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A Study on ACE, ACTN3, and VDR Genes Polymorphism in Thai Weightlifters

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Abstract

In order to excel in sport, apart from the correct training, the genetic combination of an individual may also be a factor in making an elite athlete. Weightlifting, as a popular sport, has a unique bio-mechanism dealing with muscle performance. The current study investigated the polymorphisms of the angiotensin-converting enzyme (ACE), the α-actinin-3 (ACTN3), and the vitamin D receptor (VDR) genes (individually or in combination) in Thai weightlifters. A total of 117 male and female national and junior Thai weightlifters, and 99 healthy sedentary people were recruited for this study. Genotyping was analyzed by Polymerase Chain Reaction (PCR) and Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP). When compared to the junior and non-athletes group, the genotype and allele frequencies of ACE (DD), ACTN3 (RR), and VDR (ff) were more frequent in both male and female national weightlifters. In addition, the genotype combinations between ACE (DD) + VDR (ff), ACE (DD) + ACTN3 RR, and ACTN3 RR + VDR ff presented highly in both genders of national weightlifters. Taken together, our results suggest that the ACTN3 and VDR genotype, individually or in combination, may influence muscle performance in weightlifters, appearing to significantly contribute to better weightlifting performance.

Keywords: ACE, ACTN3, VDR, gene, polymorphism

Introduction

Physical performance is a complex trait that is influenced by many factors, including body composition, level of physical activity, training status, and environment. It is estimated that approximately 20 - 80 % of the variation in a number of traits important to athletic performance is attributable to genetic factors [1]. The most common types of genetic variants that can influence inter-individual differences in any phenotype are single nucleotide polymorphisms (SNP) [2]. To date, more than 200 genes associated with athletic performance and physical fitness have been identified and investigated [3-5].

Among these genes, a genetic variation in muscular strength has recently received much attention [6]. The heritability of muscle strength has been shown to range from approximately 30 to 80 % [7]. The first genetic element that had been demonstrated to influence human physical performance is the angiotensin converting enzyme (ACE) gene. ACE is recognized as a key enzyme in the renin-angiotensin system (RAS) that plays an important role in the control of systemic blood pressure [8]. Additionally,

angiotensin II, a primary mediator of RAS, appears to enhance overload-induced skeletal muscle hypertrophy [9].

ACE gene polymorphism is characterized by the presence (insertion, I allele) or absence (deletion, D allele) of a 287 amino acid base pair Alu repeat sequence within intron 16 of the ACE gene on chromosome 17 [10]. Whilst the I allele of the ACE gene has been associated with lower ACE activity level and endurance performance, the D allele has been associated with higher concentrations of serum ACE activity and strength training [11]. Several previous reports [12], Nazarov *et al*. [13] suggested that the DD genotype is more prevalent in elite sprint athletes compared with endurance athletes and controls. The association of ACE DD genotype with a greater proportion of fast twitch fibers has also been observed [2]. Nevertheless, some studies have found no association of the ACE genotype with isometric and dynamic strength [14-16]. The basis for this discrepancy between studies is unclear, but it may stem from a difference in the physical fitness levels or ethnic backgrounds of the selected subjects.

Another candidate gene that may influence muscle strength and performance of athletes is alpha-actinin-3 (ACTN3). ACTN3 belongs to a member of family of actin-binding proteins [17]. ACTN3 is a myofibrillar protein located at the Z disk which forms a lattice structure that anchors together actin containing thin filaments. The functions of ACTN3 are likely to involve a static function in maintaining the ordered myofibrillar array in a sarcomere. It is only found in Type 2 fibers and is, thus, implicated in generating fast and strong contraction [7,18]. A genetic variation in the ACTN3 gene has been extensively studied. The R577X mutation in ACTN causes no functional ACTN protein to be made because of a premature stop codon [19]. Ben-zaken *et al*. [20] found that the ACTN3 577RR genotype was more frequent among elite sprinters compared to endurance runners, while none of the top sprinters harbored the 577XX genotype [11]. Furthermore, MacArthur and North [1] showed that the absence of ACTN3 in ACTN3 knockout mice inhibits the performance of the fast twitch muscle fibers that are important for generating force at high velocity. Other studies have reported that ACTN3 RR genotype is overrepresented in strength/sprint athletes [21,22], whereas ACTN3 XX genotype is under-represented in elite wrestlers [23]. In addition, Vincent *et al*. [24] found that men who were homozygous for the X allele had significantly fewer types 2X as compared to those homozygous for the R allele. This finding suggests that ACTN3 SNPs may influence muscle power through the control of fiber type distribution. Recently, however, Norman *et al*. [25] found no differences in fiber type composition between the ACTN3 XX and RR genotype groups.

In addition to ACE and ACTN3, there is now emerging evidence that the VDR gene may play an important role in cell proliferation, differentiation, and muscle contractile process [26,27]. More specifically, Windelinckx *et al*. [28] found that VDR gene polymorphisms are associated with quadricep strength in both males and females. In addition, Geusens *et al*. [29] reported that senior women who carry the bb allele have high isometric quadriceps and hand grip strength when compared to the control with BB allele. However, the relationship between VDR polymorphism and athletic performance is less clear. Recently, Micheli *et al*. [30] reported that the VDR (*FokI*) ff allele was more highly associated with muscular strength in soccer players than subjects who carry FF allele.

Taken together, it is more likely that the inter-individual differences in athletic performance is not just determined by one polymorphism but the interaction of several gene variants. Moreover, no studies have been conducted to examine the of ACE, ACTN3, and VDR polymorphism in weightlifters.

Weightlifting is one of the oldest sport disciplines in the Olympic games and is becoming increasingly popular around the world. During lifting performance, weight lifters, besides using technical and tactical skills, are required to generate high isometric peak force (PF) and power output (PP). Typically, weightlifters have a greater PF (15 - 20 %) and rate of force development (RFD) (13 - 16 %) in comparison to other strength and power athletes (e.g., sprinters, throwers, football players) [31,32]. The highest weight that the athletes can lift is strongly related to PF and PP. In addition, weightlifting performance is strongly correlated to type 2A percent content [33]. Other key factors that can influence athletic performance are anthropometric characteristics. In comparison with other strength and power athletes of a similar body mass, weightlifters have a shorter height and arm span [34]. These provide mechanical advantages to weightlifters when lifting maximal load. Many previous studies examining factors determining weightlifting performance have focused on biomechanical and physiological factors;

however, to our knowledge, there is little information available on the genetic influence of ACE, ACTN3 and VDR genes polymorphisms on muscle performance in weightlifters. Therefore, the purposes of this study are to determine the ACE (I/D), ACTN3 (R577X), and VDR (F/f) gene polymorphisms (individually or in combination), and to determine whether there is a preferable genetic profile which can be used as a potential predictor for successful weightlifters. Athletic performance is a heritable trait influenced by genetic and environmental factors. Research on genotypes will allow us to have a better understanding of the genetic make-up and molecular physiology of athletes. The genetic markers can be used as sports talent identification criteria and a potential predictor for successful athletes, especially in weightlifters.

Materials and methods

Subjects

Two hundred and sixteen subjects volunteered for this study. No participants had a history of any chronic injuries or diseases, and all were informed of the procedures and potential risks prior to signing an informed consent form. The younger subjects, whose ages were below 18 years, were approved by coaches or parents before participating in this study. All procedures were approved by the Mahidol Ethical Committee for Human Research (COA, approval No. 2013/086.1009). There were 117 subjects, divided into 2 groups of weightlifters, used in this study. The first group consisted of 38 national male and female weightlifters, aged between 15 to 32 years old, and recruited from a training camp used for selecting the Thailand national weightlifting team. The second group of subjects included 79 junior weightlifters of both sexes, aged between 15 and 20 years old, recruited from athletes who participated in the Thailand Youth Games. In the sport of weightlifting, competitions are organized for men and women. The athletes compete in categories, specified in the rules, according to their bodyweight. The IWF recognizes 3 age groups: 1) Youth: up to and including 17 years of age; 2) Junior: up to and including 20 years of age, and 3) Senior (IWF Technical Rules, International Weightlifting Federation. Retrieved from http://www.iwf.net/doc/Technical&CompRules2009-2012.pdf/html20/ 11/2012). The non-athletes, or the sedentary group, consisted of 99 healthy young adults (male and female) from high schools or who were 1st or 2nd year university students. No participants had been trained on a regular basis or had been involved in any vigorous exercise program for at least 3 months prior to the study.

Experimenting protocols

1) DNA sample collection and extraction

DNA samples were collected at least 1 h after meals. All subjects were asked to wash their mouth with water before buccal cells were swabbed. A cotton swab was used to collect the mouth epithelial cells from both the left and right cheeks by scraping inside of the mouth approximately 20 times. After this, the heads of the cotton swabs were cut and incubated in 200 µl of cell lysis solution before being stored at room temperature until genotyping analysis was performed. In DNA extraction, the swabs were incubated in a water bath at 65 °C for 60 min for a high yield (QAIGEN, USA.). After that, DNA concentration measurement by Nucleic acid quantification and purity was assessed using a Thermo Scientific Nano Drop 1000 Spectrophotometer, V3.7, USA.

2) Genes polymorphism identification

ACE gene polymorphism

To investigate gene ACE polymorphism, genomic DNA was extracted from the samples by using a DNA extraction kit a protocol provided by the manufacturer (QIAGEN). Polymerase chain reactions (PCR) were used to detect the insertion (I) and deletion (D) alleles of the ACE (rs1799752) gene according to the method described by Rigat *et al.* [10]. The PCR primers were as follows: forward primer was 5'-CTGGAGACC ACTCCC ATCCTTCTC-3' and the reverse primer was 5'-GATGTGGCCATCACATTCGTCAGAT-3'. PCR reaction were 10X PCR buffer, 25 mM $MgCl_2$, 10 mM dNTPs, 10pmol/ml ACE Forward, 10pmol/ml ACE Reverse, ddH_2O, *Taq* Polymerase (2.5 U/ml), and DNA template. PCR conditions were as follows: denaturation at 94 °C for 5 min, followed by 30

cycles of 94 °C for 1 min, annealing at 58 °C for 1 min, and an extension at 72 °C for 2 min and a final extension at 72 °C for 10 min. DNA products were detected by gel electrophoresis with a 1.5 % agarose and identified by ethildium bromide staining. This method yielded PCR fragments of 190 bp and 490 bp, representing the D and the I alleles, respectively.

ACTN3 gene polymorphism

To investigate polymorphism of the gene α actinin 3 (ACTN3), the templates from the extraction were used for PCR. The forward primer was 5'-CTGTTGCCTGTGGTAAGTGGG-3' and the reverse primer was 5'-TGGTCACAGTATGCAGGAGGG-3'. PCR reaction were 10X PCR buffer, 25 mM $MgCl_2$, 10 mM dNTPs, 10pmol/ml ACE Forward, 10pmol/ml ACE Reverse, ddH_2O, *Taq* Polymerase (2.5 U/ml), DNA template. The PCR conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and an extension at 72 °C for 30 s and a final extension at 72 °C for 10 min. Gel electrophoresis gel was performed to check the PCR products. The resulting PCR products were subjected for Restriction Fragment Length Polymorphisms (ACTN3) by reaction 10 X buffer, PCR product, and enzyme *DdeI* (New England Biolab). The digested PCR fragments 205 bp and the 86 bp were of the R allele, and 108 bp, 97 bp, and 86 bp fragments were of the X allele, whereas the 205 bp 108 bp, 97 bp, and 86 bp were of the RX allele. PCR products were detected by 2 % agarose gel electrophoresis and stained with ethidium bromide.

VDR gene polymorphism

To investigate polymorphism of the Vitamin D receptor (VDR) gene, the DNA templates were amplified using the PCR-RFLP. The forward primer was 5'-TTGTTCTTCCACAGATTCAGAC-3' and the reverse primer was 5'- GAAAAGG ACCTTGAACGAGAG - 3'. The PCR reaction conditions were as follows: denaturation at 95 °C for 5 min, followed by 30 cycles at 95 °C for 1 min, annealing at 58 °C for 1 min and extension at 72 °C for 5 min, and a final extension at 72 °C for 10 min. The PCR products were then digested by reaction 10 X buffer, PCR product and the restriction enzyme, *FokI* (Biolabs Inc, New England), to digest the F allele (490 bp) into 3 fragments of the 265, 196, and 69 bp. The digested products were separated by 2 % agarose gel electrophoresis.

Statistical Analysis

A $\chi 2$ test was used to confirm that the observed frequencies for ACE, ACTN3, and VDR were in the Hardy-Weinberg equilibrium. All data was confirmed as normally distributed using SPSS for Windows version 17.0 (SPSS, Chicago, IL, USA) and Microsoft Excel 2000 for Windows (Microsoft, Redmond, WA, USA). Data were presented as means ± S.D. with statistical significance defined as p values of < 0.05.

Results and discussion

Characteristics of subjects

The physical characteristics of the subjects, including age and athletic experience of the subjects, are presented in **Table 1**. The average ages of male national and junior weightlifters, as well as those of the control, were 21.7 ± 4.8 yrs, 17.5 ± 1.3 yrs, and 18.4 ± 2.5 yrs, respectively. Similar results for age and athletic experience were obtained for female national weightlifters. In addition, for national weightlifters, both males and females tended to have a slightly higher athletic experience than those in the junior group.

Table 1 Descriptive characteristics of weightlifters and sedentary controls by sex and group.

Subject	Gender (n)	Age (yrs)	Athletic experience (yrs)
National	M (19)	21.7 ± 4.8 [a, b]	6.6 ± 1.4 [b]
	F (19)	18.8 ± 3.2 [b]	6.1 ± 1.5 [b]
Junior	M (43)	17.5 ± 1.3	4.6 ± 1.7
	F (36)	16.6 ± 1.7 [a]	3.9 ± 1.6
Control	M (57)	18.4 ± 2.5[b]	N/A
	F (42)	18.9 ± 2.4[b]	N/A

Values are means ± S.D., N/A represents not available data.
[a] $p < 0.05$ when compared to control group
[b] $p < 0.05$ when compared to junior group

Angiotensin converting enzyme (ACE) Polymorphisms

Polymerase chain reactions (PCR) were used to detect the insertion (I) and deletion (D) alleles of the ACE gene polymorphisms. PCR fragments of 190 bp and 490 bp represented the D and the I alleles, respectively (**Figure 1**).

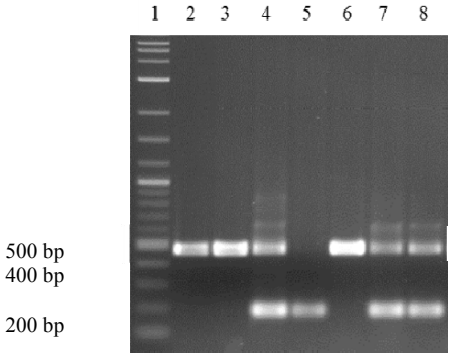

Figure 1 Genotypes for the ACE I/D polymorphism.

1) ACE genotype distribution and allele frequency of male group

The ACE genotype distributions of male subjects were in the Hardy-Weinberg equilibrium. The genotype distributions of each group were in the following pattern; male nationals' ACE II 26 %, ID 26 %, and DD 48 %, male juniors' ACE II 54 %, ID 30 %, and DD 16 %, and male controls' ACE II 62 %, ID 33 %, and DD 5 % (**Figure 2A**). The ACE allele frequencies of male nationals were I allele 39 %, D allele 61 %, male juniors I allele 69 %, D allele 31 %, and male controls I allele 78 %, D allele 22 % (**Figure 2B**). The χ^2 test indicated that the ACE DD genotype was overrepresented in male national weightlifters compared with those in male controls (**Figure 2A**). In junior weightlifters, however, the ACE genotype distribution and allele frequency were of no significant difference from both national and control groups (**Figures 2A** and **2B**). On the other hand, the control group carried the highest percentage of the II genotype and I allele.

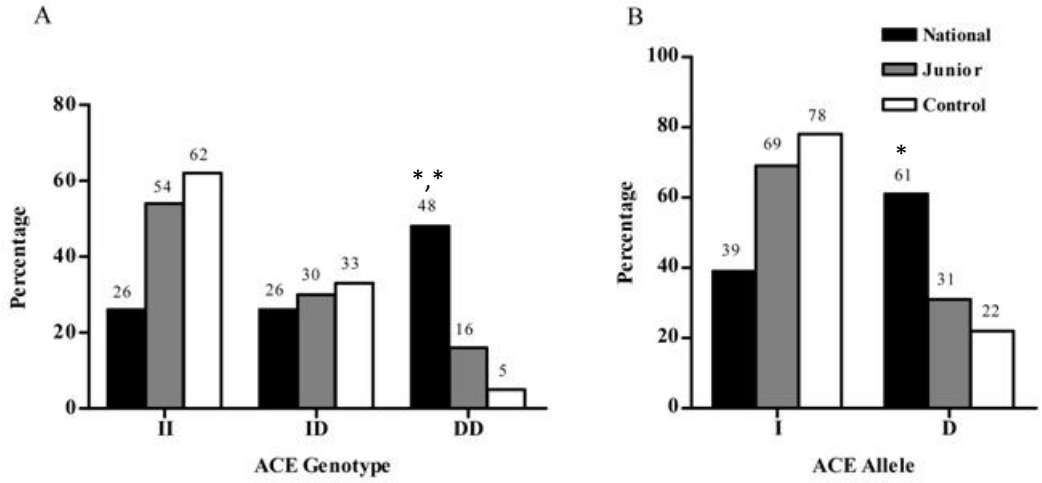

Figure 2 Genotype distribution (A) and allele frequency (B) of ACE I/D polymorphisms of male weightlifters and controls.
$\chi^2 = 19.56$, d.f. 2, $p < 0.05$ for ACE genotype frequencies in national weightlifters vs. controls.
$\chi^2 = 7.73$, d.f. 2, $p < 0.05$ for ACE genotype frequencies in national weightlifters vs. junior weightlifters.
$\chi^2 = 19.65$, d.f. 1, $p < 0.05$ for ACE allele frequencies in national weightlifters vs. controls.
The * indicates significantly different *p*-value.

2) ACE genotype distribution and allele frequency of female group

The ACE genotype distributions of female subjects were in the Hardy-Weinberg equilibrium. The genotype distribution of female nationals' ACE was II 26 %, ID 32 %, and DD 42 %, female juniors' ACE were II 47 %, ID 39 %, and DD 14 % while the female controls' ACE were II 29 %, ID 48 %, DD 23 % (**Figure 3A**). In addition, the ACE allele frequencies of female nationals were I allele 42 %, D allele 58 %, female juniors I allele 67 %, D allele 33 %, and female controls I allele 53 %, D allele 47 % (**Figure 3B**). The χ^2 test revealed that the ACE DD genotype in female national weightlifters was significantly higher ($p < 0.05$) than those of juniors and female controls (**Figure 3A**). Junior weightlifters carried the highest percentage of the ACE II genotype and I allele, as illustrated in **Figures 3A** and **3B**.

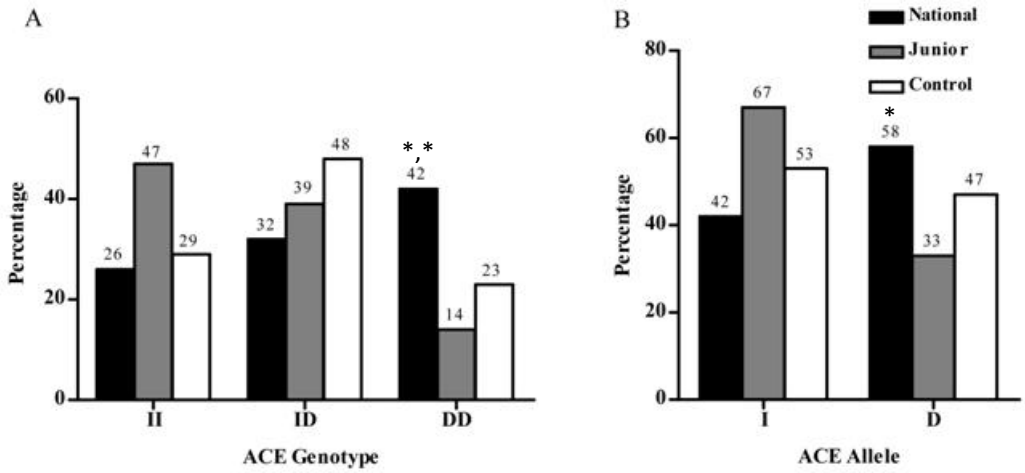

Figure 3 Genotype distribution (A) and allele frequency (B) of ACE I/D polymorphisms of female weightlifters and control.
$\chi^2 = 9.22$, d.f. 2, $p < 0.05$ for ACE genotype frequencies in national weightlifters vs. controls.
$\chi^2 = 5.73$, d.f. 2, $p < 0.05$ for ACE genotype frequencies in national weightlifters vs. junior weightlifters.
$\chi^2 = 9.58$, d.f. 1, $p < 0.05$ for ACE allele frequencies in national weightlifters vs. controls.
The * indicates significantly different *p*-value.

Alpha-actinin-3 (ACTN3) Polymorphisms

Polymerase chain reactions-Restriction Fragment Length Polymorphism (PCR-RFLP) was used to detect the fragments of ACTN3 gene polymorphisms (R and X alleles), (**Figure 4**).

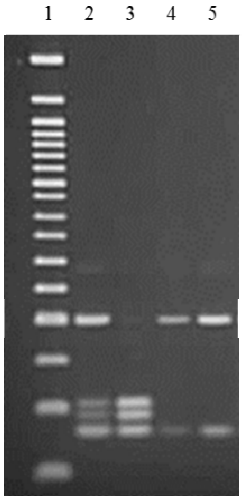

Figure 4 Genotyping for the ACTN3 R/X polymorphism. PCR-RFLP fragments of 205 bp and 86 bp representing the R allele, and 108 bp, 97 bp, and 86 bp fragments, representing the X allele. Lane 1 is the 50 base pair marker; lane 2, ACTN3 gene with R/X (heterozygous allele 205, 108, 97, and 86 bp); lane 3, X/X (homozygous X allele 108, 97, and 86 bp), and lanes 4 and 5, R/R (homozygous R allele 205, 86 bp).

1) ACTN3 distribution and allele frequency of male group

Considering the male groups, the ACTN3 genotype distributions were in the Hardy-Weinberg equilibrium. The male nationals' genotype distributions of ACTN3 were XX 16 %, RX 47 %, and RR 37 %, whereas those of the male juniors were XX 14 %, RX 49 %, and RR 37 %, and male controls were XX 33 %, RX 56 %, and RR 11 % (**Figure 5A**). The ACTN3 allele frequencies of male nationals were X allele 39 %, R allele 61 %, male juniors X allele 38 %, R allele 62 %, and male controls X allele 61 %, R allele 39 % (**Figure 5B**). The χ^2 test revealed that ACTN3 RR genotype distribution and R allele frequency in male national and male junior weightlifters differed markedly from those of male controls. However, male controls carried the highest percentage of RX genotype and X allele (**Figures 5A** and **5B**).

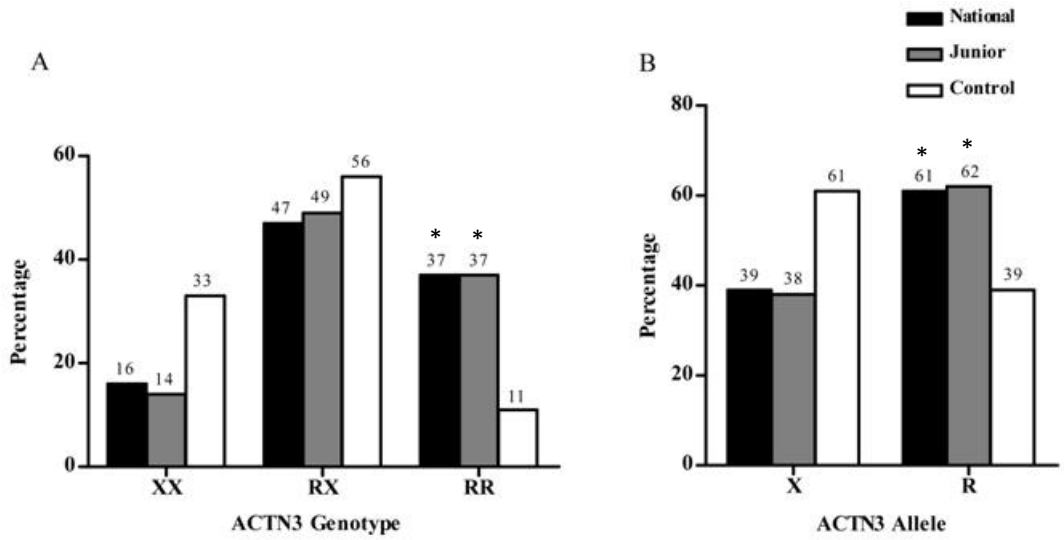

Figure 5 Genotype distribution (A) and allele frequency (B) of ACTN3 R577X polymorphisms of male weightlifters and control.
$\chi^2 = 7.49$, d.f. 2, $p < 0.05$ for ACTN3 genotype frequencies in national weightlifters vs. controls.
$\chi^2 = 11.86$, d.f. 2, $p < 0.05$ for ACTN3 genotype frequencies in junior weightlifters vs. controls.
$\chi^2 = 5.56$, d.f. 1, $p < 0.05$ for ACTN3 allele frequencies in national weightlifters vs. controls.
$\chi^2 = 10.41$, d.f. 1, $p < 0.05$ for ACTN3 allele frequencies in junior weightlifters vs. controls.
The *indicates significantly different *p*-value.

2) ACTN3 genotype distribution and allele frequency of female group

The ACTN3 genotype distributions of the female group were in Hardy-Weinberg equilibrium. The genotype distributions in female nationals' ACTN3 were XX 26 %, RX 37 %, and RR 37 %, whereas female juniors' ACTN3 were XX 14 %, RX 47 %, and RR 39 %, and female controls' ACTN3 were XX 36 %, RX 57 %, and RR 7 % (**Figure 6A**). The ACTN3 allele frequencies of female nationals were X allele 45 %, R allele 55 %, female juniors X allele 38 %, R allele 62 %, and female controls X allele 64 %, R allele 36 % (**Figure 6B**). The χ^2 test indicated that ACTN3 RX genotype distribution and X allele frequency in female controls were at higher percentage than those of female nationals and female junior weightlifters (**Figures 6A** and **6B**).

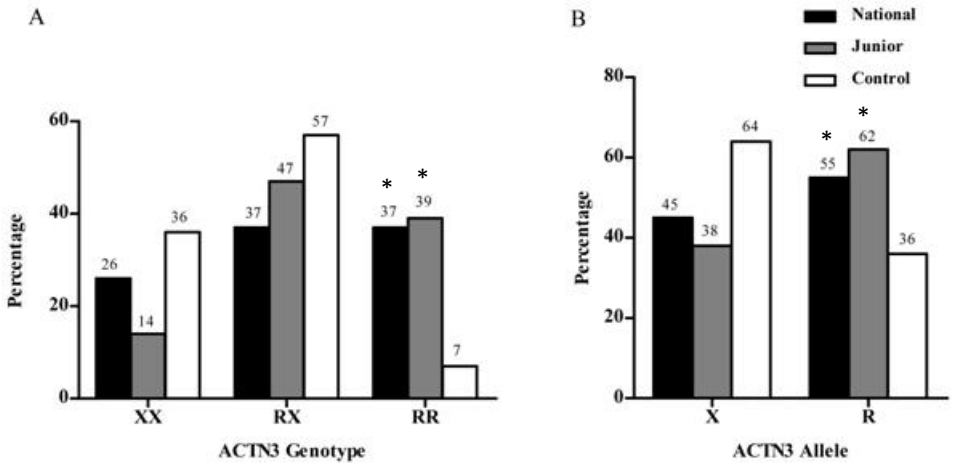

Figure 6 Genotype distribution (A) and allele frequency (B) of ACTN3 R577X polymorphisms of female weightlifters and control.
$\chi^2 = 8.45$, d.f. 2, $p < 0.05$ for ACTN3 genotype frequencies in national weightlifters vs. controls.
$\chi^2 = 12.93$, d.f. 2, $p < 0.05$ for ACTN3 genotype frequencies in junior weightlifters vs. controls.
$\chi^2 = 4.11$, d.f. 1, $p < 0.05$ for ACTN3 allele frequencies in national weightlifters vs. controls.
$\chi^2 = 11.14$, d.f. 1, $p < 0.05$ for ACTN3 allele frequencies in junior weightlifters vs. controls.
The * indicates significantly different *p*-value.

Vitamin D Receptor (VDR) Polymorphisms

Polymerase chain reactions-Restriction Fragment Length Polymorphism (PCR-RFLP) was used to detect the fragments of VDR gene polymorphisms (F and f alleles), (**Figure 7**).

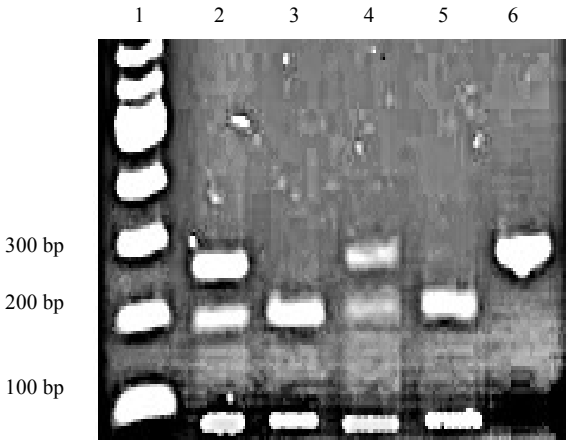

Figure 7 Genotyping of VDR polymorphism: PCR-RFLP fragments of 206, 196, and 69 bp representing the F and the f alleles, respectively. Lane 2, 4, 206, 196, 69 bp is Ff heterozygous; lanes 3 and 5, 196 and 69 bp ff homozygous, and lane 6, 265 bp FF homozygous.

1) VDR genotype distribution and allele frequency of male group

The VDR genotype distributions of the male group were in the Hardy-Weinberg equilibrium. The genotype distribution of male nationals' VDR were FF 5 %, Ff 42 %, and ff 53 %, male juniors' VDR were FF 37 %, Ff 40 %, and ff 23 %, and male controls VDR were FF 37 %, Ff 49 %, and ff 14 % (**Figure 8A**). The VDR allele frequencies of male nationals were F allele 26 %, f allele 74 %, male juniors F allele 57 %, f allele 43 %, and male controls F allele 61 %, f allele 39 % (**Figure 8B**). The χ^2 test indicated that VDR ff genotype distribution and f allele frequency in male national weightlifters differed markedly from those of male controls ($p < 0.05$) (**Figures 8A** and **8B**). However, male controls carried the highest percentage of F allele (**Figure 8B**).

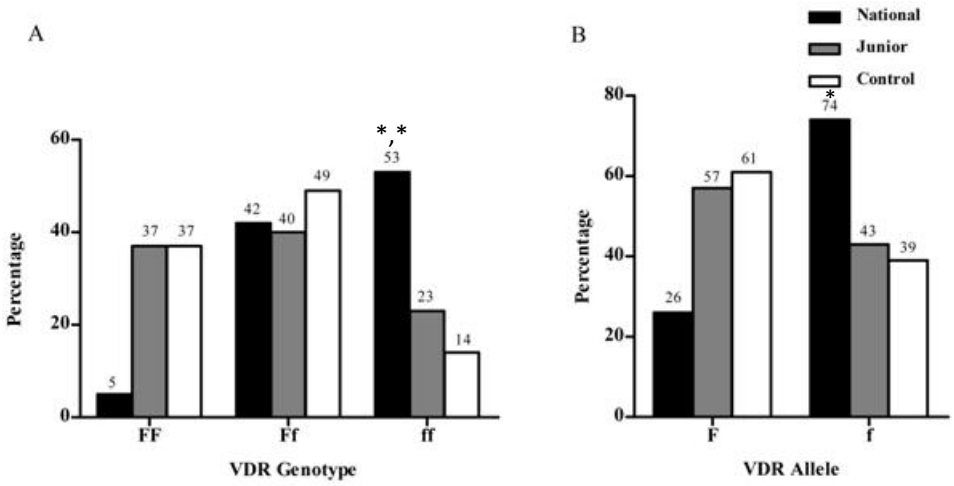

Figure 8 Genotype distribution (A) and allele frequency (B) of VDR F/f polymorphisms of male weightlifters and controls.
$\chi^2 = 14.02$, d.f. 2, $p < 0.05$ for VDR genotype frequencies in national weightlifters vs. controls.
$\chi^2 = 7.68$, d.f. 2, $p < 0.05$ for VDR genotype frequencies in national weightlifters vs. junior weightlifters.
$\chi^2 = 14.07$, d.f. 1, $p < 0.05$ for VDR allele frequencies in national weightlifters vs. controls.
The * indicates significantly different *p*-value.

2) VDR genotype distribution and allele frequency of female group

The VDR genotype frequencies of the female groups were in the Hardy-Weinberg equilibrium. The genotype distributions in female nationals' VDR were FF 32 %, Ff 32 %, and ff 36 %, female juniors' VDR were FF 36 %, Ff 47 %, and ff 17 %, and female controls' VDR were FF 33 %, Ff 53 %, and ff 14 %, as shown in **Figure 9A**. The VDR allele frequencies of nationals were F allele 47 %, f allele 53 %; female juniors F allele 60 %, f allele 40 %; and female controls F allele 60 %, f allele 40 % (**Figure 9B**). The χ^2 test revealed that VDR Ff genotype distribution and F allele frequency in female junior weightlifters differed markedly ($p < 0.05$) from other groups (**Figures 9A** and **9B**) and female national weightlifter carried the highest percentage of ff genotype when compared to female juniors and female controls (**Figure 9A**). However, female juniors and female controls carried the highest percentage of F allele (**Figure 9B**).

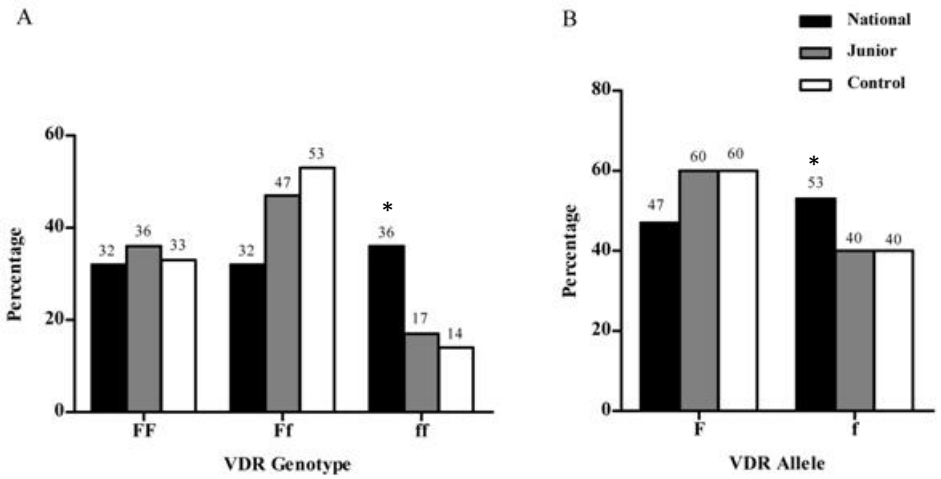

Figure 9 Genotype distribution (A) and allele frequency (B) of VDR F/f polymorphisms of female weightlifters and controls.
$\chi^2 = 4.37$, d.f. 2, $p = 0.11$ for VDR genotype frequencies in national weightlifters vs. controls.
$\chi^2 = 5.67$, d.f. 1, $p < 0.05$ for VDR allele frequencies in national weightlifters vs. controls.
The * indicates significantly different *p*-value.

Genotypes combination between ACE + VDR, ACTN3 + VDR and ACE + ACTN3

For genotype combination study, the genetic data of 3 genes (ACE + ACTN3, ACE + VDR, and ACTN3 + VDR) were analyzed in the present study. There were 9 possible combined genotypes including the dominant and recessive models for each genotype combination.

1) ACE (I/D) and VDR (F/f) genotype combinations in male and female

The frequency of combined ACE (ID) and VDR (Ff) genotype among male nationals, junior weightlifters, and male controls are presented in **Figure 10A**. It was found that the frequency of the ACE ID + VDR Ff genotype combination in male nationals highly differed from those of the controls (χ^2 = 30.34, d.f. = 8, $p < 0.05$). Such difference, however, was not observed between junior weightlifters and controls (χ^2 = 6.75, d.f. = 8, p = 0.584). Although the ACE DD + VDR ff genotype combination was more frequent in male nationals, the ACE (II) + VDR (FF) genotype combination were highly represented in male juniors and male controls. Furthermore, there was no significant difference between ACE ID + VDR ff/Ff genotype combination in male juniors and male controls. The DD + ff genotype combination, however, was highly significant ($p < 0.01$) in male nationals when compared to male controls, as shown in **Figure 10A**. In females**,** the frequency of the combined ACE (ID) and VDR (Ff) genotype among female nationals, junior weightlifters, and female controls were presented in **Figure 10B**. Both nationals and juniors had genotype combinations of ACE (ID) and VDR (Ff) that were not different from the controls (nationals; χ^2 = 13.87, d.f. = 8, p = 0.085, juniors; χ^2 = 3.45, d.f. = 8, p = 0.903). Moreover, the combined ACE DD + VDR ff genotype was highly represented in female nationals.

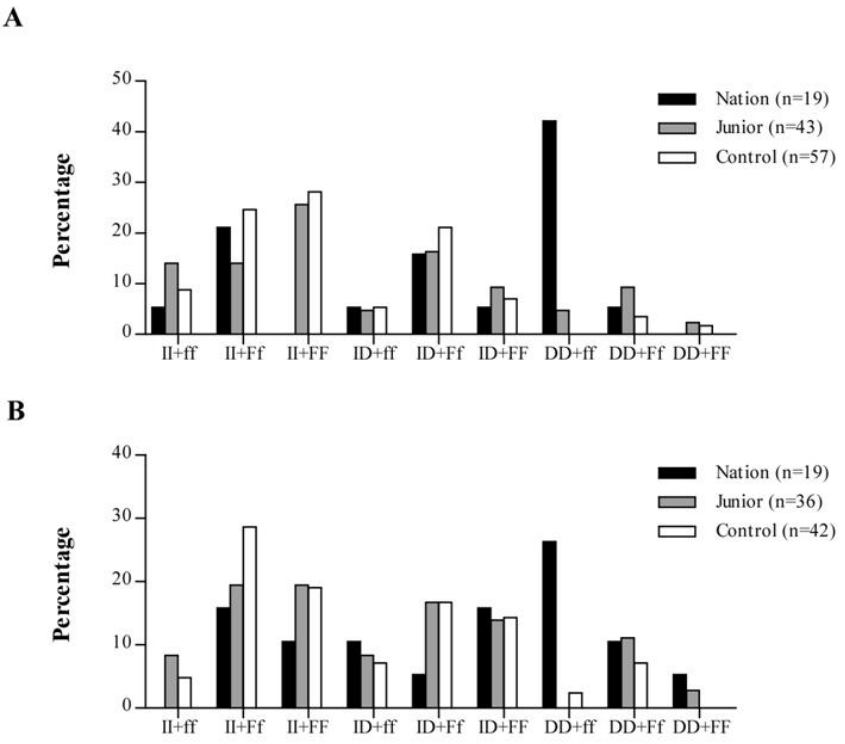

Figure 10 Combined ACE and VDR genotype frequencies of male group (A) and female group (B).

2) ACTN3 (R/X) and VDR (F/f) genotype combinations in male and female

The frequency of combined ACTN3 R577X and VDR Ff genotype among male nationals, junior weightlifters, and the controls are presented in **Figure 11A**. It was found that the ACTN3 RR and VDR ff genotype combination in male national weightlifters was more prevalent when compared to the controls ($\chi^2 = 22.20$, d.f. = 8, $p = 0.005$). Additionally, the genotype combination of ACTN3 RX + VDR ff in male nationals was higher than in the controls ($p < 0.05$). However, the ACTN3 RX + VDR Ff genotype combination in the junior group were not significantly different ($\chi^2 = 14.46$, d.f. = 8, $p = 0.071$) when compared to the controls.

In females, the frequency of the combined ACTN3 (R577X) and VDR (F/f) genotype among female nationals, junior weightlifters, and controls are presented in **Figure 11B**. Female national weightlifters possessed high amounts of ACTN3 RX + VDR Ff genotype combination when compared to juniors and controls ($\chi^2 = 14.34$, d.f. = 8, $p = 0.073$). Interestingly, however, the ACTN3 RR + VDR Ff genotype combination was higher in female juniors than in nationals and controls ($\chi^2 = 18.12$, d.f. = 8, $p = 0.020$).

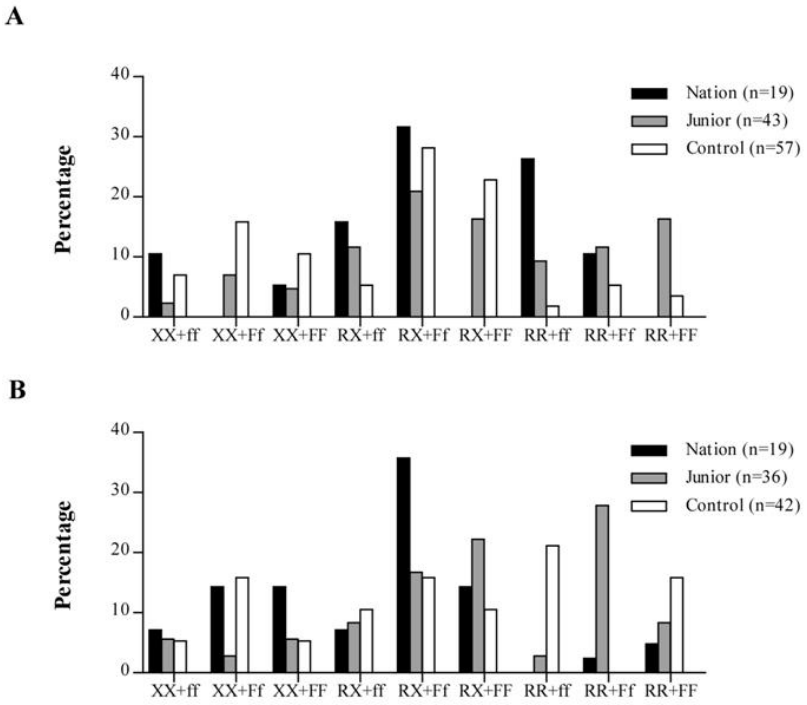

Figure 11 Combined ACTN3 and VDR genotype frequencies of male group (A) and female group (B).

3) ACE (I/D) and ACTN3(R/X) genotype combinations in male and female

The frequency of the combined ACE (I/D) and ACTN3 (R/X) genotype among male nationals, junior weightlifters, and the controls are presented in **Figure 12A**. The ACE DD + ACTN3 RR genotype combination in male nationals was highly different when compared to male juniors and controls. (χ^2 = 24.52, d.f. = 8, p = 0.002). Additionally, the combination of ACE DD + ACTN3 RX genotype in male junior weightlifters differed more than in the controls (χ^2 = 18.69, d.f. = 8, p = 0.017) (**Figure 12 A**). The frequency of the combined ACE (I/D) and ACTN3 (R/X) genotype among female nationals, junior weightlifters, and the controls are presented in **Figure 12B**. In female nationals and juniors, the ACE DD + ACTN3 RR genotype combinations were highly different from those of the juniors and controls (χ^2 = 17.65, d.f. = 8, p = 0.024). The ACE DD + ACTN3 RX was higher in juniors than in national weightlifters and controls; (χ^2 = 15.51, df = 8, p = 0.050). In the present study, the combined DD + RR genotype was highly represented in only female national weightlifters, while the ACE (II) + ACTN3 (RX) genotype combination was highly represented in female junior weightlifters and controls when compared to national weightlifters ($p < 0.05$) (**Figure 12B**).

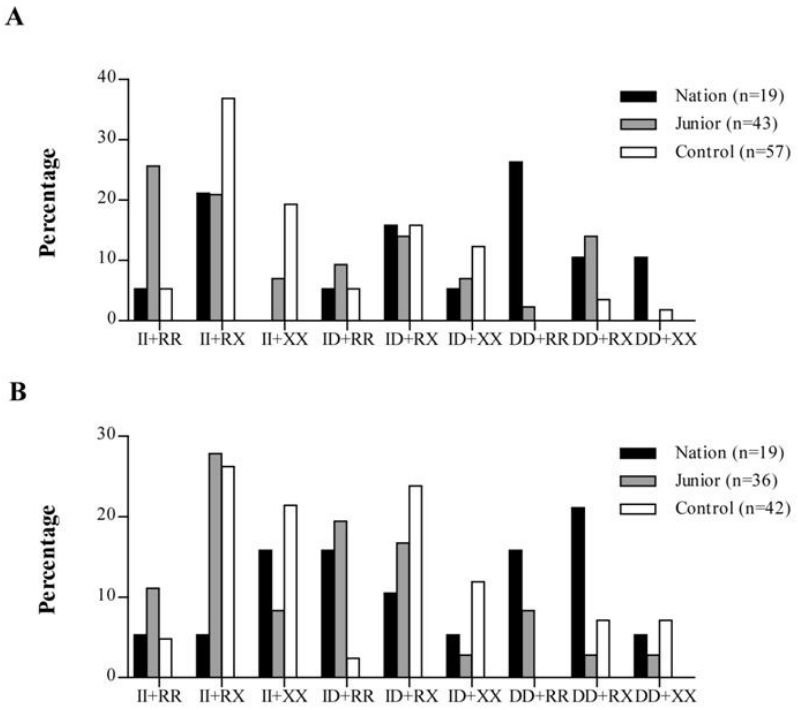

Figure 12 Combined ACE and ACTN3 genotype frequencies of male group (A) and female group (B).

Discussions

This study investigated the genotype distribution of ACE, ACTN3, and VDR polymorphisms (individual or combined) in Thai male and female weightlifters. The main finding of this study was the overrepresentation of ACE DD genotype distribution and allele frequency in both male and female national weightlifters when compared to junior weightlifters and control groups. Our findings were in agreement with previous reports. Kikhuchi *et al.* [23] demonstrated an excess of the DD genotype distribution and allele frequency in Japanese wrestlers when compared to the controls. However, some studies have provided conflicting results. For examples, Scott *et al*. [35] found no association between the ACE I/D polymorphism and athletic status in elite Jamaican and US sprinters. Moreover, Ginevi & Pranculis [36] reported the prevalence of ACE I/I and I/D genotypes in Lithuanian elite athletes compared with the general population. Clearly, this discrepancy in results may stem from differences in sport disciplines, ethnic background, and sample sizes between studies.

The ACTN gene is well known to influence muscle function and human performance. Whereas the ACTN3 RR genotype was reported to be associated with sprint and power performance [37], the X allele or ACTN3 XX genotype was related to elite endurance performance [11]. In the present study, the genetic influence of ACTN3 R/X polymorphism on muscle performance was examined in weightlifters. Our result showed that the ACTN3 genotype and allele frequencies in male national and all junior weightlifters were significantly higher than those in the controls. This finding was in line with the result of Kikhuchi *et al*. [23], which demonstrated an excess of the RR genotype distribution and allele frequency of Japanese wrestlers when compared to the controls. In addition, the study of Garatachea *et al*. [38] reported that genotype distributions did not differ between Spanish elite basketball athletes and controls. Despite several reports showing no association of that muscle performance with single ACTN3 polymorphism, investigation of genes has always been considered essential, as it is still hypothesized that understanding genetic composition can lead to the ability to predict performance and genetic screening in elite athletes or other populations [37]. A study in national or international track and field Japanese

athletes showed a higher frequency of RR + RX genotype than in the controls and that ACTN3 (R/X) was associated with sprint and power performance [39]. Altogether, these results suggest that, although the ACTN3 R/X polymorphism is a strong candidate for athletic physical fitness or exercise related phenotype, this polymorphism alone or in combination may contribute in the muscle power of weightlifters.

In this study, we also determine the association of VDR *FokI* polymorphisms. Our results showed that ff genotype was highly expressed in male national weightlifters. This is similar to the results by Micheli *et al*. [30] reporting that VDR homozygous ff genotype was more highly presented in young football players than non-exercise controls. Unfortunately, we did not observe any association in the male and female groups. In addition, Windelinckx *et al*. [28] reported that VDR F/f have been associated with quadricep muscles in females. The Vitamin D receptor gene (VDR) is a C/T transition polymorphism located in the VDR start codon. This may affect the structure and function of the encoded proteins [40-42]. Thus, it is possible that this individual gene may interact with other genes to influence physical performance.

Another major finding in the present study was the demonstration that the genotype combinations of ACE DD+ ACTN3 RR/RX, ACE DD + VDR ff/Ff, and VDR ff+ ACTN3 RR/RX were highly represented in both male and female national weightlifters when compared to controls. This finding was in accordance with those of Gunel *et al*. [43], showing a higher frequency of ACE DD/ACTN3 RR genotype combinations in elite athletes than in the controls. This finding was supported by some previous reports showing that the influence of ACTN3 and VDR gene variants may vary by sex [28,44,45]. For instance, Delmonico *et al*. [45] reported higher peak power in women with the ACTN RR genotype at baseline and higher gains in power following RT, whereas no significant differences were observed in men. Furthermore, Windelinckx *et al*. [28] showed that quadricep isometric and concentric strength were higher in female f/f homozygotes compared to F allele carries, whereas no association was observed in men.

More recently, the investigation of sex-specific differences in the genetic influence of genes and genotype, observed the influences of ACTN3 R577X on muscular strength were found to vary by sex. For example, Vincent *et al*. [46] found muscle strength at a greater level in women with ACTN3 RR genotype when compared with X allele carriers, but not in men. Massidda *et al*. [47] studied the association between the polymorphisms of the VDR gene and muscular mass and strength in elite male soccer players. They found no association of VDR polymorphisms with muscular strength or thigh muscle.

Given that weightlifting is a power sport that requires highly skillful technique, perfect body physique, and psychological factors ([48], Gunel *et al*. [43]), a further study is needed to better understand the genetic influence on physical performance in weightlifters. Furthermore, the whole genome-wide technologies approach needs to be employed sufficiently to study large cohorts of world-class athletes with adequately-measured phenotypes.

Conclusions

The result of this investigation can conclude that the frequencies of ACE D, ACTN3 R, and VDR f allele and genotypes were highly represented in both male and female weightlifters when compared to the non-athletes group. In addition, the frequencies of favorable genotype combinations between ACE (DD) + VDR (ff), ACE (DD) + ACTN3 (RR), and ACTN3 (RR) + VDR (ff) in both male and female national groups were higher than those of junior weightlifters and controls.

Based on these findings, it is suggested that favourable genotypes, individually or in combination, of ACE (DD), ACTN3 (RR), and VDR (ff) may influence muscle performance in weightlifters. This suggests the genetic potential of ACE, ACTN3, and VDR in determining muscle performance.

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