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Replication study of the vitamin D receptor (*VDR*) genotype association with skeletal muscle traits and sarcopenia

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Abstract

Polymorphisms in the vitamin D receptor (*VDR*) gene are some of the most studied in relation to skeletal muscle traits and significant associations have been observed by multiple groups. One such paper by our group provided the first evidence of a genetic association with sarcopenia in men, but that finding has yet to be replicated in an independent cohort. In the present study, we examined multiple *VDR* polymorphisms in relation to skeletal muscle traits and sarcopenia in 864 men and women across the adult age span. In addition to *VDR* genotypes and haplotypes, measurements of skeletal muscle strength and fat-free mass (FFM) were determined in all subjects and a measure of sarcopenia was calculated. We observed significant associations between FokI and BsmI genotypes and skeletal muscle strength in men and women, though these associations were modest and no significant associations were observed for these polymorphisms and muscle mass traits nor for BsmI–TaqI haplotype with muscle strength. FokI FF genotype was associated with an increased the risk of sarcopenia in older women compared to f-allele carriers (1.3-fold higher risk). These results support previous findings that *VDR* genetic variation appears to impact skeletal muscle strength and risk for sarcopenia but the influence is modest.

Keywords

Genetics; Muscle strength; Sex differences; Haplotype; Muscle mass

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Conflict of interest The authors report no conflicts of interest regarding this manuscript.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Introduction

Despite being considered a consequence of old age, sarcopenia has been reported in community-dwelling men and women below the age of 50 years [1–4], and nearly one in ten women aged 34–58 years (mid-life) has sarcopenia associated with compromised physical functioning [5]. Such variability in the losses of skeletal muscle mass and strength indicates a susceptibility for sarcopenia in some individuals. Variation in skeletal muscle traits among individuals can be attributed to environmental factors, genetic factors, or the interaction of both. Multiple investigations have shown that skeletal muscle strength and mass are highly heritable traits, indicating a strong genetic contribution [6–9]. Despite this high heritability, few genes have been conclusively identified as contributing to skeletal muscle mass or strength [10], and very few studies have examined sarcopenia in particular [10–13].

The vitamin D receptor gene (*VDR*) is among the targets of interest in explaining inter-individual differences in skeletal muscle traits and was one of the first to be studied in this context [14]. The receptor is expressed in skeletal muscle and vitamin D is required for skeletal muscle development [15, 16]. Vitamin D receptor levels are lower in skeletal muscles of older compared to younger individuals independent of 25-hydroxyvitamin D levels [17] and low vitamin D levels have been associated with low muscle strength and muscle mass measures [18]. The *VDR* gene has multiple polymorphisms (e.g., FokI, BsmI, and TaqI) that have been examined in relation to skeletal muscle traits. The FokI polymorphism affects the translational start site of *VDR* [19, 20]. The BsmI and TaqI variants are in linkage disequilibrium [21] and significant haplotype associations with skeletal muscle strength have been observed [22]. Though some inconsistencies have been noted [23–25], multiple papers have reported higher skeletal muscle strength and mass values in *VDR* FokI f/f compared to F/F genotype carriers [13, 22, 26], with a twofold higher risk for sarcopenia in F/F genotype men in previous work from our lab [13]. Findings for the BsmI site are less consistent [26, 27] but both Barr et al. [28] and Onder [29] have reported a higher risk of falls in BB genotype compared to b-allele carriers.

In a recent paper, Tan and colleagues [10] report the need to validate/replicate the genetic variants underlying sarcopenia in large human cohorts and state that further progress in understanding the genetic etiology of sarcopenia will provide valuable insights into important fundamental biological mechanisms underlying muscle physiology that will lead to improved ability to recognize individuals at risk for developing sarcopenia and our ability to treat this debilitating condition. In the present study, we sought to provide clarity on the existing literature on *VDR* genotypes and skeletal muscle traits and sarcopenia in particular by performing an extensive replication analysis in over 800 men and women. Furthermore, in addition to examining the *VDR* FokI polymorphism we extended our analyses to include the BsmI–TaqI haplotype.

Methods

Subjects

The subjects, 489 men and 375 women aged 22–90 years, included in this study were investigated as part of the Baltimore Longitudinal Study of Aging (BLSA), an ongoing NIA-

funded investigation of normal aging recruited primarily from population-based listings [30]. Only participants of Northern-European ancestry were included in the analysis as sample sizes were not adequate for inclusion of other race groups. All BLSA subjects underwent a complete medical history and physical examination, and subjects with clinical cardiovascular or musculoskeletal disorders that could be adversely affected by exercise testing were excluded. Before the study, all subjects received a complete explanation of the purpose and procedures of the investigation and gave their written informed consent. The experimental protocols were approved by the Institutional Review Boards (IRB) for Medstar Research Institute, Johns Hopkins Bayview Medical Center, and the University of Maryland. The protocols related to the analysis of genetic data were approved by the IRB at the University of Maryland.

Body composition

Body composition variables were obtained using methods previously described by the BLSA [31]. Body mass and height were measured to the nearest 0.1 kg and 0.5 cm, respectively, using a Detecto medical beam scale. Whole-body soft tissue composition was measured by dual-energy X-ray absorptiometry (DXA) with the array mode, as previously described [31]. Total body fat and soft tissue fat-free mass (FFM) and total leg fat and FFM (both legs combined) were assessed. Men and women over 50 years were classified as normal or sarcopenic by the definition of Baumgartner et al. [38]. Women with sarcopenia were defined as having appendicular FFM values $<5.45 \text{ kg/m}^2$, while men with sarcopenia were defined as having appendicular FFM values $<7.26 \text{ kg/m}^2$. Soft tissue FFM was used as a valid indicator of muscle mass based on previous work [32, 33]. The scanner was calibrated daily before testing while reliability was assessed by performing two total body scans, 6 weeks apart, on 12 older men (>65 year). The difference between the two scans was $\sim 0.01 \%$ for fat and FFM.

Peak torque

Peak torque (strength) was measured using the Kinetic Communicator isokinetic dynamometer (Kin-Com model 125E, Chattanooga Group, Chattanooga, TN) and was assessed using the Kin-Com computer software (version 3.2). Shortening phase peak torque (PT) was measured at angular velocities of 0.52 rad/s ($30^\circ/\text{s}$) and 3.14 rad/s ($180^\circ/\text{s}$) for the dominant knee extensors. The terms “shortening” and “lengthening” are substituted throughout the present study for the more commonly used terms “concentric” and “eccentric,” respectively [32]. For each test, subjects performed three maximal efforts, separated by 30 s rest intervals, from which the highest value of the three trials was accepted as the PT. Detailed procedures regarding subject positioning and stabilization, gravity correction, Kin-Com calibration, and test–retest reliability are described elsewhere [31, 34].

Genotyping

Genomic DNA was isolated from EDTA anticoagulated whole blood using standard methods (Puregene DNA Extraction kit, Gentra Systems, Inc., Minneapolis, MN). All subjects were genotyped for the VDR Fok1 (final analyzed $M=422$, $F=339$), Bsm1 ($M=457$, $F=375$), and Taq1 ($M=308$, $F=181$) polymorphisms as described previously [35]. For Taq1 the following primers were used F-5'-GGATCCTAAATGCACGGAGA and R-5'-

TCGGCTAGCTTCTGGATCAT. Amplification products were digested with TaqI for 24 h and resolved on a 2 % agarose gel for genotyping of the following products: TT 335 bp, Tt 335, 224 and 107, and tt 231 and 107. Six sequence-verified control samples from these same BLSA subjects carrying all three genotypes for each polymorphism were used for all assays as positive controls, with ambiguous samples re-genotyped. Samples with ambiguous results in two assays were excluded from all analyses. All DNA sequencing to confirm positive control samples was completed using standard procedures using an Applied Biosystems 3730xl DNA Analyzer. Haplotypes for the BsmI and TaqI polymorphisms were constructed using PHASE software (v2.1) [36, 37]. The haplotypes with frequencies of 10 % and above were analyzed for their association with muscle phenotypes.

Statistical analysis

Two types of analyses were performed, with a three-genotype comparison performed initially followed by a dominant-model two-group analysis with subjects grouped as homozygotes compared to rare-allele carriers. Analysis of variance (ANOVA) models were used to test for differences in physical characteristics among genotype groups. Physical characteristic data are presented as mean $\pm$ SE. Dependent variables were analyzed using analysis of covariance (ANCOVA) and were used to compare means among genotype groups. Analyses were performed within each sex group separately. Significant covariates were included in all models and are listed in each table caption. We initially analyzed all data with the inclusion of age, height and appendicular fat-free mass (aFFM) in the ANOVA model; in a secondary analysis we removed aFFM from the model. We determined linkage disequilibrium between BsmI and TaqI loci with multiple statistics (r , *Lewontin's D* (D') and *Yule's Q* (YQ)). Haplotypes were generated with the expectation maximum algorithm. Only the most common haplotypes (>10 %) were considered for *Chi-square* tests for differences in body composition and strength measures with and without age, height and aFFM in the model. All statistical analyses were performed in SAS® 9.12. Data presented are least squares mean $\pm$ standard error (SE). Statistical significance was defined as $p < 0.05$; when the omnibus p value was < 0.05 for the three-genotype analysis, specific contrasts were examined in two-group models and those p values are reported separately.

Results

FokI polymorphism

A total of 422 men were genotyped for FokI (allele frequencies F/C = 62.6 %, f/T = 37.4 %; FF/CC $n = 152$, Ff/CT $n = 224$, ff/TT $n = 46$); genotypes were not in Hardy–Weinberg equilibrium (HWeq; $P = 0.01$). A total of 339 women were genotyped for FokI (allele frequencies F/C = 64.2 %, f/T = 35.8 %; FF/CC $n = 143$, Ff/CT $n = 149$, ff/TT $n = 47$) and those genotypes were in HWeq ($P = 0.41$).

FokI was analyzed in both a three-genotype model and a two-genotype model (CC/FF vs T/f-allele carriers). Neither male nor female Caucasians differed in either model for any variable, including total fat-free mass (tFFM), or appendicular fat-free mass (aFFM) (Table 1). For FokI males no significant differences were observed for any strength variable measured, though isokinetic peak torque at both $30^\circ \times s^{-1}$ and $180^\circ \times s^{-1}$ s tended to be

different ($P = 0.1$, Table 2). Female ff homozygotes were different for 120° isometric force compared to all other groups, and tended to be different without aFFM included in the model ($P = 0.03$ and $P = 0.11$, respectively) (Table 2). When analyzed in the two-genotype model, significant differences were observed for isokinetic peak torque at both $30^\circ \times s^{-1}$ (CC = FF 138.4 N m^{-1} vs CT/TT 148.7 N m^{-1} , $p = 0.04$) when aFFM was included in the model and at $180^\circ \times s^{-1}$ (CC = FF 100.1 N m^{-1} vs CT/TT 105.4 N m^{-1} , $P = 0.05$) when aFFM was removed from the model. Fok1 females were not different for any of the measured strength variables in the two-genotype model (data not shown).

Bsm1 polymorphism

For Bsm1, 457 men were genotyped (allele frequencies B/A = 44.2 %, b/G = 55.8 %; BB/AA $n = 86$, Bb/AG $n = 232$, bb/GG $n = 139$) and genotypes were in HWeq ($P = 0.53$). A total of 375 women were genotyped (allele frequencies B/A = 43.5 %, b/G = 56.5 %; BB/AA $n = 70$, Bb/AG $n = 186$, bb/GG $n = 119$) and genotypes were also in HWeq ($P = 0.86$).

Bsm1 was analyzed in both a three-genotype model and a two-genotype model (AA/BB vs G/b-allele carriers). Neither males nor females differed in either model for any body composition variable, including tFFM and aFFM (Table 3). In the three-genotype model male Bsm1 GG individuals tended to have higher isokinetic peak torque values at $180^\circ \times s^{-1}$ compared to AA individuals ($P = 0.07$; Table 3). In the two-genotype model a similar relationship was observed for G-allele carriers having greater peak torque values at $180^\circ \times s^{-1}$ ($96.6 \pm 3.5 \text{ N m}^{-1}$) compared to AA individuals ($105.4 \pm 1.7 \text{ N m}^{-1}$, $P = 0.02$). Similarly, female Bsm1 GG carriers had higher isokinetic peak torque values at $30^\circ \times s^{-1}$ ($P < 0.01$; Table 4) and this was true in the two-genotype model as well, with G-allele carriers exhibiting higher isokinetic peak torque values at $30^\circ \times s^{-1}$ ($100.6 \pm 1.8 \text{ N m}^{-1}$ vs 89.8 ± 4.1 , $P < 0.01$).

Taq1 polymorphism

For Taq1, 308 men were genotyped (allele frequencies T/t = 61.5%, C/T = 38.5%; TT/TT $n = 120$, Tt/TC $n = 186$, tt/CC $n = 49$) and genotypes were in HWeq ($P = 0.41$); 181 women were genotyped (allele frequencies T = 57.2 %, C = 42.8 %; TT/TT $n = 54$, Tt/TC $n = 99$, tt/CC $n = 28$) and genotypes were also in HWeq ($P = 0.12$). No significant differences were observed in the three-group analyses for any descriptive, body composition, or strength variable for Taq1 in either men or women (data not shown).

Bsm1–Taq1 haplotype analysis

Since BsmI and TaqI *VDR* polymorphisms have previously been shown to be linked by Windelinckx et al., we tested for linkage disequilibrium in the BLSA cohort. For women ($n = 176$), no linkage disequilibrium was detected by a Chi-square test (Chi = 0.01729, $P = 0.6698$); however, in males ($n = 301$) strong linkage disequilibrium was detected (Chi = 151.077, $P < 0.001$). We calculated r , Lewontin's D (D') and Yule's Q (YQ) statistics for females (LD coefficient = 0.0027; $r = 0.0111$; $D' = 0.0121$; $YQ = 0.0225$) and males (LD coefficient = 0.1712; $r = 0.7134$; $D' = 0.7887$; $YQ = 0.9520$). The data indicated weak linkage in females and strong linkage in males. We then predicted Bsm1–Taq1 haplotypes,

which resulted in four different haplotype alleles and nine [9] haplotype genotypes (data not shown). Only the most common haplotypes (frequencies of >11 % for men and >13 % for women) were analyzed in relation to skeletal muscle traits.

We performed a three-haplotype group analysis using Bt-bT (AC/GT), bT-bT (GT/GT), Bt-Bt (AC/AC) in the males and Bt-bT (AC/GT), Bt-BT (AC/AT), and BT-bT (AT/GT) in females. No significant haplotype differences were observed for any variable in women (data not shown). In men, total FFM was significantly different for haplotype GT/GT (74.4 ± 1.6 kg) vs AC/AC (69.2 ± 2.3 kg) and Bt/bT (70.0 ± 1.3 kg), but this association was lost when age was included as a covariate ($p = 0.19$; GT/GT: 54.9 ± 0.7 , AC/AC: 56.9 ± 1.0 , AC/GT: 54.8 ± 0.6 kg).

Logistic regression and sarcopenia risk

Women with sarcopenia ($n = 106$) represented 37.6 % of the female cohort, while men with sarcopenia ($n = 101$) represented 31.8 % of the male cohort. For women, 41 % of the FF group ($n = 49$) were sarcopenic, while 37 % of the Ff group ($n = 46$) and 27.5 % of the ff group ($n = 11$) were sarcopenic. A Chi-square analysis revealed that the frequency of FF sarcopenic women was significantly different from the expected (Chisq = 108.964, $P < 0.001$). In men, 30 % of the FF group ($n = 36$), 33 % of the Ff group ($n = 56$), and 32 % of the ff group ($n = 9$) were sarcopenic; no significant difference was observed in the men (Chisq = 2.24 $P = 0.3248$). We then analyzed the effect of having the FF (CC) FokI genotype compared to the f(T) allele on risk of sarcopenia in women via age-adjusted logistic regression. This analysis revealed that having the FF genotype gave significant increased odds of sarcopenia of 1.3 (95 % CI 1.2–1.4). The BsmI genotype was not associated with risk of sarcopenia in men or women.

Discussion

The present study provides a detailed replication analysis of the association of the *VDR* gene with sarcopenia risk in older men [13] and extends those original findings to women as well. Though we observed some significant associations between specific *VDR* polymorphisms and both skeletal muscle traits and sarcopenia, the data indicate a generally modest contribution of this gene with sarcopenia risk. When examined in relation to the existing literature on *VDR* genotype and skeletal muscle, the findings argue for continued study of the underlying biology but provide some uncertainty about the likely clinical significance of genotype testing currently in relation to predictive risk for sarcopenia.

Substantial evidence points to the importance of vitamin D in maintaining skeletal muscle structure. With advancing age, vitamin D appears to maintain the function of type II fibers, which contributes to preserving muscle strength and may reduce the risk of falls [15, 16]. Vitamin D supplementation appears important for restoring muscle strength and function in those with low 25-hydroxyvitamin D levels, though any benefits in otherwise healthy elderly are uncertain [39]. *VDR* genotypes were some of the first to be examined in relation to skeletal muscle traits beginning in the 1990s, though inconsistencies have been observed likely owing to sample size and incomplete analysis of the several polymorphisms in the *VDR* gene. Initial studies focused on the BsmI polymorphism. While Geusens et al. [14]

first reported higher quadriceps and grip strength in BsmI b/b compared to B/B genotype carriers, Grundberg and colleagues [40] and Wang et al. [41] found higher strength in B/B compared to b/b carriers. Subsequent studies identified the importance of *VDR* haplotype, encompassing the BsmI, ApaI, and/or TaqI polymorphisms [21]. For example, Windelinckx and coworkers [22] reported that BsmI/TaqI Bt/Bt haplotype homozygotes exhibited greater strength than bT haplotype carriers. Van Pottelbergh et al. [42] reported associations between TaqI/ApaI haplotypes and lean mass in older men, with the highest lean mass observed in the At-At haplotype group. This relationship was not observed, however, in a group of younger men. In the present study, we found that BB genotype carriers exhibited lower quadriceps strength compared to b-allele carriers in both men and women, though no differences in FFM were noted. But in combination with the TaqI polymorphism as a BsmI–TaqI haplotype, no associations were observed in females in the present study and no significant differences remained in men after accounting for differences in age across haplotype groups. In total, these findings point to a small contribution of this *VDR* haplotype to skeletal muscle traits that may not be clinically relevant.

The other polymorphism of focus in *VDR* is the FokI polymorphism, which is known to affect the translational start site of the gene [19, 20] thus making it a potentially functional polymorphism, though it is not in linkage disequilibrium with the BsmI or TaqI polymorphisms. In our previous work, we observed higher knee extensor strength in FokI f/f carriers compared to F/F carriers, but this difference was fully accounted for by higher FFM in the f/f carriers [13]. It was that same study that identified the significant association with sarcopenia, the risk for which was twofold higher in F/F genotype men. Similarly in the present study, the odds of sarcopenia were significantly, if moderately, higher in F/F women compared to f-allele carriers. Though no significant differences in sarcopenia risk were observed in men in the present study, men f-allele carriers exhibited significantly higher quadriceps strength compared to FF genotype carriers. Both Windelinckx et al. [22] and Hopkinson et al. [26] have also reported higher strength levels in FokI f/f compared to F/F genotype carriers. Thus, several studies have now provided evidence that the FokI polymorphism in *VDR* is associated with skeletal muscle traits, with the F-allele appearing to be a risk allele for reduced muscle strength and sarcopenia.

The present study is not without limitations. We were not able to address *VDR* genotype interactions with vitamin D levels in the present analysis, though this has recently been shown to be important in prostate cancer [43, 44]. Though the sample size was large in comparison to most gene association studies for skeletal muscle traits, much larger studies are common when investigating the genetics of other complex traits. Importantly, the present study replicates previous findings in an independent cohort and such replication is considered a gold standard for determining when a gene is important for a trait, especially for genes of generally weak influence [45].

The present study extends the existing literature in this area by providing additional support for a modest role for *VDR* genotypes in altering skeletal muscle traits and sarcopenia risk. Such replication analyses are critical for identifying polymorphisms with potential clinical significance in genomic or personalized medicine. That said, the existing literature shows considerable variability in results owing to an incomplete understanding of the various

interacting factors that influence the *VDR* genotype and skeletal muscle relationship. Future clinical studies should examine the interaction of vitamin D levels, which are known to contribute independently to muscle strength and function, with *VDR* genotype to determine the clinical importance of genetic screening of *VDR* polymorphisms as part of age-related skeletal muscle interventions.

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Table 1Descriptive statistics by *VDR* Fok1 polymorphism

Men, three-genotype model Fok1				
<i>VDR</i> -Fok1	CC/FF	CT/FF	TT/ff	<i>P</i> value
<i>N</i>	152	224	46	
Age (years)	68.8 ± 1.3	69.1 ± 1.1	68.5 ± 2.4	0.9
Height (cm)	176.2 ± 0.6	176.0 ± 0.5	175.5 ± 1.1	0.9
Total fat mass (kg)	24.6 ± 0.9	26.0 ± 0.7	23.8 ± 1.8	0.3
Fat-free mass (kg)	55.6 ± 0.6	56.0 ± 0.5	54.5 ± 1.3	0.6
Appendicular fat-free mass (kg)	24.1 ± 0.4	24.1 ± 0.3	23.8 ± 0.7	0.9
Women, three-genotype model Fok1				
<i>VDR</i> -Fok1	CC/FF	CT/FF	TT/ff	<i>P</i> value
<i>N</i>	143	149	47	
Age (years)	62.0 ± 1.5	61.6 ± 1.5	62.5 ± 2.6	0.9
Height (cm)	163.9 ± 0.6	163.0 ± 0.6	162.1 ± 1.0	0.3
Total fat mass (kg)	27.0 ± 1.0	28.7 ± 1.0	27.0 ± 1.6	0.4
Fat-free mass (kg)	39.8 ± 0.4	40.0 ± 0.4	39.1 ± 0.7	0.7
Appendicular fat-free mass (kg)	16.0 ± 0.3	16.0 ± 0.3	16.0 ± 0.5	0.9

Data are presented as means ± standard error

Table 2

Strength variables by *VDR* Fok1 polymorphism

Men, three-genotype model Fok1				
<i>VDR</i> -Fok1	CC/FF	CT/FF	TT/ff	<i>P</i> value
Dominant quad 30°/s (N m ⁻¹)	138.4 ± 3.9	149.1 ± 3.3	146.3 ± 8.4	0.1
	142.2 ± 3.4	151.1 ± 2.8	145.4 ± 6.2	0.1
Dominant quad 180°/s (N m ⁻¹)	98.2 ± 2.5	104.2 ± 2.1	102.55 ± 5.4	0.19
	100.1 ± 2.2	105.9 ± 1.9	103.0 ± 4.0	0.13
Dominant quad 120° isometric (N m ⁻¹)	438.5 ± 12.9	450.9 ± 11.2	438.0 ± 25.8	0.7
	431.3 ± 12.7	453.1 ± 11.2	444.0 ± 24.0	0.4
Dominant quad 140° isometric (N m ⁻¹)	322.5 ± 9.7	308.6 ± 8.5	312.9 ± 19.4	0.6
	317.0 ± 9.4	309.2 ± 8.3	318.2 ± 17.7	0.8
Grip strength right (kg)	36.5 ± 0.7	36.4 ± 0.6	37.6 ± 1.4	0.7
Grip strength left (kg)	34.7 ± 0.6	34.4 ± 0.5	35.0 ± 1.3	0.9
Women, three-genotype model Fok1				
<i>VDR</i> -Fok1	CC/FF	CT/FF	TT/ff	<i>P</i> value
Dominant quad 30°/s (N m ⁻¹)	97.4 ± 2.7	94.5 ± 2.7	101.9 ± 4.8	0.4
	100.2 ± 2.5	99.8 ± 2.4	102.8 ± 4.4	0.8
Dominant quad 180°/s (N m ⁻¹)	66.7 ± 1.9	63.7 ± 1.9	68.5 ± 3.3	0.4
	67.8 ± 1.8	66.5 ± 1.8	69.6 ± 3.1	0.7
Dominant quad 120° isometric (N m ⁻¹)	350.5 ± 9.5	337.7 ± 8.9	387.7 ± 16.8	0.03
	343.9 ± 9.8	340.5 ± 9.4	382.2 ± 17.7	0.11
Dominant quad 140° isometric (N m ⁻¹)	257.5 ± 8.4	253.4 ± 7.8	252.9 ± 14.8	0.9
	252.9 ± 8.5	255.5 ± 8.2	250.2 ± 15.4	0.9
Grip strength right (kg)	22.7 ± 0.5	23.6 ± 0.5	24.8 ± 0.9	0.08
Grip strength left (kg)	21.6 ± 0.5	22.1 ± 0.5	22.8 ± 0.9	0.5

Data are presented as least square means ± standard error. *Top values* results when covaried for age, height, and appendicular FFM (aFFM). *Bottom values* results when aFFM is removed from the model

Table 3Descriptive statistics by *VDR* Bsm1 polymorphism

Men, three-genotype model				
<i>VDR</i> -Bsm1	AA/BB	AG/Bb	GG/bb	<i>P</i> value
<i>N</i>	86	232	139	
Age (years)	67.0 ± 1.8	67.2 ± 1.1	70.6 ± 1.4	0.13
Height (cm)	176.6 ± 0.8	176.0 ± 0.5	176.1 ± 0.7	0.9
Total fat mass (kg)	25.1 ± 1.2	25.0 ± 0.7	26.2 ± 1.0	0.6
Fat-free mass (kg)	56.8 ± 0.9	56.3 ± 0.5	55.0 ± 0.7	0.2
Appendicular fat-free mass (kg)	24.5 ± 0.5	24.4 ± 0.3	23.9 ± 0.4	0.5
Women, three-genotype model				
<i>VDR</i> -Bsm1	AA/BB	AG/Bb	GG/bb	<i>P</i> value
<i>N</i>	70	186	119	
Age (years)	61.3 ± 2.1	61.0 ± 1.3	61.7 ± 1.6	0.9
Height (cm)	163.5 ± 0.8	163.4 ± 0.5	163.0 ± 0.6	0.9
Total fat mass (kg)	29.3 ± 1.4	27.4 ± 0.8	26.2 ± 1.1	0.2
Fat-free mass (kg)	39.4 ± 0.6	39.4 ± 0.4	40.1 ± 0.5	0.5
Appendicular fat-free mass (kg)	16.0 ± 0.4	16.0 ± 0.3	16.1 ± 0.3	0.9

Data are presented as least square means ± standard error

Table 4

Strength measures by *VDR* Bsm1 polymorphism

Men, three-genotype model				
<i>VDR</i> -Bsm1	AA/BB	AG/Bb	GG/bb	<i>P</i> value
Quadriceps 30°/s (N m ⁻¹)	141.5 ± 5.4	149.2 ± 3.3	149.0 ± 4.5	0.45
	144.8 ± 4.7	149.4 ± 2.8	151.9 ± 3.7	0.49
Quadriceps 180°/s (N m ⁻¹)	96.6 ± 3.5	105.0 ± 2.1	106.2 ± 2.9	0.07
	99.5 ± 3.1	104.8 ± 1.8	109.2 ± 2.4	0.05
Quadriceps 120° isometric (N m ⁻¹)	447.5 ± 19.1	449.9 ± 11.4	448.2 ± 14.9	0.9
	442.5 ± 19.1	450.5 ± 11.2	451.2 ± 14.4	0.9
Quadriceps 140° isometric (N m ⁻¹)	321.0 ± 13.8	323.2 ± 8.2	312.6 ± 10.8	0.7
	317.7 ± 13.7	323.1 ± 8.0	314.8 ± 10.3	0.8
Grip strength right (kg)	37.9 ± 0.9	37.4 ± 0.6	36.6 ± 0.8	0.5
Grip strength left (kg)	34.9 ± 0.8	35.0 ± 0.5	35.0 ± 0.7	0.9
Women, three-genotype model				
<i>VDR</i> -Bsm1	AA/BB	AG/Bb	GG/bb	<i>P</i> value
Quadriceps 30°/s (N m ⁻¹)	89.8 ± 4.1	97.5 ± 2.3	105.2 ± 2.3	0.006
	95.6 ± 3.8	101.1 ± 2.1	105.1 ± 2.6	0.11
Quadriceps 180°/s (N m ⁻¹)	65.7 ± 2.8	65.3 ± 1.6	70.0 ± 1.9	0.15
	67.8 ± 2.8	66.9 ± 1.6	70.2 ± 1.9	0.4
Quadriceps 120° isometric (N m ⁻¹)	337.0 ± 18.6	372.7 ± 11.2	355.7 ± 13.9	0.2
	330.8 ± 20.1	366.1 ± 11.7	354.9 ± 14.4	0.3
Quadriceps 140° isometric (N m ⁻¹)	255.2 ± 13.3	267.9 ± 8.0	261.7 ± 10.0	0.7
	250.4 ± 14.5	263.7 ± 8.4	261.8 ± 10.4	0.7
Grip strength right (kg)	23.8 ± 0.7	23.2 ± 0.4	23.4 ± 0.5	0.8
Grip strength left (kg)	22.3 ± 0.7	21.9 ± 0.4	21.6 ± 0.6	0.8

Data are presented as least square means ± standard error. *Top values* results when covaried for age, height, and appendicular FFM (aFFM). *Bottom values* results when aFFM is removed from the model