Vitamin D receptor genotypes influence quadriceps strength in chronic obstructive pulmonary disease

Nicholas S Hopkinson, Ka Wah Li, Anthony Kehoe, Steve E Humphries, Michael Roughton, John Moxham, Hugh Montgomery, and Michael I Polkey

ABSTRACT

Background: Quadriceps weakness is an important complication of chronic obstructive pulmonary disease (COPD) and is associated with impaired exercise capacity and greater mortality. Its etiology is multifactorial, and evidence is growing that it is partly determined by genetic susceptibility.

Objective: Using an established cohort, we tested whether quadriceps weakness in patients with COPD is influenced by common variations in the gene for the vitamin D receptor.

Design: Vitamin D receptor FokI and BsmI genotypes and the (I/D) angiotensin-converting enzyme (ACE) and bradykinin receptor (+9/-9) genotypes were identified in 107 patients with stable COPD [FEV1: 34.5 ± 16.5] and 104 healthy, age-matched control subjects. Quadriceps maximum voluntary contraction force and fat-free mass assessed by bioelectrical impedance analysis were measured.

Results: After adjustment for covariates, both patients and control subjects who were homozygous for the C allele of the FokI polymorphism had less quadriceps strength than did those with ≧1 T allele [41.0 ± 11.8 compared with 46.0 ± 13.2 kg (P = 0.01) and 32.5 ± 11.2 compared with 36.2 ± 13.1 kg (P = 0.005), respectively]. The b allele of the BsmI polymorphism was associated with greater quadriceps strength in patients—37.0 ± 13.3, 33.8 ± 11.6, and 33.8 ± 11.6 kg for bb, bb, and BB, respectively (P = 0.0005)—but had no effect in healthy control subjects. The effect of BsmI on quadriceps strength was least apparent in patients with the ACE II genotype (P = 0.003).

Conclusions: The FokI common variants in the VDR gene are associated with skeletal muscle strength in both patients and control subjects, whereas the BsmI polymorphism is associated with strength only in patients. Am J Clin Nutr 2008;87:385–90.

KEY WORDS Muscle, quadriceps, angiotensin-converting enzyme, bradykinin

INTRODUCTION

Quadriceps weakness is an important systemic consequence of chronic obstructive pulmonary disease (COPD) and is associated with a decreased exercise capacity (1–4). Moreover, reduced quadriceps bulk (5) and strength (6) are associated with greater mortality. Several etiologic factors have been proposed, including disuse atrophy, systemic inflammation, hormonal dysfunction, and systemic corticosteroid treatment (7).

Twin studies have shown that there is a significant genetic component to muscle strength (8, 9). A study of lower-limb strength in elderly male twins found that genetic factors appeared to be more significant in the poorest quartile of performance, and environmental factors appeared more significant as determinants of good performance (9). It seems reasonable to assume that susceptibility to the systemic effects of COPD should to some extent be genetically determined. In support of this, we have shown associations between quadriceps strength and both the I/D polymorphism of the angiotensin-converting enzyme (ACE) and the +9/-9 polymorphism of the bradykinin receptor (BK,R) in patients with COPD (10, 11). Vitamin D has a well-described role in calcium metabolism, increasing the absorption of calcium and phosphate from the intestine and increasing renal calcium reabsorption. It has been suggested that vitamin D also may have an important role in influencing skeletal muscle function (12), and vitamin D receptors have been identified in this tissue (13).

Several VDR polymorphisms exist that are associated with a range of biological effects on variables, including bone mineral density, immune response, and susceptibility to a range of diseases such as osteoarthritis, diabetes, cancer, cardiovascular disease, and tuberculosis (14). FokI, a polymorphism involving a T→C transition in exon 2 of the VDR gene, changes the site at which translation is initiated. Persons with the C allele (sometimes designated “F”) have a shorter 424-amino acid VDR than do persons with the T (“f”) allele, which is 427 amino acids long. The shorter VDR appears to have enhanced transactivation capacity as a transcription factor (15–17). The C allele of FokI has been associated with reduced fat-free mass (FFM) and quadriceps strength in otherwise healthy elderly men (18).

BsmI is a restriction fragment length polymorphism at the 3′ end of the VDR gene that has also been found to be associated

1 From the Respiratory Muscle Laboratory, Royal Brompton Hospital, London, United Kingdom (NSH and MIP); the Centre for Cardiovascular Genetics, Royal Free and UCL Medical School, Rayne Institute, London, United Kingdom (KWL and SEH); the UCL Institute for Human Health and Performance, London, United Kingdom (AK and HM); the National Heart and Lung Institute, Imperial College, London, United Kingdom (NSH and MIP); the Centre for Cardiovascular Genetics, Royal Free and UCL Medical School, Rayne Institute, London, United Kingdom (NSH and MIP); and the Department of Respiratory Medicine, King’s College Hospital, London, United Kingdom (JM).

2 Supported by The Wellcome Trust (to NSH), by grant no. QLK6-CT-2002-02285 from the European Union (to MIP), and by grant no. PG2005/014 from the British Heart Foundation (to KWL and SEH).

3 Reprints not available. Address correspondence to NS Hopkinson, Royal Brompton Hospital, Fulham Road, London, SW3 6NP United Kingdom. E-mail: n.hopkinson@ic.ac.uk.

Received August 29, 2007.
Accepted for publication September 19, 2007.

with skeletal muscle function. The \( b \) (rather than \( B \)) allele was associated with lower FFM and hamstring (but not quadriceps) strength in healthy young women aged 20–39 y (19), whereas, in nonobese elderly women, both handgrip and quadriceps strength were higher in those with the \( bb \) genotype (20).

Given the association of impaired skeletal muscle function with COPD, and the association of VDR genotype with muscle function in other population samples, we sought a similar association of VDR \( FokI \) and \( BsmI \) genotypes with muscle strength in a group of COPD patients and age-matched healthy subjects in whom the association of \( ACE \) and \( BK_{R} \) genotypes with strength was shown previously (10, 11).

SUBJECTS AND METHODS

Subjects

Data from this group of subjects were published previously (10, 11). Briefly, patients with COPD, defined in accordance with the criteria of the Global Initiative for Chronic Obstructive Pulmonary Disease (Internet: www.goldcopd.com), were recruited from hospital clinics. Subjects with significant comorbidity including heart failure, diabetes, malignancy, and neuromuscular disease were excluded. Healthy age-matched control subjects were recruited by advertisement in local newspapers.

All participants provided written informed consent. The study was approved by the Ethics Committee of The Royal Brompton and Harefield NHS Trust.

Methods

FFM was measured by using a bioelectrical impedance device (Bodystat 1500; Bodystat, Douglas, United Kingdom). For patients, a disease-specific regression equation was used (21), and, for healthy subjects, the device’s internal algorithm was used. Pulmonary function (gas transfer, plethysmographic lung volumes and spirometry in patients, and spirometry only in controls) was assessed by using a CompactLab system (Jaeger, Wuerzburg, Germany). Quadriceps strength, calculated as quadriceps maximal voluntary contraction force (QMVC), was measured while subjects were seated in a custom-designed chair with their legs bent at the knees to 90 degrees. An inextensible strap connected the subject’s ankle to a strain gauge. The signal was amplified and passed to a computer running LABVIEW software (version 4.1; National Instruments, Austin, TX). The force generated was visible online, and subjects received vigorous encouragement. The best of \( \geq 3 \) efforts was taken.

In control subjects, handgrip strength also was measured by using a Jamar handgrip dynamometer (Sammons Preston Rolyan, Bolingbrook, IL). Subjects performed 6 maximum contractions \( \geq 30 \) s apart, alternating hands. The mean of the peak value for each hand was recorded.

Genotyping

\( VDR \) \( FokI \) genotypes (rs10735810) were identified by using the forward primer \( 5^\prime \) GGCCCTGCTTTGCTTCTTAC 3\’ and reverse primer \( 5^\prime \) TGCTTCTTCTCCCTCCCTT 3\’. \( VDR \) \( BsmI \) genotypes (rs1544410) were identified by using the forward primer \( 5^\prime \) CCTCAGCCCTAGCTCTG 3\’ and reverse primer \( 5^\prime \) CCATCTCTCAGGCTTCAAG 3\’. Polymerase chain reaction (PCR) was performed by using a thermal cycler (PTC-225; MJ Research, Watertown, MA), and digestion products were run on 7.5% polyacrylamide gels. All genotypes were scored by 2 independent technicians (blinded as to subject status), and any discrepancies were resolved by repeat genotyping. \( ACE \) \( VD \) and \( BK_{R} \) \( R + 9/9 \) genotypes were identified as reported previously (10, 11).

Statistical analysis

Analyses were performed with STATVIEW software (version 5.0; Abacus Concepts Inc, Berkeley, CA); they focused on the effect of the \( VDR \) genotype on QMVC. A hierarchy of models was analyzed in all subjects including potential confounders [ie, age, sex, FFM, and forced expiratory volume in 1 s (FEV\(_1\))] and a term to test for differences between patients and control subjects and also including the various genotypes to test for significant interactions. Interactions between the \( VDR \) genotype and \( ACE \) and \( BK_{R} \) \( R \) genotype were also sought. An effect of the \( ACE \) genotype was considered across all 3 genotypes. For the \( BK_{R} \) \( R \) genotype, a binary approach comparing \( +9 \) homozygotes to the other 2 genotypes was used (10, 11). For \( FokI \), \( C \) homozygotes were compared with the other 2 genotypes, whereas \( BsmI \) was analyzed across all 3 genotypes, as is consistent with previous studies (18, 20). In the COPD population, gas transfer [carbon monoxide transfer factor (TLco)] was used instead of FEV\(_1\) as an index of disease severity, because it is known that muscle wasting in COPD patients is correlated with the degree of emphysema more strongly than is the severity of airflow limitation (22, 23). Continuous variables are expressed as means \( \pm \) SDs. \( P < 0.05 \) was taken as statistically significant.

RESULTS

Data were available for 104 healthy control subjects and 107 COPD patients. \( BsmI \) genotyping failed in 1 patient. COPD patients had a significantly (\( P < 0.05 \)) lower FFM index and QMVC than did controls (Table 1). The allele frequencies of the \( VDR \), \( ACE \) and \( BK_{R} \) \( R \) polymorphisms did not differ significantly between patients and control subjects, and the distribution of genotypes was as expected for Hardy-Weinberg proportions. There was no evidence of linkage disequilibrium between \( FokI \) and \( BsmI \) polymorphisms (\( D^\prime = -0.03; R^2 = -0.005; P = 0.6 \)).

\( FokI \) genotype

In the entire sample, \( FokI \) \( C \) homozygous subjects had significantly weaker quadriceps than did subjects with 1 or 2 \( T \) alleles: \( 36.5 \pm 12.2 \) kg compared with \( 41.2 \pm 14.0 \) kg (\( P = 0.01 \), Scheffe test) (Figure 1). This difference became more significant (\( P = 0.0002 \)) in a model that included age, sex, FFM, FEV\(_1\), and \( ACE \) genotypes. There was no evidence that being a patient or a control subject affected the relation between \( FokI \) genotype and QMVC. In the control group, there was a trend for \( C \) homozygotes to have a lower QMVC than did those with \( \geq 1 \) \( T \) allele: \( 41.0 \pm 11.8 \) kg compared with \( 46.0 \pm 13.2 \) kg (\( P = 0.052 \), Scheffe test). This difference became significant (\( P = 0.007 \)) in a model including age, FFM, FEV\(_1\), sex, and \( ACE \) genotype.

In the COPD group, \( C \) homozygotes had significantly lower QMVC than did those with \( \geq 1 \) \( T \) allele: \( 32.5 \pm 11.2 \) kg compared with \( 36.2 \pm 13.1 \) kg (\( P = 0.02 \)). Univariate analysis in the COPD patient group showed a relation between QMVC and FFM (\( R = 0.62; P < 0.001 \)), TLco (% of predicted) (\( R = 0.23; P = 0.02 \)), and age (\( R = 0.18; P = 0.06 \)) but not with FEV\(_1\) (% of predicted).
or arterial blood gas variables. If TLCO (% of predicted) was used as a measure of disease severity instead of FEV₁ in the model above, the effect of C homozygosity on QMVC became even more significant (P = 0.005). No evidence of an interaction between C homozygosity on QMVC and other polymorphisms was found either in an analysis of the whole population or in separate analyses of patients and control subjects.

**BsmI genotype**

In the whole population studied, there was a trend for the b allele of the BsmI polymorphism to be associated with greater or lesser QMVC (P = 0.09 for linear trend) (Figure 2). In the entire population, the effect of patient group × genotype interaction for QMVC was not significant (P = 0.38). In control subjects alone, there was no significant relation with QMVC. A highly significant association (P = 0.0005) existed between BsmI genotype and QMVC in COPD patients in a model including cofactors and the other genotypes studied (Table 2 and Table 3). This model including FFM, age, VDR allele C homozygosity, and ACE genotype explained 45% of the variation in QMVC. QMVC values were 37.0 ± 13.3, 33.8 ± 11.6, and 32.6 ± 12.7 kg for patients with the bb, bB, and BB BsmI genotypes, respectively. The ACE genotype × effect of BsmI interaction for QMVC was significant (P = 0.003); BsmI had the least effect in patients with the ACE II genotype. BK₂R genotype did not interact significantly with either VDR variant studied.

**TABLE 1**

Characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 104)</th>
<th>COPD patients (n = 107)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>61.8 ± 8.5</td>
<td>63.5 ± 9.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Male (%)</td>
<td>46</td>
<td>70</td>
<td>0.004</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 ± 3.9</td>
<td>24.0 ± 4.8</td>
<td>0.23</td>
</tr>
<tr>
<td>FFM (kg/m²)</td>
<td>17.3 ± 2.8</td>
<td>16.4 ± 2.2</td>
<td>0.01</td>
</tr>
<tr>
<td>FEV₁ (% of predicted)</td>
<td>103.2 ± 14.7</td>
<td>34.5 ± 16.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>QMVC (kg)</td>
<td>44.0 ± 12.8</td>
<td>34.6 ± 16.5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**FIGURE 1.** Mean (±SEM) influence of the FokI allele of the vitamin D receptor (VDR) on quadriceps strength [quadriceps maximal voluntary contraction force (QMVC)] in patients with chronic obstructive pulmonary disease and control subjects. Patients (■; n = 104) were significantly weaker than control subjects (●; n = 104) (P < 0.0001, unpaired t test). In both groups, after correction for covariables, homozygosity for the C allele of the FokI VDR polymorphism was associated with lower QMVC than was seen in subjects with the other genotypes. *P = 0.005, †P = 0.007 (ANOVA). The effect of FokI polymorphism on QMVC did not differ significantly between patients and control subjects.

**FIGURE 2.** Mean (±SEM) influence of the BsmI allele of the vitamin D receptor (VDR) on quadriceps strength [quadriceps maximal voluntary contraction force (QMVC)] in patients with chronic obstructive pulmonary disease and control subjects. Patients (■; n = 106) were significantly weaker than control subjects (●; n = 104) (P < 0.0001, unpaired t test). In patients, QMVC varied significantly according to the BsmI allele (*P = 0.0005 (ANOVA)) in a model containing fat-free mass, age, sex, angiotensin-converting enzyme genotype, and C homozygosity for the FokI genotype. No significant relation between genotype and QMVC existed in control subjects.

**TABLE 2**

Characteristics of chronic obstructive pulmonary disease patients by BsmI genotype

<table>
<thead>
<tr>
<th></th>
<th>bb</th>
<th>bB</th>
<th>BB</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>36</td>
<td>45</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>64.8 ± 8.7†</td>
<td>63.7 ± 11.2</td>
<td>61.1 ± 6.9</td>
<td>0.32</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3 ± 5.0</td>
<td>24.2 ± 5.1</td>
<td>24.1 ± 3.50</td>
<td>0.62</td>
</tr>
<tr>
<td>FFM (kg/m²)</td>
<td>15.8 ± 2.4</td>
<td>16.8 ± 2.2</td>
<td>16.2 ± 1.6</td>
<td>0.11</td>
</tr>
<tr>
<td>FEV₁ (% of predicted)</td>
<td>34.6 ± 16.5</td>
<td>35.4 ± 19.5</td>
<td>26.9 ± 11.3</td>
<td>0.03†</td>
</tr>
<tr>
<td>TLCO (% of predicted)</td>
<td>36.9 ± 19.7</td>
<td>39.9 ± 19.0</td>
<td>32.6 ± 14.5</td>
<td>0.29</td>
</tr>
</tbody>
</table>

**TABLE 3**

Characteristics of chronic obstructive pulmonary disease patients by BsmI genotype

<table>
<thead>
<tr>
<th></th>
<th>bb</th>
<th>bB</th>
<th>BB</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects (n)</td>
<td>43</td>
<td>47</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>61.5 ± 9.8</td>
<td>62.2 ± 8.0</td>
<td>61.0 ± 7.2</td>
<td>0.86</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.8 ± 3.4</td>
<td>25.2 ± 4.6</td>
<td>25.5 ± 2.9</td>
<td>0.19</td>
</tr>
<tr>
<td>FFM (kg/m²)</td>
<td>17.1 ± 2.8</td>
<td>17.3 ± 2.9</td>
<td>17.5 ± 2.8</td>
<td>0.90</td>
</tr>
<tr>
<td>FEV₁ (% of predicted)</td>
<td>102.1 ± 16.1</td>
<td>103.0 ± 13.9</td>
<td>107.3 ± 12.7</td>
<td>0.52</td>
</tr>
</tbody>
</table>

1 FFMI, fat-free mass index; FEV₁, forced expiratory volume in 1 s; TLCO, carbon monoxide transfer factor.
2 † ± SD (all such values).
3 †P < 0.05, ANOVA.

Downloaded from https://academic.oup.com/ajcn/article-abstract/87/2/385/4633386 by guest on 30 October 2018
Vitamin D deficiency is associated with weakness, which tends to be proximal (24), and several cross-sectional studies have shown an association between low vitamin D stores and skeletal muscle impairment (25–27). Biopsies in a handful of cases showed predominantly Type II fiber atrophy and fiber necrosis and fatty infiltration (28–30). Possible mechanisms are reduced calcium uptake by the sarcoplasmic reticulum and phosphate depletion that impairs glycolysis (31). Vitamin D has effects on muscle through genomic and nongenomic mechanisms. It binds to a nuclear receptor, and the ligand receptor complex causes mRNA transcription and protein synthesis, which in turn influence calcium uptake, phosphate transport, and phospholipid metabolism. Receptor binding also drives cell proliferation and differentiation into mature muscle fibers (32) through a mechanism involving the mitogen-activated protein kinase pathway (33, 34). Data from knockout mice suggest that the VDR plays a role in muscle differentiation (35). In addition, nongenomic signal transduction occurs more rapidly through binding to a membrane-bound VDR, which enhances calcium uptake (36, 37). VDR expression decreases with age (38), and that change may play an important role in the loss of strength that occurs with aging. Systemic inflammation is a feature of COPD, and vitamin D is involved in the immune response, inhibiting lymphocyte proliferation and the secretion of cytokines, including interleukin-2, interferon-γ, and interleukin-12 (14).

Our data support a significant role for the BsmI variant in determining quadriceps strength in patients with COPD. The BsmI site is not itself thought to have a functional effect, and, thus, its effects on phenotype must be due to its being in linkage disequilibrium with another functional allele. Of note, several studies have failed to show the FokI polymorphism to be in linkage disequilibrium with any of the other known VDR polymorphisms, which agrees with the findings in the present study (14). The DNA change creating the BsmI is in strong linkage disequilibrium with the polyA variable number of tandem repeats (VNTR) in the 3′ untranslated region (UTR) of the regulatory region of the VDR gene, so that the b allele is associated with a long polyA stretch and the B allele is associated with a short one (19, 39). The 3′ UTR is known to play an important role in regulating gene expression—eg, polymorphisms in this area of the glucocorticoid receptor alpha have been shown to influence mRNA stability (40). There is some evidence that the long polyA stretch may be associated with higher VDR activity in combination with the C allele of FokI. This greater activity could result from increased translational activity, greater mRNA stability, or both (16). These mechanisms remain to be elucidated, however, and studies measuring a range of vitamin D–dependent outcomes have provided conflicting data, as reviewed by Uitterlinden et al (14).

The BsmI effect that we found in COPD patients is consistent with the finding in previous studies of greater quadriceps strength in nonobese elderly women with the b allele (20). It is interesting that the authors of that study found a nonsignificant trend in the opposite direction in obese [body mass index (in kg/m²) > 30] subjects. By contrast, in a study of younger women, the b allele was associated with lower weight and FFM and with lower hamstring strength, although there was no difference in quadriceps strength (19). Another study found lower quadriceps peak torque...
in young Chinese women with the bb genotype (41). This discrepancy suggests that the VDR polymorphism may exert different influences in different stages of life or, alternatively, that larger studies are required to resolve the issue.

We previously found an association between quadriceps strength and the I/D polymorphism of the ACE and the +9/−9 polymorphism of BK2R in this group of patients (10, 11). In COPD, the D allele of the ACE gene (associated with greater tissue ACE) was associated with preserved quadriceps strength in a linear fashion across genotype, whereas patients homozygous for the +9 allele (associated with reduced expression of BK2R) had less FFM. Neither polymorphism influenced strength or FFM in the control group. In the present study, we found that the effect of BsmI genotype was lowest in patients who were homozygous for the I allele of the ACE gene. Both the ACE and BsmI polymorphisms affected strength in patients with COPD but not in control subjects. The ACE polymorphism has known effects on fiber type: the D allele favors Type II fast-twitch fibers and a strength phenotype, whereas the I allele is associated with a higher proportion of Type I slow-twitch, fatigue-resistant fibers (42) and a greater response to inflammation (43). This association may be relevant because it is implicated in the loss of skeletal muscle strength in COPD patients, and because vitamin D influences inflammatory responses (14). Thus, the variation in strength associated with the BsmI variant may be fiber type-specific, or it may be influenced by the presence of a greater systemic or local inflammatory response. A recent study showed a relation between ACE gene polymorphism and secondary hyperparathyroidism in patients on hemodialysis; higher parathyroid concentrations and greater cumulative vitamin D requirements were found in patients with the DD genotype than in those ≥1 I allele (44). The VDR may act by influencing the regulation of insulin-like growth factor (IGF-1)–binding protein (45), which is of interest because muscle-specific expression of IGF-1 has been shown to block angiotensin II–induced muscle wasting (46). Vitamin D itself acts as a potent suppressor of the rennin-angiotensin system (47), which indicates that an effect of the VDR would be less pronounced when ACE concentrations are lower.

The VDR is widely distributed and has several different effects. An interesting observation is that the VDR is co-activated by peroxisome proliferator–activated receptor (PPAR) γ coactivator-1α (PGC-1α), which is expressed in skeletal muscle and which promotes mitochondrial biogenesis (48). PGC-1α activates various transcription factors including PPARα, PPARδ, and PPARγ. The PPARs mediate lipid and carbohydrate metabolism in skeletal muscle and may determine fiber type (49, 50). PPARγ is the target of the glitazone drugs recently introduced to treat insulin resistance, and the b allele has been shown to be associated with lower fasting glucose concentrations in young men who did not take part in high levels of physical activity (51).

We acknowledge that data from the subjects in the present study were reported previously. Apart from the polymorphisms described here, no other genetic testing has been carried out, and the decision to investigate the VDR polymorphisms in an existing, well-phenotyped cohort was driven by published data and biological plausibility.

In conclusion, we have identified associations between VDR polymorphisms and strength in patients with COPD. This finding suggests that this receptor has a significant influence on one of the important complications of this disease and that it may be a useful future area for research into mechanisms of skeletal muscle impairment in COPD and other diseases.

The authors’ responsibilities were as follows—NSH, HM, MIP, and JM: study conception; NSH: patient studies and collection of specimens for analysis; KWL, AK, and SHE: genotyping of the samples; NSH, SHE, and MR: statistical analysis; NSH: writing of the first draft of the manuscript; and all authors: contributions to revision of the manuscript and approval of the final version. None of the authors had a personal or financial conflict of interest.

REFERENCES


19. Grundberg E, Brandstrom H, Ribom EL, Ljunggren O, Mallmin H, Kindmark A. Genetic variation in the human vitamin D receptor is


