

Vitamin D receptor gene polymorphisms and musculoskeletal injuries in professional football players

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Abstract. The aim of the present study was to investigate the association between vitamin D receptor (VDR) gene polymorphisms and musculoskeletal injury (MI) in elite football players. In total, 54 male professional football players were recruited from an official Italian professional championship team between 2009 and 2013. The cohort was genotyped for the *ApaI*, *BsmI* and *FokI* polymorphisms and MI data were collected over four football seasons. No significant differences were identified among the genotypes in the incidence rates or severity of MI ($P=0.254$). In addition, no significant associations were observed between VDR polymorphisms and MI phenotypes ($P=0.460$). However, the results of the casewise multiple regression analysis indicated that the *ApaI* genotypes accounted for 18% of injury severity ($P=0.002$). Therefore, while the *BsmI* and *FokI* polymorphisms did not appear to be associated with the severity or incidence of MI, the *ApaI* genotypes may have influenced the severity of muscle injury in top-level football players.

Introduction

The vitamin D receptor (VDR) gene is located on human chromosome 12 (12q12-q14) and is 100 kb in length, with >100 restriction endonuclease cutting site polymorphisms (1). VDR is a member of the steroid superfamily of nuclear receptors, which serve key functions in the regulation of the transcriptional activity of the vitamin D metabolite $1\alpha, 25$ -dihydroxyvitamin D₃. Vitamin D performs an established role in calcium metabolism, increasing the absorption of calcium and phosphate from the intestines and the reabsorption of calcium in the kidneys. Furthermore, vitamin D is hypothesized to be crucial in skeletal muscle function, and VDRs have been identified in this tissue (2,3). In addition,

vitamin D has been hypothesized to influence myocytes via VDRs, resulting in myocyte proliferation, differentiation, growth and inflammation (4).

The VDR gene contains numerous polymorphisms. A polymorphic start codon in the 5' end of the gene is identified by the restriction enzyme *FokI*. There are three polymorphisms at the 3' end of the VDR gene, generating the *BsmI*, *ApaI* and *TaqI* restriction sites (5).

These functional genetic polymorphisms in the VDR gene may be associated with tissues that respond to vitamin D, including muscle cells and adipocytes. Skeletal muscle is established to be a target organ for vitamin D, and vitamin D metabolites directly affect muscle cell metabolism via a number of pathways (6). Furthermore, previous studies have associated vitamin D deficiency with muscular weakness (6,7).

Numerous cohort and cross-sectional studies have investigated the associations between vitamin D status and various parameters of neuromuscular performance. Prior studies have indicated that vitamin D exerts a beneficial effect on these parameters (7-10); however, certain other studies have contradicted these findings (11-14).

Muscle injury is a major problem for football players and has been reported to account for 20-37% of all injury-associated time loss at the male professional level and 18-23% at the amateur level (15,16). Muscle weakness may be one of the primary factors determining the outcome of athletic injuries (17).

In a published abstract, Shindle *et al* (18) assessed vitamin D levels and skeletal muscle injuries in 89 professional American football players and observed that players who suffered at least one muscle injury throughout the prior season exhibited significantly lower vitamin D levels compared with players that sustained no muscle injury during the same period. A recent study aimed to assess vitamin D levels in professional American football players and to evaluate the association between vitamin D levels and ethnic group, fracture history, and the ability to obtain a contract position (19). The study indicated that professional football players of African descent exhibit a higher rate of vitamin D deficiency compared with Caucasian players. Furthermore, professional football players with higher vitamin D levels were more likely to obtain a contract position in the National Football League. The authors hypothesized that professional football players deficient in

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Table I. Characteristics of the players by VDR polymorphism and respective genotypes (*ApaI*: AA, Aa or aa; *BsmI*: BB, Bb or bb; and *FokI*: FF, Ff or ff).

Polymorphism	Age (years)	Height (cm)	Weight (kg)	Seasons played (n)
<i>ApaI</i> AA	25.0±5.3	180.1±5.6	71.7±3.7	2.0±1.0
<i>ApaI</i> Aa	25.8±3.3	184.5±3.7 ^{ab}	76.2±5.5 ^a	1.7±1.0
<i>ApaI</i> aa	27.6±4.3	177.8±6.2	72.8±4.2	2.2±1.2
<i>BsmI</i> BB	26.3±5.4	180.6±4.8	72.0±3.6	2.0±1.2
<i>BsmI</i> Bb	25.3±3.9	184.0±4.4	75.1±5.9	1.8±1.0
<i>BsmI</i> bb	26.4±4.1	180.0±6.3	73.8±4.6	2.0±1.0
<i>FokI</i> FF	25.5±2.9	180.9±5.5	73.4±4.5	1.6±0.9
<i>FokI</i> Ff	27.0±5.2	181.6±5.4	73.8±5.7	2.1±1.1
<i>FokI</i> ff	24.5±4.9	185.4±4.8	76.0±5.3	2.2±1.2

Data are expressed as the mean ± standard deviation. ^aP<0.01 vs. AA and ^bP<0.01 vs. aa. VDR, vitamin D receptor.

vitamin D level may be at a greater risk of bone fracture. In addition, a number of previous studies have suggested associations between certain genetic markers and the susceptibility of players to injury and performance while participating in sport (20,21). Thus, the performance of professional football players and their susceptibility to injury may be influenced by genetic factors (22-34).

The present study aimed to analyze the associations between the VDR gene polymorphisms *FokI*, *BsmI* and *ApaI* and indirect musculoskeletal injury (MI) rates in professional football players over the course of four competitive seasons.

Materials and methods

Participants. The sample population contained 54 male professional football players (age, 25.9±4.3 years; weight, 73.9±5.0 kg; height, 181.7±5.5 cm) from an Italian professional championship team. All the subjects were of Caucasian descent for at least three generations. The data for 25 players (46.2%) were collected between 2009 and 2010; data for 15 players (27.7%) were collected between 2009 and 2011; and data for 14 players (25.9%) were collected between 2009 and 2013.

Players trained for ~six weeks (two sets of 120 min per day; five days per week) pre-season and ~32 weeks during the competitive season (520 min per week). Players joining/leaving the cohort were included/excluded from the date of joining/leaving. Players with an existing injury at the start of the study were not excluded from the study; however, their existing injuries were not included in the study. Training exposure was defined as any team-based or individual physical activity, conducted under the control or guidance of the team's coaching and fitness staff, that was aimed at maintaining or improving players' football skills or physical condition. Matches between teams were considered to be training exposure. Any match activity that was a part of a player's rehabilitation from injury was not recorded as match exposure.

Experiments were undertaken with the written informed consent of each participant and the study protocol was approved by the Cagliari Calcio ethics committee (Cagliari, Sardinia). The present study was conducted in accordance

Table II. Reaction mix for PCR analysis, µl.

Reagent	<i>FokI</i>	<i>ApaI</i>	<i>BsmI</i>
Reaction buffer	2.5	3.5	1.5
MgCl ₂	0.8	3.5	0.75
dNTPs (200 µM each)	1	1	1
Primer F (0.5 µM)	0.2	0.2	0.4
Primer R (0.5 µM)	0.2	0.2	0.4
H ₂ O	14.2	14.5	18.85
Taq polymerase	0.1	0.1	0.1
DNA	2	2	2
Total volume	21	25	25

PCR, polymerase chain reaction; dNTP, deoxynucleotide triphosphate.

with the Declaration of Helsinki for Human Research of 1974 (last modified in 2000).

Study design. Recruitment, genotyping and statistical analyses were performed following the guidelines described in 'replicating genotype-phenotype associations' (35). The study design followed the consensus on definitions and data collection procedures in studies of football injury as outlined in the consensus document (36) and by the Union of European Football Associations (37).

Injury data collection. MI was defined as any physical condition that occurred during practice that prevented a player from participating in training or match play for at least one day following onset (38). Injuries were categorized under four degrees of severity based on the number of days absence incurred: Minimal (code 1), 1-3 days; mild (code 2), 4-7 days; moderate (code 3), 8-28 days; and severe (code 4), >28 days. The registration of an MI was based on a clinical examination by the football team medical staff. Ultrasound and magnetic resonance imaging scans were used to morphologically classify the injuries. Time loss due to injury was recorded

Table III. Injury incidence and severity between VDR genotypes.

A, Injury incidence									
Parameter	<i>ApaI</i>			<i>BsmI</i>			<i>FokI</i>		
	AA	Aa	aa	BB	Bb	bb	FF	Ff	ff
Injury incidence	0.19±0.34	0.21±0.60	0.80±0.93	0.17±0.27	0.22±0.64	0.45±0.72	0.30±0.60	0.37±0.62	0.20±0.60
Athletes (n)	20	26	8	12	23	19	25	21	8
B, Injury severity									
Parameter	<i>ApaI</i>			<i>BsmI</i>			<i>FokI</i>		
	AA	Aa	aa	BB	Bb	bb	FF	Ff	ff
Injury severity	2.62±1.06	3.2±0.44	3.6±0.54	2.4±1.44	3.25±0.51	3.33±0.70	3.25±0.88	3.11±0.60	3.0±0.33
Athletes (n)	8	5	5	5	4	9	8	7	3

Data are expressed as the mean ± standard deviation. VDR, vitamin D receptor.

on a weekly basis by the medical staff using a standardized injury report form during the preseason and regular season. Structural-mechanical injuries (such as tendon ruptures and total/partial muscle ruptures) and functional injuries (such as fatigue-induced or neurogenic muscle hardening/hypertonia) or cramps, were included in the injury group, whereas contusions and hematomas were excluded. MI incidence was calculated per 1,000 h of training exposure (training and matches).

DNA analysis. Genomic DNA was extracted from buccal swab samples using a QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany). Extracted DNA was analyzed by quantitative polymerase chain reaction (Gene Amp® PCR System 2720; Applied Biosystems Life Technologies, Foster City, CA, USA) using the primers suggested by Rezende *et al* (39).

The reaction was performed as reported in the Table I. The running conditions were as follows: Predenaturation at 95°C for 5 min, followed by 35 cycles of denaturation (94°C for 1 min), annealing (59°C for 30 min), extension (72°C for 1 min) and a final extension at 72°C for 5 min.

Amplified products were digested with specific restriction enzymes (*FokI*, *ApaI* and *BsmI*), that generated the following fragments: *FokI*, 272 bp for the wild-type allele (allele F) or 198 and 74 bp for the polymorphic variant (allele f); *ApaI*, 740 bp for the wild-type allele (allele A) or 515 and 225 bp for the polymorphic variant (allele a); *BsmI*, 825 bp for the wild-type allele (allele B) or 650 and 175 bp for the polymorphic variant (allele b). Fragments were separated by electrophoresis in 8% polyacrylamide gel and visualized by silver staining (15).

Statistical analysis. Analysis of variance (ANOVA) was used to compare genotypes and continuous data. Scheffé post hoc

analysis was used to identify significant differences between the three genotypes. Factorial ANOVA was used to examine associations among the three polymorphisms and injury parameters.

Multivariate analysis was used to determine the model that most accurately predicted MI incidence and severity. Factors that were significantly associated with MI incidence (age and height) and severity (min of match exposure) and VDR polymorphisms were included in the model. The Hardy-Weinberg equilibrium was calculated using Genepop software, version 4.0.10 (genepop.curtin.edu.au). Data were analyzed using Statistica software, version 7.0 (StatSoft, Inc., Tulsa, OK, USA).

Results

Height and weight. The VDR *BsmI* (BB, 22.2%, n=12; Bb, 42.5%, n=23; and bb, 35.1%, n=19), *ApaI* (AA, 37%, n=20; Aa, 48.1%, n=26; and aa, 14.8%, n=8) and *FokI* (FF, 46.3%, n=25; Ff, 38.8%, n=21; and ff, 14.8%, n=8) polymorphisms concurred with the Hardy-Weinberg equilibrium ($P=0.325$). Age, height, weight and the number of seasons played were not observed to be significantly different between the *BsmI* ($P=0.295$) and *FokI* ($P=0.203$) genotypes (Table II). However, carriers of the Aa genotype for the *ApaI* polymorphism exhibited higher height and weight compared with carriers of the AA and aa genotypes ($P=0.002$).

Muscle injury. No significant differences were identified in MI incidence/severity between genotypes ($P=0.254$) (Table III).

Factorial analysis suggested no significant association between the *BsmI*, *ApaI* and *FokI* polymorphisms and the incidence or severity of MI ($F=0.557$; $P=0.460$).

The age, height and VDR polymorphisms of the players were assessed for their association with MI incidence. VDR

polymorphisms and match exposure were assessed for their contribution to MI severity variance using a casewise multiple regression model. The optimum model for MI incidence, which accounted for 12% ($P=0.171$; standard error=2.69) of the variance, included height and age. However, only height ($R^2_{adj}=6.83$; $P=0.034$) was significantly associated with the total incidence of injury model. The model for MI severity, which accounted for 28% ($P=0.035$, standard error=23.06) of the variance, included the *ApaI* genotypes and mins of match exposure, but only *ApaI* genotypes ($R^2_{adj}=18.72$; $P=0.041$) contributed significantly to the severity of injury model.

Discussion

To the best of our knowledge, the present study is the first to analyze the influence of VDR polymorphisms on MI in professional football players.

The results suggested that *BsmI* and *FokI* polymorphisms were not associated with MI incidence or severity in elite football players. However, it is possible that the *ApaI* polymorphism may be associated with the severity of MI. The forward stepwise multiple regression model indicated that the *ApaI* polymorphism of the VDR gene contributed significantly to the MI severity variance ($P<0.05$).

Hormonally active forms of vitamin D mediate their effects via agonist action at VDRs, which are transcription regulators principally located in the nuclei of target cells. Vitamin D deficiency has been associated with muscle weakness (6,7), predominantly type II fibre atrophy, accompanied by fibre necrosis and fatty infiltration. This may occur due to reduced calcium uptake by the sarcoplasmic reticulum and the impairment of glycolysis by phosphate depletion.

VDR polymorphisms are associated with differences in strength in premenopausal females. Furthermore, VDR expression levels reduce with age and VDR genotypes have been associated with fat-free mass and strength in elderly individuals (40). Few studies have specifically examined the association between VDR polymorphisms and exercise training, and to the best of our knowledge only one study has analyzed the association between VDR polymorphisms and football performance (23). A previous study (41) observed a significant increase in the expression levels of VDR at day 7 of muscle regeneration. These findings confirmed the expression of VDR *in vivo* and indicated that vitamin D may be involved in skeletal muscle regeneration following injury.

The present study identified no associations between the *BsmI* and *FokI* genotypes and the incidence or severity of MI. However, the *ApaI* polymorphism contributed significantly to the MI severity variance, suggesting that *ApaI* may affect the timing of skeletal muscle regeneration following injury. Studies involving larger cohorts are required to fully understand the association between VDR polymorphisms and the risk of MI in football players.

Therefore, the present study indicates the importance of the VDR *ApaI* polymorphism for the assessment of the risk of football players incurring musculotendinous injuries and to improve their recovery period following injury.

Predictive genomic DNA profiling for injury predisposition may be used by football professionals, in addition to other more common parameters, to aid in the formulation of

personalized training to achieve optimal performance within safety limits for each athlete and to adopt individual protocols for the prevention of injury.

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