Polymorphisms in VDR gene in Tunisian postmenopausal women are associated with osteopenia phenotype

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Key words: VITAMIN D RECEPTOR, VDR, ApaI, TaqI, LUMBAR SPINE BONE MINERAL DENSITY, FEMORAL BONE MINERAL DENSITY, VERTEBRAL FRACTURES, OSTEOPENIA, OSTEOPOROSIS, POSTMENOPAUSAL TUNISIAN POPULATION

ABSTRACT

Objectives Osteopenia is characterized by intermediate values of bone mineral density (BMD) as compared to normal and osteoporotic subjects. BMD, a surrogate phenotype for osteoporosis, is influenced in part by genetic factors. Among the genes associated with BMD, the vitamin D receptor (VDR) was the first gene studied as a potential candidate associated with BMD in adult and postmenopausal bone loss. However, results are controversial.

Methods To determine whether VDR polymorphisms ApaI and TaqI are associated with BMD, osteopenia, osteoporosis and low-impact fracture risk in North Africans, these genotypes were analyzed in 566 postmenopausal Tunisian women.

Results In postmenopausal Tunisian women, the GT ApaI genotype seems to be protective against osteoporosis development ($p = 0.02$; odds ratio = 0.54). Moreover, the presence of the combined GT/TT genotype of ApaI and TaqI polymorphisms is more frequent in normal BMD women than in osteoporotic women ($p = 0.00$; odds ratio = 0.41). Interestingly, the GG ApaI genotype is associated with osteopenia development ($p = 0.02$; odds ratio = 1.86) and also the TT TaqI polymorphism ($p = 0.02$; odds ratio = 1.53). The GG ApaI genotype is associated with a three times risk of vertebral fracture.

Conclusions The ApaI polymorphism showed an association with osteopenia and low-impact vertebral fracture incidence but not with osteoporosis. The TaqI polymorphism is associated specifically with the osteopenia phenotype. The presence of the two polymorphisms increases the risk to develop osteopenia in postmenopausal Tunisian women. Osteopenia seems to be genetically determined. However, osteoporosis is the result of interaction between genetic and environmental factors.

BACKGROUND

Osteoporosis is defined as bone fragility often revealed though low-impact fractures and confirmed measurement of bone mineral density (BMD). This feature remains the single most clinically useful risk factor for osteoporotic fracture and is the metric on which most therapeutic decisions are based[1,2]. Therefore, it is often used as a surrogate phenotype for osteoporosis[3]. A clinical situation defined as osteopenia is characterized by intermediate BMD values as compared to normal and osteoporotic values. It is accepted that osteopenia is transient and it is expected that osteopenic subjects would mostly evolve toward clear osteoporosis. As it is known, osteoporosis is a multifactor disease that happens frequently in women during postmenopause and that is under the effect of several behavioral factors such as calcium intake or parity that could influence BMD. However, this quantitative trait could be determined as well by genetic factors[4–8].

The number of candidate genes associated with BMD and consequently with osteoporosis is large, ranging from those

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ORIGINAL ARTICLE

DOI: 10.3109/13697137.2015.1007123

Received 11-12-2014
Accepted 08-01-2015

regulating calcium homeostasis to the several locally involved genes in bone cell recruitment and activity. The vitamin D receptor (VDR) was the first gene studied as a potential candidate associated with BMD in adult and postmenopausal bone loss[9,10]. Several association studies between VDR alleles, BMD and fractures have subsequently been performed with conflicting results.

The vitamin D endocrine system is pleiotropic and plays an important role in bone metabolism. The effect of vitamin D is mediated through binding to its receptor VDR. The VDR is a nuclear transcription factor that regulates gene expression by interacting with vitamin D response elements in target genes, inducing skeletal cell stimulation and bone turnover regulation[3,11]. The VDR gene is localized on 12q12–14, is composed of 11 exons, and is approximately 75 kb in length[11]. Several polymorphisms in the VDR gene have already been reported. Those in the 3' end region have been the first and the most extensively studied genetic markers in relation to BMD in adult women and postmenopausal bone loss[9,10,12–14]. The previous studies have also identified two other adjacent restriction fragment length polymorphisms for ApaI and TaqI. The ApaI site is located in intron 8 and displays a G/T transversion that does not affect any splicing site and/or transcription factor binding site. The TaqI site, which is localized in exon 9, presents a T to C transition with no change of the amino acid sequence of the encoded protein[12]. The ApaI and TaqI polymorphisms have also been very commonly studied and have shown association with BMD variations. These controversial results in terms of association with osteoporosis could be due to the impact of different populations, since the combination of risk factors could vary according to the sample or to the considered population. In the current study, we have investigated the association between the VDR gene TaqI and ApaI polymorphisms with BMD, fracture and osteoporotic and osteopenic status in a population of postmenopausal Tunisian women. Considering such well-defined groups according to genotypes, our results suggest that osteopenia should probably be considered in terms of risk factors as a specific physiopathological entity.

METHODS

Study population

A total of 566 postmenopausal women, all with Tunisian ancestry, were recruited for osteoporosis consultation from the Rabta and Charles Nicolles Hospitals of Tunis and were classified according to World Health Organization criteria as osteoporotic ($n = 141$), osteopenic ($n = 194$) and normal ($n = 231$). BMD measurements at the lumbar (L2–4) spine and total hip were obtained by dual-energy X-ray absorptiometry. Detailed interviews were performed. Information about history fractures was obtained. Only fractures caused by low impact were included. Our study was approved by the National Ethics Committee and all participants provided written informed consent.

Single nucleotide polymorphism genotyping

Genomic DNA was extracted from blood by the phenol chloroform procedure. The VDR fragment involving the site of the two polymorphisms was amplified by the following primers (designed by Primer blast software): 5′ TGCACG GAGAAGTCACTGGAGGG 3′, 5′ GGACCGGGGAAAA GCCCGCA 3′.

PCR products were digested with TaqI (65°C, 4 h) and ApaI (37°C, 2 h) restriction endonuclease enzymes and subjected to electrophoresis in 3% agarose gel.

Statistical analysis

The categorical variable data are given as mean ± standard deviation and were compared using the Pearson χ^2-test. For genotype distribution, Hardy–Weinberg equilibrium was tested for each single nucleotide polymorphism (SNP) by use of the standard χ^2 test. The associations between polymorphisms and BMD were calculated by ANOVA using the Snedecor *F*-test. Assessment of the risk of fracture, of osteopenia or of osteoporosis according to genotypes was performed using the χ^2-test followed by odds ratio calculation if $p < 0.05$. All statistical analysis were examined by SPSS software.

RESULTS

The age, body mass index (BMI), and BMD of the normal, osteopenic, and osteoporotic postmenopausal Tunisian women included in the study are shown in Table 1. As expected, the osteoporotic group was older than the other two groups. Osteopenic and osteoporotic women had the lowest BMI. It is, however, noteworthy that the BMI of the three groups indicated that the women were to be considered as overweight according to the World Health Organization classification. The osteoporotic women had a late menarche and multiple pregnancies. No statistically significant differences were observed among the three groups with respect to menopause.

The distribution of the ApaI and TaqI polymorphism genotypes and the allele frequencies are shown in Table 2. No significant deviation from the Hardy–Weinberg equilibrium was observed for either of these two polymorphisms.

The mean values of baseline BMD at the lumbar spine and femoral neck according to *ApaI* and *TaqI* genotypes in postmenopausal women are shown in Table 3. No significant difference was detected for the two VDR SNPs.

Table 4 shows the association analysis of VDR genotypes with fracture incidence. The GG *ApaI* genotype seems to be associated with vertebral fracture risk (odds ratio, OR = 3.14).

Comparison of the distribution of *ApaI* genotypes and alleles between osteopenic and normal women, under the co-dominant and dominant models, revealed a significant association of the GG genotype with development of osteopenia

Table 1 Age, body mass index, age at menarche, age at menopause and parity of the postmenopausal Tunisian women studied. Data are given as mean ± standard deviation

Parameters	*Normal* ($n = 23$)	*Osteopenic* ($n = 194$)	*Osteoporotic* ($n = 141$)	p^*	$p^\dagger$	$p^\ddagger$	p^{**}
Age (years)	58.02 ± 6.81	59.74 ± 7.64	62.0 ± 8.03	0.000	NS	0.000	0.014
Body mass index (kg/m^2)	29.76 ± 4.66	28.48 ± 4.39	28.46 ± 5.04	0.006	0.015	0.028	NS
Age at menarche (years)	12.6 ± 1.58	12.9 ± 1.77	13.1 ± 1.71	0.042	NS	0.049	NS
Age at menopause (years)	48.82 ± 4.43	48.19 ± 4.71	48.40 ± 4.53	NS	NS	NS	NS
Parity	3.00 ± 1.47	3.14 ± 1.56	4.31 ± 2.67	0.000	NS	0.000	0.000

NS, non-significant (Student's *t*-test)

*, Comparison between the three groups; †, comparison of normal vs. osteopenic groups; ‡, comparison of normal vs. osteoporotic groups; **, comparison of osteoporotic vs. osteopenic groups

($p < 0.05$, OR = 1.86). For the *TaqI* polymorphism, we also found a significant association under the co-dominant and dominant analysis models (Table 5). The TT genotype seems to be associated with a 1.53 risk of developing osteopenia.

Comparison of the distribution of *ApaI* genotypes and alleles between osteoporotic and normal women revealed under the co-dominant model that the GT genotype seems to be protective, with an OR = 0.54. For the *TaqI* polymorphism, no association with osteoporosis was found under the three analysis models (Table 5).

To investigate further the association of VDR polymorphisms, we determined the distribution of the combined (*ApaI/TaqI*) genotypes in the three groups. Data are shown in Table 6. For osteoporosis, we found a statically significant difference only in carriers of the GT/TT genotype. This combined genotype seems to be protective against osteoporosis development (OR = 0.41). The contribution of the VDR combined genotypes is different for the osteopenic group. In fact, the GG/TT genotype conferred a 2.3 times risk of developing osteopenia and the TT/CC or TC carriers seem to protect from osteopenia (OR = 0.51).

DISCUSSION

More than 150 studies have been reported on associations between VDR gene polymorphisms and bone-related traits[15,16]. A great deal of attention has been focused on the relationship of the BsmI, ApaI and TaqI polymorphisms and BMD, and these polymorphisms were the most extensively studied genetic markers. However, as noted by Ferrari and colleagues[17], the results from numerous studies have been highly controversial, as there are probably as many positive[10,18] as negative[19,20] studies. Most of these studies were performed on Caucasian subjects or Asian people. No data about people with North African or sub-Saharan origin are available.

The ApaI and TaqI polymorphisms are located in intron 8 and exon 9 at the 3′ untranslated region (3′UTR) of the VDR gene, respectively. This region is part of the ligand-binding domain of the VDR and therefore any structural variation in the protein might lead to a differential binding specificity of vitamin D, although the functional effects of the ApaI and TaqI SNPs remain unknown. In this study, we focused on the association between ApaI and TaqI polymorphisms and BMD-related traits in Tunisian postmenopausal women. This is the first study to investigate the association between TaqI and ApaI polymorphisms and osteoporosis.

Our sample consisted of 566 postmenopausal Tunisian women, who were randomly selected and representative of the Tunis Region population. Regarding anthropometric and obstetric parameters, we found that advanced age, low BMI, late menarche and multiparity to be risk factors for osteoporosis in our cohort. This result is consistent with those of several other studies[21]. However, the osteopenic group seems to be characterized by different features. As expected, osteopenic women are younger than osteoporotic women. This observation would be consistent with the idea that osteopenia is a transient stage toward osteoporosis. Moreover, osteopenic women displayed lower parity than osteoporotic ones and were merely close to normal women.

For the ApaI G/T (rs7975232) polymorphism, the TT, GT, and GG genotype frequencies that we reported in our Tunisian postmenopausal women were respectively 0.389, 0.456, and 0.155. These frequencies are similar to those reported by Tizaoui and colleagues[23] and are also similar to those seen in sub-Saharan African and European populations. For the TaqI T/C (rs731236) polymorphism, we determined the TT, TC, and CC genotype frequencies as, respectively, 0.473, 0.394 and 0.133. These frequencies are similar to those reported

Table 2 Vitamin D receptor genotypes and allele frequencies distribution in the 566 postmenopausal women

	Genotypes (%)			*Allele (%)*	
ApaI	*GG*	*GT*	*TT*	*G*	*T*
n	88	258	220	434	698
%	15.5	45.6	38.9	38.3	61.66
TaqI	*TT*	*TC*	*CC*	*T*	*C*
n	268	223	75	759	373
%	47.3	39.4	13.3	67	32.9

Table 3 Association of vitamin D receptor (VDR) genotypes with bone mineral density (BMD), expressed as mean ± standard deviation

	VDR polymorphisms							
	ApaI genotypes				*TaqI genotypes*			
Bone mineral density	*GG*	*GT*	*TT*	*p**	*TT*	*TC*	*CC*	*p**
Spine (g/cm^2)	0.848 ± 0.139	0.871 ± 0.144	0.861 ± 0.143	0.414	0.865 ± 0.143	0.865 ± 0.135	0.856 ± 0.165	0.873
Right hip (g/cm^2)	0.863 ± 0.118	0.884 ± 0.126	0.878 ± 0.133	0.423	0.883 ± 0.130	0.878 ± 0.122	0.864 ± 0.136	0.515
Left hip (g/cm^2)	0.860 ± 0.160	0.880 ± 0.121	0.877 ± 0.128	0.442	0.879 ± 0.137	0.875 ± 0.118	0.863 ± 0.126	0.624
Total hip (g/cm^2)	0.859 ± 0.160	0.878 ± 0.122	0.877 ± 0.122	0.444	0.878 ± 0.137	0.875 ± 0.118	0.862 ± 0.127	0.628

*, One-way ANOVA test

by Tizaoui and colleagues[23] and also similar to those seen in sub-Saharan African and European populations.

The distribution of the ApaI genotype between the normal, osteopenic and osteoporotic groups revealed a significant association of the GG ApaI genotype with osteopenia risk ($p = 0.02$; OR = 1.86). The GT genotype seems to be protective (OR = 0.54) against the osteoporosis phenotype. The last result is consistent with studies in Italian, Belarusian and Indian populations[24–26] and in contradiction from other studies which failed to determine any significant association with osteoporosis[22,25–29]. The effect of the ApaI genotype on BMD is not detected at any BMD site, as described in other studies[7,9,30]. For low-impact fracture incidence, the GG genotype seems to be associated with fracture incidence. The GG genotype carriers have three times the risk of developing vertebral fracture risk. This result is consistent with other studies detecting an association of the ApaI polymorphism with vertebral fracture[27].

For the TaqI polymorphism, we have not detected any statistically significant difference between the osteoporotic and normal groups. In fact, this polymorphism seems not to be associated with the osteoporosis phenotype. For BMD, our results are in contradiction with other studies detecting an association of the TaqI polymorphism with femoral neck BMD and femoral neck + lumbar spine BMD[31,32] but are consistent with many others that failed to detect an association with VDR[28,33]. This discordance in VDR gene contribution between populations in BMD level and osteoporosis development seems to be caused by the difference in genetic distribution between ethnic groups. Interestingly, the TT TaqI genotype seems to be more frequent in osteopenic than in normal women. This genotype seems to be associated with the development of osteopenia in Tunisian postmenopausal women.

Using a combined distribution of the two polymorphisms between osteoporotic and normal women, we detected a significant difference between the combined genotypes only for the GT/TT genotype ($p = 0.003$). The GT/TT genotype carriers are protected against osteoporosis in our Tunisian sample. The GG/TT genotype is associated with the osteopenia phenotype. This result is in accordance with other studies suggesting that the presence of the two polymorphisms simultaneously is associated with low BMD phenotype[34].

Osteopenia is often defined as a demineralized skeletal aspect without fracture[35]. Only osteopenic women with vertebral fracture are considered for osteoporosis treatment[36]. In this study, we have determined the association of VDR polymorphisms with osteopenia phenotype and osteoporosis development. All case/control studies are interested in osteoporosis and not in osteopenia. The new aspect in our work is that we have considered osteopenia

Table 4 Association of vitamin D receptor genotypes with low-impact fracture incidence. Data are given as number of women (%)

	Fractures ($n = 66$)				
	Vertebral fracture ($n = 22$)	*Peripheral fracture* ($n = 44$)	*No fracture* ($n = 500$)	*p*	*Odds ratio (95% confidence interval) for vertebral fracture*
ApaI					
GG	8 (36.4%)	3 (3.8%)	77 (15.4%)	0.02	3.14 (1.27–7.74)
GT	5 (22.7%)	23 (52.3%)	230 (46.0%)		0.35 (0.13–0.96)
TT	9 (40.9%)	18 (40.9%)	193 (38.6%)		1.1 (0.46–2.62)
TaqI					
TT	4 (18.2%)	6 (13.6%)	65 (13.0%)	0.165	
TC	6 (27.3%)	24 (54.6%)	193 (38.6%)		
CC	12 (54.5%)	14 (31.8%)	242 (48.4%)		

Table 5 Vitamin D receptor genotypes and allele frequency distribution in the three groups. Data are given as number of women (%)

Model	*Genotypes*	*Alleles*	*Osteoporotic (%) (n = 141)*	*Osteopenic (%) (n = 194)*	*Normal (%) (n = 231)*	*p**	*OR (95% CI) Osteopenic vs. normal*	*p†*	*OR (95% CI) Osteoporotic vs. normal*
ApaI									
Co-dominant	GG		25 (17.7)	37 (19.1)	26 (11.3)	0.02	1.86 (1.08–3.2)	0.07	
	GT		53 (37.6)	90 (46.4)	115 (49.8)	0.48		0.02	0.54 (0.35–0.83)
	TT		63 (44.7)	67 (34.5)	90 (39)	0.34		0.27	
Recessive	GG + GT		78 (55.3)	127 (65.5)	141 (61.0)	0.34		0.27	
	TT		63 (44.7)	67 (34.5)	90 (39.0)				
Dominant	GG		25 (17.7)	37 (19.1)	26 (11.2)	0.02	1.86 (1.08–3.2)	0.08	
	GT + TT		116 (82.3)	157 (80.9)	205 (88.7)		0.54 (0.31–0.93)		
		G	103 (36.5)	164 (42.3)	167 (36.1)	0.06		0.90	
		T	179 (63.5)	224 (57.3)	295 (63.8)				
TaqI									
Co-dominant	TT		58 (41.1)	107 (55.2)	103 (44.6)	0.02	1.53 (1.04–2.25)	0.51	
	TC		57 (40.4)	71 (36.6)	95 (41.1)	0.34		0.89	
	CC		26 (18.4)	16 (8.2)	33(14.3)	0.06		0.28	
Recessive	TT + TC		115 (81.6)	178 (91.8)	198 (85.7)	0.06		0.28	
	CC		26 (18.4)	16 (8.2)	33 (14.3)				
Dominant	TT		58 (41.1)	107 (55.2)	103 (44.6)	0.02	1.53 (1.04–2.25)	0.51	
	CC + TC		83 (65.9)	87 (44.8)	128 (55.4)		0.65 (0.44–0.96)		
		T	173 (61.3)	285 (73.4)	301 (65.1)	0.00	1.48 (1.10–1.99)	0.29	
		C	109 (38.7)	103 (26.6)	161 (34.9)		0.68 (0.51–0.91)		

OR, odds ratio; 95% CI, 95% confidence interval

*, Comparison of normal vs. osteopenic groups; †, comparison of normal vs. osteoporotic groups

and osteoporosis as two different entities. Considering osteopenia as transient, not all osteopenic women should go on to develop to osteoporosis.

Regarding the VDR polymorphisms, we have demonstrated that osteopenia is associated with the GG genotype in ApaI and the TT genotype in TaqI. This result suggests that osteopenia, as a primary natural physiological status, seems to be mostly determined by genetic factors. However, for osteoporosis, we have not detected any association with ApaI and TaqI VDR polymorphisms. Osteoporosis should be the consequence of interaction between genetic and environmental risk factors[37]. In fact, environmental factors such as calcium intake, physical activity, and smoking habits may confound the VDR genotypes[38–40].

In conclusion, we have detected a significant association for ApaI polymorphism in Tunisian postmenopausal women with osteopenia and low-impact vertebral fractures. However, the lack of an association between the VDR gene polymorphisms and BMD suggests different ways in which the VDR contributes to fracture incidence. This requires further investigation. In addition, further investigation is needed to identify osteopenia risk factors and to determine the osteopenic carriers. This phenotype is different from that in normal and osteoporotic groups and could be considered as a different entity.

Table 6 Association of vitamin D combined genotypes with osteoporosis and osteopenia. Data are given as number of women (%)

Genotypes ApaI/TaqI	*Osteoporotic (%) (n = 141)*	*Osteopenic (%) (n = 194)*	*Normal (%) (n = 231)*	*p**	*OR (95% CI) Osteopenic vs. normal*	*p†*	*OR (95% CI) Osteoporotic vs. normal*
GG/CC or TC	9 (6.5%)	7 (3.6%)	9 (3.9%)	0.88		0.28	
GG/TT	16 (11.3%)	30 (15.5%)	17 (7.3%)	0.00	2.3 (1.23–4.31)	0.18	
GT/CC or TC	37 (26.2%)	51 (26.3%)	60 (26.0%)	0.94		0.95	
GT/TT	16 (11.3%)	39 (20.1%)	55 (23.8%)	0.35		0.00	0.41 (0.22–0.75)
TT/ CC or TC	37 (26.2%)	29 (14.9%)	59 (25.5%)	0.00	0.51 (0.31–0.84)	0.88	
TT/TT	26 (18.5%)	38 (19.6%)	31 (13.4%)	0.08		0.19	

*, Comparison of normal vs. osteopenic groups; †, comparison of normal vs. osteoporotic groups

ACKNOWLEDGEMENTS

The authors thank all the staff of the Genetics, Immunology and Human Pathologies Laboratory, Faculty of Mathematical, Physical and Natural Sciences of Tunis, Tunis El Manar University, Tunisia and the staff of the Osteoporosis and Arthritis Laboratory, Rabta Hospital, Faculty of Medicine of Tunis, Tunis El Manar University, Tunisia for their skillful technical assistance. R.S. conceived and performed the molecular analysis and wrote the manuscript. H.S. was involved in clinical measurement. S.L.F. was involved in scientific and medical support. C.S. was involved in technical support and assistance. B.Z. performed statistical analysis. A.B.E.G. and S.S. designed the study. All authors read and approved the final manuscript.

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

Source of funding Nil.

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