubertal boys but not in girls participating in a 1-year longitudinal study [18]. In the same study, this polymorphism was also associated with decreased lumbar spine bone mineral content and projected bone area and there was a trend toward lower BMD in the spine [18].

The implication of Ala1330Val and Val667Met polymorphisms in BMD variation and risk of post-menopausal osteoporosis may differ between populations of various origins. However in North Africa, no association study was reported. To investigate whether allelic variations of the *LRP5* gene contribute to bone mass and osteoporosis in Tunisian population, we studied the association of Ala1330Val and Val667Met polymorphisms with BMD variation and osteoporosis risk in 566 Tunisian post-menopausal women.

Methods

Study population

Five hundred and sixty six postmenopausal Tunisian women, all with Tunisian ancestry, were recruited for osteoporosis consultation from the Rabta and Charles Nicolles Hospitals of Tunis. Women were prospectively recruited and included in the study regardless of their BMD values. Subjects with a history of bone disease, metabolic or endocrine diseases, hormone replacement therapy, and a corticotherapeutical history were excluded. Our study was approved by the National ethic committee and all participants provided written informed consent.

Clinical measurements

The BMD was measured for the lumbar spine (L2-L4) and femoral neck (dual femur) by dual energy X-ray absorptiometry (DXA) using a GE-Lunar PRODIGYTM device.

The prevalence of clinical fractures was estimated by obtaining a fracture history for each subject including the age at the time of fracture, the site of fracture and the trauma intensity. Only fractures caused by low impact were included. All the characteristics of the studied population as age, anthropometric and obstetric parameters are shown in Table 1. The osteodensitometric results of our population are summarized in Table 2 and the classification of osteopenian and osteoporosis women were made according to T-SCORE < -1, respectively <2.5 at spine or hip.

SNP genotyping

Genomic DNA was extracted from blood by Phenol Chloroform procedure. Ala1330Val and Val667Met SNPs were genotyped by the pyrosequencing method, consisting of a series of four enzymatic steps resulting in sequencing a short region that include the SNP [23]. The specific primers used in the PCR are reported by Ferrari et al study [18].

Statistical analysis

Hardy-Weinberg equilibrium was tested for each SNP by use of the standard χ^2 test. Data are represented as means \pm SD. The categorical variables were compared between genotypes using the χ^2 test. The associations between polymorphisms and BMD were assessed using Student *t* test. Due to the extremely low frequency (<10%) of the minor alleles, all analysis were performed under the assumption of a dominant model (combining homozygote for the minor allele and heterozygote in one group). Logistic regression models were applied, after controlling for several potential covariates (age, weight, height, menopause age, menarche age, parity and fractures). Association study and logistic regression were examined by SPSS software.

Results

Our study comprised 566 Tunisian post-menopausal women with a mean age of 59.5 ± 7.7 years (Table 1).

Table 1 Characteristics of the studied Tunisian post menopausal women

Variable	Mean \pm SD	n
Age (yr)	59.5 \pm 7.7	
weight (kg)	72.0 \pm 12.7	
BMI	28.9 \pm 5.0	
Age at menarche (yr)	12.8 \pm 1.6	
Age at menopause (yr)	48.4 \pm 4.5	
Parity	3.4 \pm 2.0	
Fracture		55
No fracture		511

Table 2 Mean BMD by WHO diagnostic group

Variable	Mean ± SD		
	Osteoporotic n = 141	Osteopenic n = 194	Normal n = 231
LS BMD (g/cm ²)	0.695 ± 0.065	0.827 ± 0.070	0.997 ± 0.092
RFN BMD (g/cm ²)	0.769 ± 0.103	0.843 ± 0.096	0.971 ± 0.101
LFN BMD (g/cm ²)	0.775 ± 0.096	0.837 ± 0.092	0.966 ± 0.113

LS, lumbar spine, RFN, right femur neck, LFN, left femur neck.

59.1% had low BMD (24.9% osteoporotic and 34.1% osteopenic (Table 2). Among them, 55 women had a history of low trauma after the age of 45 years (Table 1). Val667Met is a G/A substitution in exon 9, with genotype frequencies GG: 88.0%, GA: 11.7%, and AA: 0.4%. Ala1330Val is a C/T substitution in exon18 with genotype frequencies CC: 79.9%, CT: 18.9%, and TT: 1.2%. The genotype frequencies of the 2 SNPs, studied in our population, did not significantly deviate from Hardy Weinberg equilibrium ($\chi^2 = 0.54$ and 0.88 respectively for Val667Met and Ala1330Val). Association analysis are summarized in Table 3. A significant difference in LS BMD T-scores was found between CC and TC/TT genotypes at spine ($p = 0.047$), but not at hip. Moreover, the TT/TC genotypes tended to be more frequent in women with osteopenia and osteoporosis than in normal women

($p = 0.066$) (Table 3). In contrast, the Val667Met genotypes were not associated with aBMD and their distribution was similar between normal and osteoporotic/osteopenic women. Distribution of *LRP5* genotypes did not differ between women with and without fragility fractures (Table 3).

To further evaluate the independent contribution of *LRP5* polymorphisms to BMD in our population, we performed a logistic regression analysis including age, weight, age at menarche, age at menopause, parity, fractures and *LRP5* genotypes. As shown in Table 4, Age ($p = 0.001$) and weight ($p = 0.0001$), were the most significant contributors to BMD, followed by a non-significant contribution of parity ($p = 0.073$), and A1330Val genotypes ($p = 0.11$).

Discussion

For Tunisian women, a lumbar and femoral BMD analysis showed that their average values are close to those of the Asian and Caucasian women. At femoral neck, the lowest values were noted in European population. The osteoporosis prevalence was higher in Asian than in Tunisian and Caucasian population [24]. The differences observed between various populations can be caused by many environmental factors such as diet, physical activity, smoking alcohol and the geographical

Table 3 Association of LRP5 genotypes with BMD^a

Parameter	Polymorphisms					
	Val667Met			Ala1330Val		
	GG	GA + AA	P	CC	CT + TT	P
	n = 498	n = 68	value ^{b, e, f}	n = 452	n = 114	value ^b
Osteopenia ^c	87.5	12.5		77.6	22.4	
+osteoporosis (%) ^d						
Normals (%)	88.7	11.3	0.373	83.1	16.9	0.066
fractured women (%)	89.1	10.9		85.5	14.5	
Non fractured women (%)	87.9	12.1	0.499	79.3	20.7	0.182
BMD spine (g/cm ²)	0.869 ± 0.144	0.854 ± 0.134	0.438	0.873 ± 0.145	0.841 ± 0.134	0.072
Tscore spine	-1.085 ± 1.339	-1.327 ± 1.305	0.170	-1.044 ± 1.338	-1.388 ± 1.297	0.047*
BMD right hip (g/cm ²)	0.880 ± 0.127	0.872 ± 0.136	0.62	0.882 ± 0.127	0.867 ± 0.131	0.359
Tscore right hip	-0.451 ± 1.066	-0.648 ± 1.225	0.176	-0.433 ± 1.060	-0.639 ± 1.183	0.164
BMD left hip (g/cm ²)	0.877 ± 0.129	0.870 ± 0.127	0.685	0.879 ± 0.129	0.865 ± 0.127	0.448
Tscore right hip	-0.514 ± 1.019	-0.635 ± 1.131	0.380	-0.499 ± 0.999	-0.645 ± 1.155	0.350
BMD total hip	0.857 ± 0.120	0.851 ± 0.132	0.823	0.859 ± 0.120	0.847 ± 0.126	0.430
Tscore total hip	-0.652 ± 1.001	-0.801 ± 1.182	0.522	-0.635 ± 0.994	-0.807 ± 1.135	0.232

^aContinuous variables (BMD values) are expressed as mean standard deviation, and categorical variables (osteopenia, osteoporosis, presence osteoporotic fracture) are expressed as percentages (%).

^bBased on Student t test for continuous variables.

^cWorld Health Organization definition: 1 standard deviation \geq T score \leq 2.5 standard deviation (29).

^dWorld Health Organization definition: T score \leq 2.5 standard deviation (29).

^eBased on χ^2 test between two groups OP + O and Normales.

^fBased on χ^2 test between two groups fractured and non fractured women.

* $p \leq 0,05$ significant in bold character.

Table 4 Logistic regression analysis of spine

Variable	B	t	P value
Age (yr)	-0.003	-3.471	0.001*
Weight (kg)	0.005	4.522	0.000
Age at menarche (yr)	-0.005	-1.71	0.942
Age at menopause (yr)	-0.002	-1.197	0.232
Parity	0.005	1.798	0.073
Fracture	-0.026	-1.333	0.183
Ala1330Val genotype	0.032	1.57	0.117

* $p \leq 0,05$ significant in bold character.

variability of vitamin D status. However, genetic determinism may also contribute to those differences.

During the past two decades, it has become clear that many genes contribute to the variation in BMD in the general population; however, candidate gene association studies have led to conflicting and contradictory findings [25]. Nevertheless, there is clear evidence for the association between genetic variation of *LRP5* and BMD in the general Caucasian and Chinese populations [18,19,21,26-30]. In African and North African populations, no information seems to be available about *LRP5* and BMD variation.

In our Tunisian post-menopausal women, *LRP5* polymorphisms frequencies were similar with previous studies reported in women with Caucasian background [22,31,32], but different from those observed in Asian population [19]. It should be noted that the association of the Ala1330Val variant with BMD and osteoporosis/osteopenia was in the same direction and had similar effect sizes in our study as in Europeans and Asians. However the absence of an association between BMD and the Val667Met variant in our population was distinct from previously consistent associations in other ethnic groups. This difference could represent a truly different genetic determination of osteoporosis risk between ethnicities or be due to a false negative result in our limited sample of Tunisian women, considering that the A allele is quite rare (6.1% < 10%).

Based on the physiological role of *LRP5* in bone biology, Val667Met and Ala1330Val should, in principle, result in decreased Wnt signaling. Val667Met resides at the top of the third propeller module in the extracellular domain of the receptor, a domain that is thought to interact with the Wnt-inhibitor Dkk1. Therefore, it is possible that the Val667Met mutant may have a higher binding affinity for Dkk1 as compared with the wild-type protein [33]. Ala1330Val is located within the second low-density lipoprotein (LDL) domain. Although the exact molecular mechanism remains to be clarified, the Ala1330Val mutant was shown to have a significantly reduced Wnt-signaling capacity as compared with the

wild type molecule in two independent TCF-Lef reporter assays [31,34].

The Ala1330Val seemed to be associated with lower spine bone density in post-menopausal Tunisian women. In fact, the TT/TC genotype could be more frequent in osteoporotic and osteopenic compared to women with normal BMD ($p = 0.066$). However the effect of this variant was detected only at the lumbar spine and not at the hip. Our results are concordant with those found in Asian and Caucasian populations. Thus Bich et al. [25] have demonstrated by a Bayesian meta-analysis that the A1330V variant was mainly associated with lumbar spine BMD in Asian populations, whereas in Caucasian populations associations occurred at both the lumbar spine and femoral neck [25,35,36]. The association of A1330V with lumbar spine than with femoral neck BMD is consistent with recent genome-wide association studies [11] and may reflect biological differences between the sites, a lower heritability for the femoral neck site [37,38], or higher measurement error at this site.

Richards et al. [11] noted that the *LRP5* polymorphisms were also significantly associated with fracture risk. But no significant difference was found between fractured and non-fractured women in our population. Considering the small number of fractured women in our sample ($n = 55$), our study is likely to be underpowered to detect such an association.

The logistic regression analysis for all the parameters including *LRP5* genotypes reported a significant effect of age and weight, while the association with Ala1330Val genotypes was reduced. Since body size is strongly correlated with bone size, and therefore aBMD, this observation suggests that the association of *LRP5* polymorphisms with aBMD is at least partly mediated through *LRP5* effects on bone size. This hypothesis is further supported by a previous study in Swiss young boys and girls in whom *LRP5* polymorphisms were associated with decreased lumbar spine bone mineral content (BMC) and projected bone area [18].

Conclusions

In conclusions, the Ala1330Val variant seems to be weakly associated with lower lumbar spine BMD in Tunisian post-menopausal women, contributing to the notion that *LRP5* genetic variation is a ubiquitous determinant of osteoporosis risk among various ethnicities. More investigation about the interaction of *LRP5* polymorphisms and other factors notably hormonal, environmental and nutritional as well as physical activity are needed. These latter factors obviously vary from one individual to the next and from one population to the other, which can further influence the contribution of *LRP5* polymorphisms to BMD in diverse ethnic groups.

Competing interests

We wish to confirm that there are no known competing interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Authors' contributions

RS conceived and performed the molecular analysis and wrote the manuscript. HS is involved in clinical measurement. HEM and CS are involved in technical support and assistance. BZ performed statistical analysis. ABE, SS and SLF designed the study. All authors read and approved the final manuscript.

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