ANGIOTENSIN-CONVERTING ENZYME/VITAMIN D RECEPTOR GENE POLYMORPHISMS AND BIOELECTRICAL IMPEDANCE ANALYSIS IN PREDICTING ATHLETIC PERFORMANCES OF ITALIAN YOUNG SOCCER PLAYERS

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ABSTRACT

Micheli, ML, Gulisano, M, Morucci, G, Punzi, T, Ruggiero, M, Ceroti, M, Marella, M, Castellini, E, and Pacini, S. Angiotensin converting enzyme/vitamin D receptor gene polymorphisms and bioelectrical impedance analysis in predicting athletic performances of Italian young soccer players. *J Strength Cond Res* 25(8): 2084–2091, 2011—We evaluated the association between 2 genetic polymorphisms known to be involved in fitness and performance, and anthropometric features, body composition, and athletic performances in young male soccer players with the goal of identifying genetic profiles that can be used to achieve maximal results from training. One hundred twenty-five medium–high-level male soccer players were genotyped for angiotensin-converting enzyme (ACE) I/D, and vitamin D receptor (VDR) *Fok*I gene polymorphisms and scored for anthropometric measurements, body composition, and athletic performance. Body mass index, fat mass, fat-free mass, resistance, reactance, impedance, phase angle (PA), and body cell mass were measured. Athletic performance was evaluated by squat jump, countermovement jump (CMJ), 2-kg medicine ball throw, 10- and 20-m sprint time. We observed that the homozygous *ff* genotype of the VDR gene was significantly more represented in young soccer players than in a matched sedentary population. Values of reactance and PA were differently distributed in ACE and VDR genotypes with high mean values in subjects with DD (ACE) and FF (VDR) genotypes. No correlation was observed between ACE or VDR genotypes and 2-kg medicine ball throw, 10- and 20-m sprint times. The ID genotype of ACE was associated with the best performances in squat jump and CMJ. Our results suggest that determination of ACE and VDR genotypes might help select those young athletes harboring the most favorable genetic potential to succeed in soccer.

KEY WORDS genetics, athletic performance, soccer, training

INTRODUCTION

It is widely accepted that athletic performance is the result of a combination of the most favorable genotypes with exposure to highly specialized training environments (8). Among the genes involved in predicting athletic phenotype and individual response to training, the angiotensin-converting enzyme (ACE) and the vitamin D receptor (VDR) genes play a crucial role because of their involvement in a variety of performance-related functions (1,18). In previous studies, polymorphisms of the ACE gene were associated with cardiovascular and muscle physiology and with a number of performance-related traits typical of professional athletes (7). Also, the polymorphisms of the VDR gene were associated with differences in individual response to training (24) thanks to the pivotal role of vitamin D in a number of metabolic pathways related to physiology of the cardiovascular system and muscle contraction (25).

Even though genetics might be the tool of the future to predict athletic performance and design individualized training, as of today, evaluation of physical response to exercise and training is still routinely determined by laboratory and field tests such as jump test (squat jump [SJ], countermovement jump [CMJ]), medicine ball throw, and

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sprint tests. Field tests provide results that are specific for each sport and are regarded by many as more accurate than laboratory tests (27,30). Anthropometric characteristics related to physiological characteristics (29) and body composition (16) are also commonly measured to have a more complete assessment of nutritional and health status. Body composition is commonly evaluated by bioelectrical impedance analysis that estimates the size of different body compartments such as fat-free mass (FFM), fat mass (FM), and body cell mass (BCM) (10,13). Furthermore, some "pure" bioelectrical parameters such as resistance (R), reactance (X_c), and phase angle (PA), without the use of regression equations specific for population, are considered valid tools to assess the nutritional and metabolic status of athletes and to estimate the changes of soft tissue hydration (2,9,10,15,20,21,23).

In this study, we investigated the association between ACE and VDR gene polymorphisms, bioelectrical impedance analysis parameters, anthropometric features, and athletic performance in young male soccer players; from a gene analysis perspective, the goal of this study is to identify genetic profiles that are associated with performance and can be used to select the young athletes harboring the most favorable genetic potential to succeed in soccer and to train them according to their characteristics.

Methods

Experimental Approach to the Problem

The distribution of ACE and VDR gene polymorphisms in a group of young Italian medium–high-level soccer players was determined, and the results were compared to the distributions of such polymorphisms in sedentary control populations. Furthermore, the association between anthropometric features, bioelectrical impedance analysis parameters, field physical tests, and genotypes within the group of young soccer players was evaluated by means of statistical analysis.

Subjects

One hundred twenty-five medium–high-level soccer players (Caucasian men under 17 years of age) were enrolled by the Training Methodology and Applied Biomechanics Laboratory, Technical Division; *Federazione Italiana Gioco Calcio* (the Italian soccer federation) *FIGC* of Coverciano, Firenze, Italy. The study was performed in accordance with the required ethical standards. Written informed consent to participate in the study was obtained from the interested subject and from a parent or a tutor according to current Italian law; signed informed consent forms are archived at the Department of Anatomy, Histology, and Forensic Medicine, Università degli Studi di Firenze, Viale Morgagni 85, Italy. The study protocol was in accordance with the Declaration of Helsinki for Human Research.

The subjects trained on average 3 times a week (90–120 minutes per sessions) and played 1 match per week. Collection of hair for genotyping, measurement of anthropometry, body composition, and athletic performance reported in this study were measured once, about 1 month after the beginning of the regular season, that is, in the month of October. All subjects had been training and playing in the previous regular season. Measurements were performed at 9 AM, about 1 hour after a typical light Italian breakfast mainly composed of carbohydrates (1 glass of fruit juice, bread, and marmalade, or bread and chocolate spread, following the example of the National Football Team). All measurements were performed in a temperature-controlled room with temperature set at 24°C.

Data for sedentary control populations were obtained from current literature.

Table 1. Distribution of ACE I/D genotype in young soccer players and comparison with control population.*

		Genotype frequency (athletes)	Genotype frequency (control)	p
ACE	DD	0.51 (64/125)	0.44 (67/152)	
	ID	0.42 (52/125)	0.43 (66/152)	0.09
	II	0.07 (9/125)	0.13 (19/152)	

*Control population: 152 healthy subjects (26).

Procedures

Anthropometric Measurement, Body Composition Assessment, and Physical Performance Evaluation. Height, weight, and date of birth were recorded. Body composition was assessed by single frequency (50-kHz) bioelectrical impedance analysis with the standard hand–foot electrode placement (2) (BIA 101 Anniversary Akern Srl, Firenze, Italy). Results of the conventional bioelectrical impedance analysis such as FM, FFM, and BCM were obtained by the algorithms of the software Bodygram PRO (Akern Srl). The R and X_c values

Table 2. Distribution of VDR *Fok*I genotype in young soccer players and comparison with control population.*

		Genotype frequency (athletes)	Genotype frequency (control)	p
VDR	*FF*	0.52 (64/125)	0.54 (81/150)	
	Ff	0.34 (43/125)	0.39 (59/150)	0.005
	ff	0.14 (18/125)	0.07 (10/150)	

*Control population: 150 healthy subjects (17).

were normalized by the standing height (*H*), then plotted on the *R*–X_c graph and evaluated with bioelectrical impedance vector analysis (11,21,23). The PA was calculated by the mathematical formula arctan (X_c/R).

Athletic performance was studied by standard functional performance field tests (4–6,27,28,30) that is, the SJ and the CMJ, evaluated by the "Bosco System" force platform, Globus Italia Srl, Treviso, Italy), and 10- and 20-m sprint time (recorded by TAC photocells; TT Sport, Srl, *Repubblica di San Marino*). Furthermore, a specific test for soccer players with a 2-kg medicine ball throw was used to evaluate the upper limbs explosive strength. All these tests were performed in the gymnasium of the Training Methodology and Applied Biomechanics Laboratory at about 10 AM.

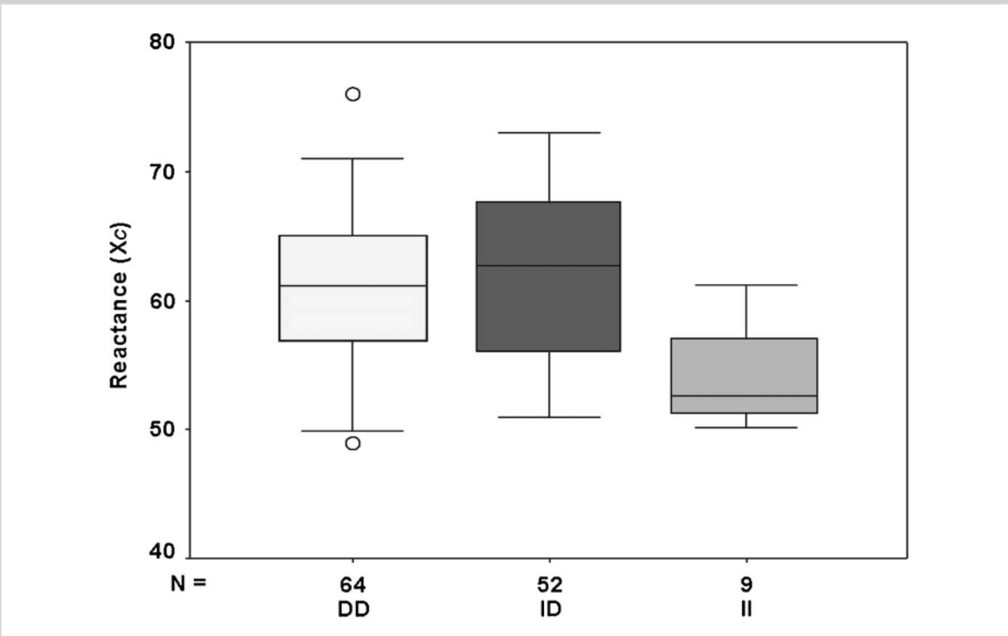

Figure 1. Box plot representation of the distribution of X_c values for each angiotensin-converting enzyme (ACE) I/D genotype.

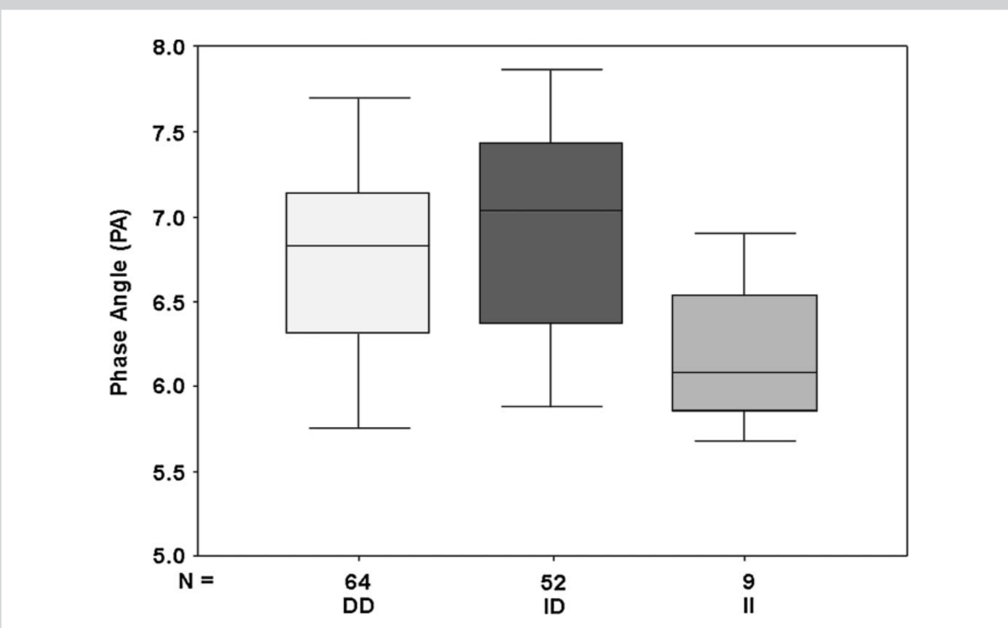

Figure 2. Box plot representation of the distribution of phase angle (PA) values for each angiotensin-converting enzyme (ACE) I/D genotype.

Vitamin D Receptor and Angiotensin-Converting Enzyme Genotyping. DNA was obtained from the hair of each subject using a QIAamp® DNA Tissue Mini Kit (Qiagen S.p.A., Milano, Italy) and amplified by a polymerase chain reaction (PCR). Amplification was performed in a final volume of 50 μL containing 200 ng of genomic DNA, 240 ng of each primer (Genenco M-medical S.r.l., Milano, Italy), and 25 μL of HotStarTaq Master Mix Kit (Qiagen S.p.A.), using standard conditions on a Mini Cycler (Mj Research Inc. Genenco M-medical S.r.l., Milano, Italy). For detection of the polymorphic *Fok*I restriction enzyme site, 2 primers were used: downstream primer 5′-AGC TGG CCC TGG CAC TGA CTC TGC TCT, and upstream primer 5′-ATG GAA ACA CCT TGC TTC TTC TCC CTC. Samples were then digested at 55°C for 3 hours with the appropriate restriction enzyme (*Fok*I; Fermentas M-medical S.r.l., Milano, Italy). The digested samples were then analyzed on 2% agarose gels (Shelton Scientific Corporation, Peosta, IA, USA) after ethidium bromide staining. Absence or presence of the *Fok*I restriction site was denominated "*F*" and "*f*," respectively.

For ACE polymorphism analysis, each genotype was identified by the amplification of a sequence in the intron 16, using appropriate primers (1). Because the D allele in heterozygous subjects is preferentially amplified, each DD genotype was confirmed by a second independent PCR with a primer pair that amplified the insertion-specific sequence (1). Primers and restriction enzyme were purchased from Fermentas M-medical Srl. The DNA fragments were analyzed, on 2% agarose gels (Shelton Scientific Corporation).

Statistical Analyses

The differences between observed and expected frequency genotype distributions were evaluated by the χ^2 test. The χ^2 test was used to test association of categorical data. Differences in frequency genotype distributions between athletes and control population were considered statistically significant when p was $\leq$0.05.

Comparison of value distribution of X_c, PA, BCM/FFM, SJ, and CMJ in each ACE and VDR genotype was analyzed by analysis of variance F-test (performed by SAS 9.1 software) because >2 groups were compared at the same time. The graphic representation of the value distribution of each parameter according to each genotype was performed by box plot charts.

In the box plot charts, the box bounds the first and third quartiles (interquartile range, corresponding to the box width), encompasses 50% of the data and includes the median (line within the box). Dispersion of the data above and below this range is marked by 'whiskers' that extend to the most extreme values within a "fence" at 1.5 times the interquartile range The "outliers" observations, indicated as rings, lie >1.5 times the interquartile range from the first and third quartiles. Differences in value distribution were considered statistically significant when p was $\leq$0.05.

Mean ± SD was calculated for each parameter in each genotype.

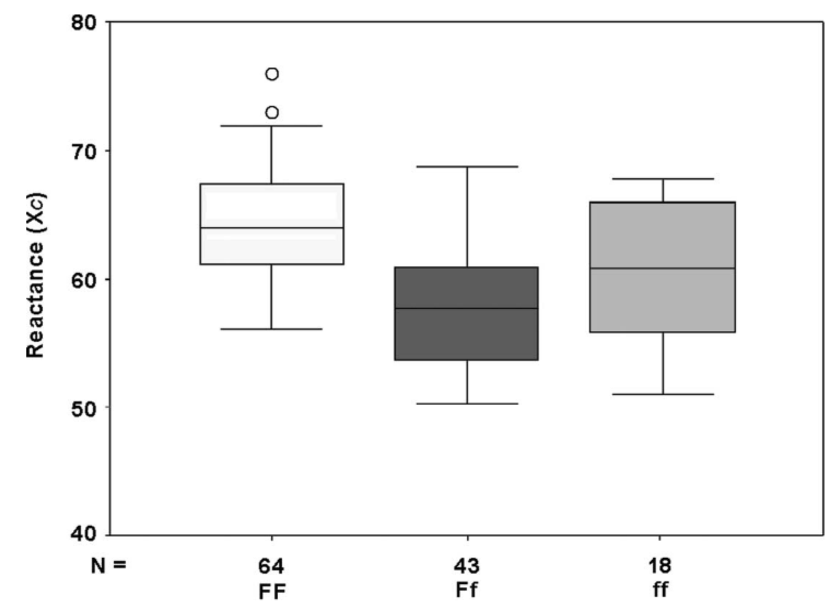

Figure 3. Box plot representation of the distribution of X_c values for each vitamin D receptor (VDR) *Fok*I genotype.

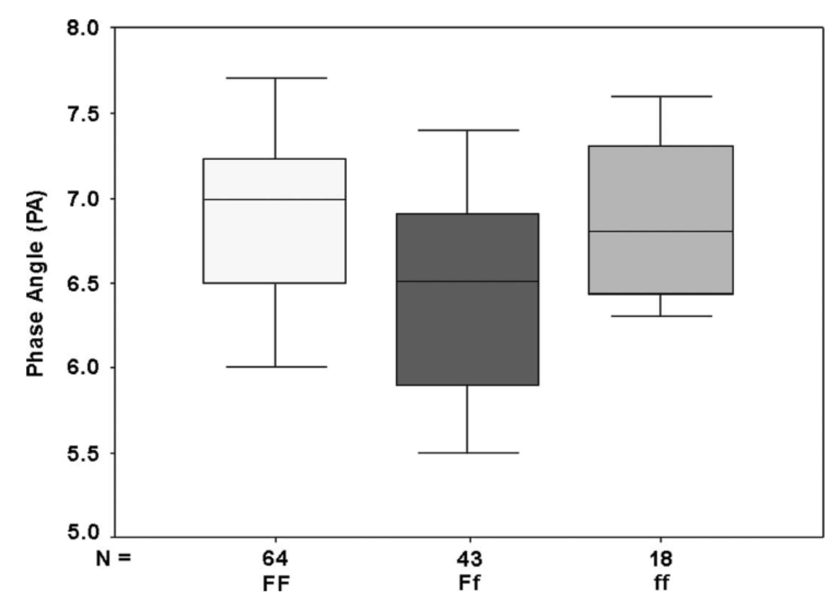

Figure 4. Box plot representation of the distribution of phase angle (PA) values for each vitamin D receptor (VDR) *Fok*I genotype.

Results

Genotype ACE I/D frequency distribution in young soccer players (Table 1) did not significantly vary from the genotype frequency distribution observed in the sedentary population taken as control (26). In contrast, VDR *Fok*I polymorphism distribution was significantly different in young soccer players in comparison to that observed in the sedentary control population (17). The frequency of the homozygous *ff* genotype was considerably higher in the young soccer players (0.14) as opposed to those in the control group (0.07) (Table 2). These results can be interpreted as if those subjects harboring the homozygous *ff* genotype were more predisposed to successful soccer playing and they are consistent with the data reported in the literature demonstrating that quadriceps isometric and concentric strength are higher in *ff* homozygotes (28).

By analyzing values of body composition, we observed that reactance (X_c) and PA values were differently distributed in the 3 ACE genotypes (DD, ID, and II) with the X_c and PA mean value higher in athletes harboring the D allele. Mean values of X_c were: 61 ± 5.1 Ω in subjects with the DD genotype, 63 ± 4.7 Ω in subjects with the ID genotypes and 53 ± 4.1 Ω in subjects with the II genotype (Figure 1). Mean values for PA were 6.8 ± 0.6° in subjects with the DD genotype, 6.7 ± 0.6° in subjects with the ID genotype and 6.2 ± 0.3° in subjects with the II genotype (Figure 2). Differences for value distribution of X_c and PA were statistically significant with $p = 0.04$.

No significant differences between ACE genotypes were found in the mean values of the other anthropometric and bioelectrical impedance analysis parameters studied (data not shown). These results appear to demonstrate that, among anthropometric and bioelectrical impedance values, only X_c and PA were associated with the different ACE genotypes, and in particular with the presence of the D allele; such a selective association strongly suggests that this association is not a mere chance but has a physiological significance.

Concerning the studied VDR genotypes (*FF*, *Ff*, and *ff*), we observed association of genotypes with X_c, PA, and BCM/FFM ratio as shown in the box plot charts in Figures 3–5. Considering the mean values of these 3 parameters, we observed that subjects with the *FF* genotype exhibited mean values of X_c, PA, and BCM/FFM higher than those observed in subjects with *Ff* and *ff* genotypes. Specifically, mean value ± *SD* for X_c was 65 ± 4.9 Ω in subjects with the *FF* genotype; 57 ± 4.7 Ω in subjects with the heterozygous *Ff* genotype, and 61 ± 5.2 Ω in subjects with the *ff* genotype. The mean value ± *SD* of PA was 7.1 ± 0.4° in subjects with the FF, 6.4 ± 0.6° in subjects with the *Ff* genotype, and 6.9 ± 0.5° in subjects with the *ff* genotype. The mean value of

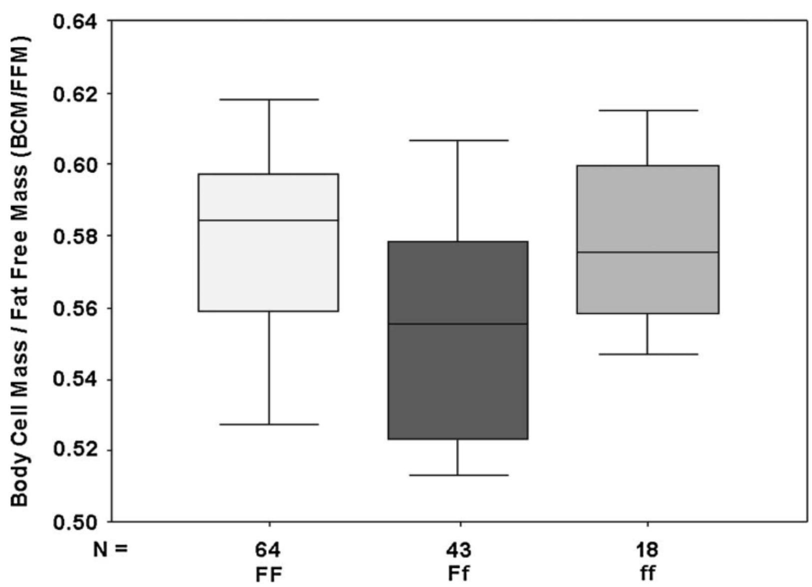

Figure 5. Box plot representation of the distribution of BCM/FFM values for each vitamin D receptor (VDR) *FokI* genotype. BCM = body cell mass; FFM = fat-free mass.

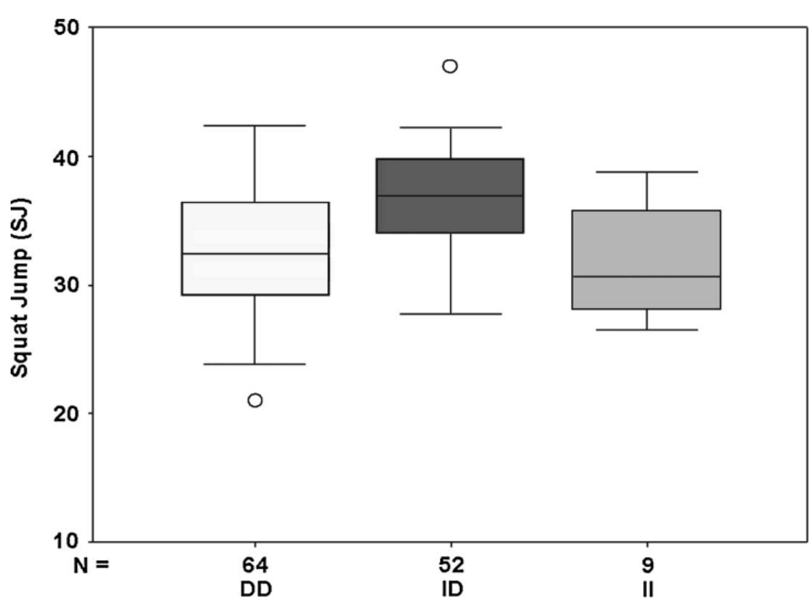

Figure 6. Box plot representation of the distribution of squat jump values for each angiotensin-converting enzyme (ACE) I/D genotype.

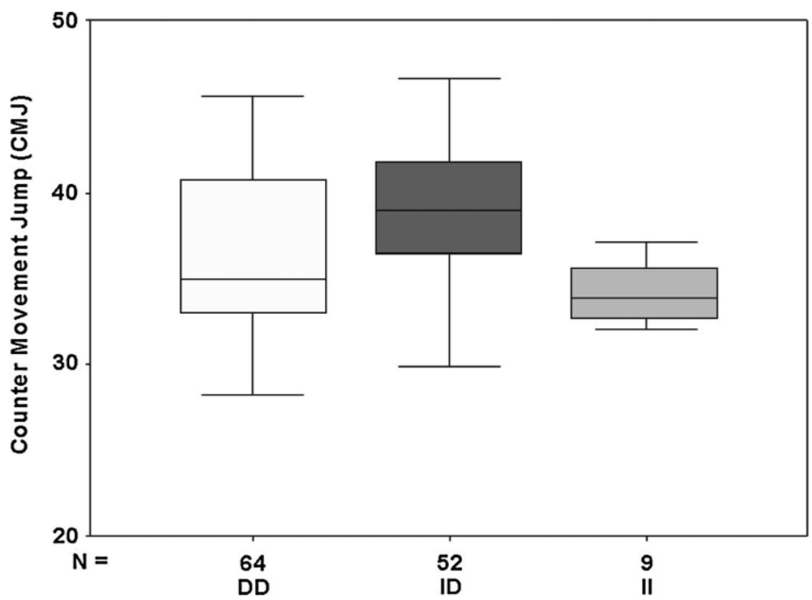

Figure 7. Box plot representation of the distribution of countermovement jump (CMJ) values for each ACE I/D genotype.

BCM/FFM ± *SD* was 0.59 ± 2 in subjects with *FF*, 0,55 ± 2 in subjects with *Ff* genotype, and 0.58 ± 1 in subjects with the *ff* genotype. Differences in value distribution were statistically significant with $p = 0.02$ (for X_c), $p = 0.03$ (for PA) and $p = 0.03$ (for BCM/FFM).

Next, we studied the association between genotypes and athletic performance. Subjects with the ID genotype displayed a significantly different distribution of SJ values in comparison with subjects with DD and II genotypes with $p = 0.02$. The SJ mean ± *SD* were higher in athletes with the ID genotype (37.4 ± 4.0 cm) than in subjects with the DD (33.1 ± 3.9 cm) and II genotypes (30.4 ± 4.6 cm) (Figure 6).

Distributions of CMJ values were significantly different in the ACE I/D genotypes with $p = 0.04$ (Figure 7). Also in this case, the mean ± *SD* values of CMJ were higher in subjects with the ID genotype (38.8 ± 3.6 cm) than in subjects with the DD (35.9 ± 4.1 cm) and II genotypes (34.7 ± 1.9 cm). Taken together, these results seem to indicate that the heterozygous ID genotype is associated with the best performances in SJ and CMJ.

No significant difference was observed in athletic performances among the athletes with different VDR genotypes (data not shown). Also, no significant difference was observed in performances related to 10- and 20-m sprint times or medicine ball throw.

Discussion

This is the first study evaluating the distribution of ACE and VDR *Fok*I polymorphisms in young soccer players and their association with athletic performances, bioelectrical impedance analysis parameters, and anthropometric characteristics. We found a significant difference in the distribution of VDR *Fok*I polymorphisms between our group of medium–high-level soccer players and a sedentary control population. From a gene analysis perspective, these results can be interpreted considering the known association between VDR polymorphisms and quadriceps isometric and concentric strength (28), that is, a trait of fundamental importance in playing soccer. In other words, it is feasible that untrained children having the genetic advantage of stronger quadriceps tend to perform better in their early attempts and are thus encouraged to pursue their passion. Because of this, the *ff* homozygous genotype (i.e., the one associated with quadriceps strength) is overrepresented in the population considered in this study. It also appears, however, that the initial advantage provided by the *f* allele is overcome by intense training; in fact, in the well-trained population that we studied, no difference in athletic performance was observed between athletes harboring the 3 different VDR genotypes. From a practical point of view, these results can be used by coaches to identify those young athletes harboring the less favorite VDR genotypes (i.e., *Ff* and *FF*) to train them adequately with the goal of increasing their strength, thus compensating the relative genetic disadvantage.

Concerning the role of ACE polymorphisms, this study demonstrates that the heterozygous genotype (ID) was associated with certain athletic performances, such as SJ and CMJ, but not with others. Because soccer is a stop and go sport involving a wide spectrum of exercise tasks at various intensities, this association could be exploited to identify those athletes that could better perform in roles involving jumping or tackling.

As far as the relationships between X_c (and PA), anthropometric measures, genotypes, and performances, are concerned, our study demonstrates that in a well-trained athlete population X_c and PA and anthropometric values were not correlated with athletic performance. In fact, we observed significant differences in X_c and PA values and in the BCM/FFM ratio in athletes harboring the 3 VDR genotypes, whereas, in the same athletes, no differences in performances could be detected. There was, however, a correlation between X_c and PA values, SJ and CMJ performance and the presence of the D allele in the ID and DD genotypes as if the D allele played a dominant role over the I allele. Taken together, these results at first may appear at odds with some data reported in the literature highlighting the association between physical performance and PA; in fact PA was reported to be correlated with values of maximum strength with isometric grip (19), and it was higher in trained subjects compared to in untrained controls (14,22). The PA also increased with age in children (3) and decreased with age in adults and elderly (9,12). However, it should be noticed that in the studies reported above, PA values were compared in different populations (e.g., trained vs. untrained). Thus, our data can be interpreted as if training compensated for differences in PA and trained athletes with different PA values nevertheless did not show differences in physical performances.

Practical Applications

It is well assessed that many sport- and exercise-related traits are inherited; nevertheless, much of exercise physiology research has been focussed predominantly on environmental factors. As a consequence, questions concerning the role of genetic profiles associated with sport- and exercise-related traits and the application of such knowledge are still largely unanswered. There are different reasons that would render extremely useful identifying genetic profiles associated with athletic performances. First, they could provide information about the genetic and molecular mechanisms underlying athletic performance. Second, such information could be related to physiology and pathology and thus become useful in sport medicine and in general medical practice. Third, the knowledge of the variations (polymorphisms) in DNA sequence associated with athletic performance may represent a tool to develop genetic tests designed at predicting the potential for performance. Individuals, their tutors and their coachers might use these tests to make decisions such as to become a professional athlete or to choose a given sport. Because most people commit to a discipline while young and require prolonged training during their growing years to become elite athletes, it is foreseeable that in the near future genetic performance tests will be used to identify the most appropriate athletic discipline for each individual and prevent minors from choosing to embark on an eventually fruitless training program.

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