維他命D受體基因起始譯碼多形性

與

台省男女性骨代謝率及骨密度之關係

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Original Article

The Vitamin D Receptor Start Codon Polymorphism (*Fok*1) and Bone Mineral Density in Premenopausal Women in Taiwan

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Abstract. The vitamin D receptor gene (VDRG) polymorphism as a factor of bone turnover rate or bone mineral density (BMD) is a controversial issue, especially in different ethnic populations. In addition to intron 8 (*Bsm*1, *Taq*1) and exon 9 (*Apa*1), VDRG polymorphism is present at its translation initiation site on exon 2. The VDRG has two translation initiation sites. The first shows a thymine/cytosine polymorphism and can be detected by restriction fragment length polymorphism (RFLP) using the endonuclease *Fok*1. This start codon polymorphism (SCP) of the VDRG was detected by polymerase chain reaction and then by RFLP with *Fok*1. While the f allele was assigned for the presence of the restriction site, the F allele was assigned for the absence of the restriction site, and the encoded vitamin D receptor is shorter by three amino acids. We examined the association between this SCP of the VDRG and bone turnover as well as BMD in 101 premenopausal Taiwanese women aged 40–53 years. Total body bone mineral content and BMD of proximal femur and lumbar spine were measured by dual-energy X-ray absorptiometry. We found a prevalence of 39.6% for the f allele of the VDRG. The frequencies of FF, Ff and ff genotypes were 35.6%, 49.5% and 14.9%, respectively. There was no statistically significant difference in BMD at any site or bone turnover markers among the three *Fok*1 genotypes (FF, Ff and ff). The SCP is independent of *Bsm*1, *Apa*1 or *Taq*1 polymorphisms of the VDRG at intron 8 and exon 9. In conclusion, the SCP polymorphism detected by endonuclease *Fok*1 does not significantly influence BMD or bone turnover in premenopausal women in Taiwan.

Keywords: Bone markers; Bone mineral density; Start codon polymorphism; Vitamin D receptor gene

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Introduction

The vitamin D receptor gene (VDRG) has two potential translation initiation sites. The first initiation site shows a thymine/cytosine polymorphism [1–3]. The VDRG with ACG instead of the first ATG codon probably initiates translation from the second ATG codon, which results in a VDR three amino acids shorter. Thus, in addition to the *Bsm*1, *Apa*1 and *Taq*1 polymorphisms at exon 8 and intron 9 [4], there is another polymorphic site. This start codon polymorphism (SCP) may potentially affect the functions of the VDR and thereby influence bone turnover and bone mineral density (BMD). A restriction endonuclease, *Fok*1, recognizes the presence of the first ATG codon as designated by the f genotype, and the absence of the first ATG codon designated by the F genotype [1–3].

Three published studies have reported the presence of an association between BMD and SCP of the VDRG [1,3,5], but another study reported no association [2]. Racial differences may partly explain the discrepancy. In this study we examined the SCP in 101 premenopausal women in Taiwan in order to discover: (1) What is the distribution frequency of SCP of the VDRG in Taiwan? (2) Does SCP of the VDRG determine BMD? (3) Does SCP of the VDRG influences bone turnover markers? (4) Are SCP of the VDRG and other VDRG polymorphisms linked?

Statistical Analysis

Statistical analysis was performed with SAS for Windows (version 6.12, SAS Institute, Cary, NC). We used one-way analysis of variance (one-way ANOVA) to compare the mean BMD among the VDRG SCP genotypes. Ninety-five percent confidence intervals (95% CI) of the means were calculated also. We also used one-way ANOVA to examine the mean biochemical markers among the three SCP genotypes. The linkage between two polymorphic VDRG regions was examined by a chi-squared test. To investigate the effect of the combination of *Fok*I and *Bsm*I genotypes on BMD and bone markers, we performed a *t*-test between the subjects with FFbb genotypes and those with f and B alleles (with FfBB, FfBb, ffBB or ffBb genotypes). The same procedures were applied for *Apa*I genotypes but not for *Taq*I genotypes, because the frequencies of Tt and tt were very low (only 4 of the 101 subjects were Tt or tt). To meet the assumptions of ANOVA [11], logarithmic transformation was performed on variables that were not normally distributed, including femoral neck BMD, BGP, ICTP, BAP, NTx, daily calcium intake, and adjusted daily calcium intake to normalize their distributions.

Results

The allelic frequency of f haplotypes of SCP of VDRG is 39.6%. This distribution of the SCP subgroups was in Hardy–Weinberg equilibrium. The frequencies for the FF, Ff and ff genotypes were 35.6%, 49.5% and 14.9%, respectively. Mean age, height, weight and calcium intake of the study population, as shown in Table 1, did not differ among the SCP genotypes (one-way ANOVA). The SCP genotypes and the genotypes at *Bsm*I, *Apa*I and *Taq*I alleles, as analyzed by chi-squared analysis, showed no significant linkage (data not shown).

The t-test showed no statistically significant difference in BMD and bone markers between FFbb and fxBx genotypes, where 'x' means 'unspecified' (for example, Bx could be BB or Bb, and fx could be Ff or ff). There was no significant difference in either BMD or bone markers between FFaa and fxAx genotypes (data not shown).

One-way ANOVA showed no significant difference in BMD at any skeletal site among the SCP genotypes (Table 1). The levels of bone metabolic markers did not show a significant difference among the SCP genotypes either (Table 1).

Table 1. Bone mass and bone markers among the *Fok*I genotypes of the start codon polymorphism of the vitamin D receptor gene in 101 premenopausal women in Taiwan

	Genotype						*p* value[a]
	FF (*n*=36, 35.6%)		Ff (*n*=50, 49.5%)		ff (*n*=15, 14.9%)		
N(%)	Mean	95% CI	Mean	95% CI	Mean	95% CI	
Basic data							
Age (years)	45.2	51.3–39.1	45.3	52.3–38.3	45.1	52.0–38.2	0.97
Body height (cm)	156.4	166.0–146.8	157.0	165.7–148.3	156.9	168.8–145.0	0.82
Body weight (kg)	57.6	69.1–46.1	56.3	69.3–43.2	59.0	75.1–42.9	0.34
Calcium intake[b]							
(mg/kcal)	0.26	1.15–0.06	0.27	1.23–0.06	0.21	1.07–0.04	0.52
(mg/day)	497	1408–175	509	1795–144	492	2003–121	0.97
BMD (g/cm²)							
Lumbar spine	1.02	1.32–0.71	1.03	1.32–0.74	1.01	1.26–0.76	0.88
Femoral neck	0.83	1.05–0.60	0.85	1.08–0.61	0.84	1.14–0.54	0.73
Femoral trochanter	0.68	0.89–0.48	0.68	0.86–0.51	0.67	0.88–0.45	0.84
Femoral Ward's triangle	0.64	0.86–0.41	0.66	0.90–0.41	0.65	0.98–0.31	0.78
Total BMC (g)	2382	3021–1744	2368	2888–1847	2362	2988–1736	0.97
Bone markers							
BGP (ng/ml)[b]	6.8	17.8–2.6	6.6	18.5–2.3	6.6	22.2–2.0	0.96
PICP (ng/ml)	82	122–43	84	128–40	84	122–46	0.94
BAP (IU/l)[b]	14.2	26.9–7.5	16.2	34.0–7.7	15.3	32.6–7.2	0.29
ICTP (ng/ml)[b]	3.1	7.4–1.0	3.4	7.8–1.2	3.8	6.6–2.1	0.29
NTx/creat	2.2	5.3–0.6	2.5	6.8–0.6	2.7	8.2–0.5	0.41
(nm/mm creatinine)[b]							

[a] *p* value from analysis of variance.
[b] Natural logarithmic transformation was performed before analysis and then the means and 95% CI were reversed by exponential transformation.
BMD, bone mineral density; BMC, bone mineral content; CI, confidence interval; BGP, bone gla protein (= osteocalcin); PICP, carboxyl terminal propeptides of type I procollagen; BAP, bone isoenzyme of alkaline phosphatase; ICTP, pyridinoline crosslinked telopeptide domain of type I collagen; NTx, amino terminal crosslinked fragment of type I collagen.

Eccleshall et al. [2] reported that urinary NTx was higher in the ff group in French women. However, we did not find this difference to be significant in our study. With the current case number, the statistical powers ($1-\beta$) of the apparent differences seen in ICTP and NTx in this study were 35% and 20% respectively [16]. The apparent differences in ICTP and NTx among the SCP groups, even if they exist, may not be important physiologically and clinically in the Taiwanese, since BMD and other bone markers did not show any trend of differences.

We have calculated the statistical power of this study to detect the differences in BMD. Based on the studies of Gross et al. [1] and Harris et al. [3], the case number in this study should have a statistical power of 90% to detect a 10% difference in femoral neck BMD and an 80% power to detect a 12% difference in lumbar spine BMD at the $p = 0.05$ level. However, the apparent differences in BMD among the SCP genotypes were only around 1–2%. It would take a sample size larger than 6000 to make them significant (with $\alpha = 0.05$ and $1-\beta = 0.9$).

In conclusion, we found no significant influence of SCP of the VDRG on BMD at any skeletal site or on bone turnover rate in premenopausal Taiwanese women. The frequencies of the genotype of the VDRG start codon are about the same among Taiwanese, Japanese and Caucasians, and higher than that of African-Americans. The distribution of SCP genotypes of the VDRG is independent of other VDRG polymorphisms defined by *Bsm*I, *Apa*I or *Taq*I. For postmenopausal Taiwanese women, the effect of SCP polymorphism on BMD and bone turnover needs further study.

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