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Original Article

The comparison of the genetic profile of ACTN3 single-nucleotide polymorphism in elite weightlifters and non-athletes

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Abstract
Background and aims: The α-actinin-3 (*ACTN3*) gene plays a key role in muscle signaling pathways and sarcomere contraction. Iranian weightlifters are among the most successful world and Olympic champions. Therefore, the present study was performed to compare the genetic profile of the single-nucleotide polymorphism of the *ACTN3* gene in elite Iranian male weightlifters vs. non-athletes.
Methods: This cross-sectional study was of a qualitative-quantitative type. Subjects included 30 volunteer elite male weightlifters, including all of Iran's premier league, members of the national team, Olympic, world, and Asian champions of Isfahan province, with a mean age of 21.77±7.11, the height of 179.87±6.98, and the weight of 96.87±22.73 (Mean±SD) versus 30 volunteers available healthy non-athletes who were the same in age, height, and weight as weightlifters. After completing the consent form and physical health measurement questionnaire, participants' saliva samples were collected, and DNA was extracted accordingly. Genotypes were determined after performing the polymerase chain reaction via the Tetra-Arms method and electrophoresis. Data were analyzed by SPSS (version 20) and the chi-square test ($P<0.05$).
Results: The prevalence of the RR genotype in selected weightlifters (56.7%) was significantly higher than that of other genotypes ($\chi^2=13.40$, $P=0.03$). There was a significant difference in ACTN3 R/X genotype distribution ($P=0.039$, $\chi^2=6.48$) between weightlifters and non-athletes.
Conclusion: A higher prevalence of the RR genotype of the *ACTN3* gene in selected elite male weightlifters versus non-athletes can be likely considered for selecting genetically predisposed individuals.
Keywords: Sport genomic, Single-nucleotide polymorphism, Weightlifting, Elite, Non-athletes

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Introduction

Sports Scientists and researchers in related disciplines unquestionably agree on the fact that the level of physical development and the process of adaptation to exertions are due to the genetic makeup of individuals so that the genetic characteristics of the Olympic athletes allow them to perform at a high level (1). In other words, exercise performance is a highly complex, multifaceted phenomenon. It is characterized by intrinsic (e.g., genetics, motor behavior, physiology, and psychology) and environmental (e.g., exercise and nutrition) factors, as well as the interaction between them. The evidence of genetic factors underlying the propensity to exercise in humans has been demonstrated in a number of studies (2). These are the source of individual differences due to variability in body strength, strength, physical morphology, and endurance, which form the basis of bodily function. Therefore, these genetic factors are probably a highly important aspect of genetic variability that underlies sports excellence (3). For example, in twin studies, the heritability of muscle strength is about 30-80% in different phenotypes such as isometric knee strength, hand strength, and elbow flexion. It is well established that sprint/power and endurance capacities are influenced by genetics. Among the candidate polymorphisms potentially associated with muscle function and physical performance, the most extensively studied are the angiotensin-converting enzyme (ACE) and the R577X variant of the α-actinin-3 (*ACTN3*) gene (4). Previous studies have shown that individuals with the ACE I/I genotype are likely to maintain higher arterial oxygen saturation at rest and when exercising at altitude, thus this feature provides an advantage for improving performance in hypoxic conditions such as altitude.

Probably the most fundamental question is whether the structural diversity of DNA affects the expression of genes and proteins at the tissue level. If a polymorphism affects the structure of a protein (e.g., an annoying polymorphism or a mutated form), it is usually easier to observe its effect on physiology and phenotype. Rapid advances in technologies in the field of genomics such as high throughput DNA sequencing, large data processing by machine learning algorithms, and gene-editing techniques are expected to make precision medicine and gene therapy a greater reality. The field of exercise genomics has also advanced by incorporating these innovative technologies (5). More recently, genetic markers, including several single-nucleotide polymorphisms (SNPs), have been correlated with enhanced aerobic capacity, strength, and an overall increase in athletic ability (6). A literary study showed that at least 43 genetic markers are associated with an athlete's strength/power status (7). To develop specific gene tests that identify a specific trait, the DNA of samples is extracted to find the SNP. The results indicate which pattern is most likely associated with the fitness factor gene (8). Thus, SNPs can be used to generate genomic maps with high accuracy (9). The best-known polymorphism affects the physical function of R577X in the *ACTN3* gene, which is a polymorphism that results in dysfunctional protein synthesis. This is due to the absence of alpha-actin-3 protein in humans carrying both polymorphic alleles. Lean specimens are reduced, providing a highly exciting model for evaluating how protein deficiency affects muscle structure, muscle metabolism, and function. The gene for this protein encodes *ACTN3*. The *ACTN3* protein is part of the fast-twitch muscle contraction fibers in the contractile system.

Rapid contraction fibers cause muscular and rapid contractions (e.g., throwing and lifting loads). ACTN3 is a protein located within the skeletal muscle with a key role in the production of sarcomeric force (10). The two types of isoforms of ACTN2 and 3 are encoded by two different genes called ACTN2 and 3. The contractile system is in the muscles (11).

Background literature demonstrates several studies on how the SNPs of the *ACTN* gene (having XX, RX, and RR genotypes) are related to athletic performance in sports groups or those examining the correlation between these SNPs with the physiological characteristics of skeletal muscle. The results of these studies have been different; thus some results have shown an association between the genotypes of the subjects regarding this gene with athletic performance or muscle strength. For example, Yang found that XX genotype frequency was higher in female endurance athletes than in the control group, while Ahmetov and Fedotovskaya reported that XX genotype frequency was higher than the control group for both female and male endurance athletes (12). However, some studies represented that the frequency of genotype XX (which leads to the non-production of *ACTN3*) is lower in athletes in speed-strength disciplines than in athletes in other disciplines and improves the performance of athletes in performances with high cardiovascular endurance needs; nonetheless, the presence of *ACTN3* (lack of genotype XX) is essential for speed-strength training.

Weightlifting is one of the oldest sports that has an international structure and has a significant presence in the sports Olympics (13). In addition, it is a discipline based on muscle strength and power, and the main structure of its exercises is characterized by high intensity and frequent endurance movements (14). Iranian weightlifting champions have also won valuable world and Olympic medals in various periods of the world and Olympic events. A survey of the background literature indicates that no research has specifically examined the prevalence of the ACTN3 X/R genotype in elite Iranian adult weightlifters.

Honarpour et al examined the relationship between the SNPs of the *ACTN3* gene with the athletic performance status of elite Iranian soccer athletes. The results of genotype distribution (RR = 41 and RX = 37%) were significantly more than the control group, highlighting the relationship between ACTN3 genotypes and high levels of athletic performance in Iranian male football players (15).

Khaledi et al reported that there was no significant difference in the frequency of genotypes of 5 genes between athletes and non-athletes (16). Further, Mirzaei et al concluded that the highest prevalence was in genotype RX = 63%, and the prevalence of genotypes was % RR = 31%, XX = 6% (17).

To the best of our knowledge, no study in the country has so far investigated the association between *ACTN3* gene polymorphism in adult elite male weightlifters. Therefore, given the importance of this sport for our country in winning medals in the Olympics and world championships, and the fact that the basis of genetics is a regional issue, there is a gap about "What is different about the *ACTN3* gene of Iranian male weightlifters compared to non-athletes? And basically, can genetics play a role in success in weightlifting?" Thus, the aim of this study was the comparison of *ACTN3* gene profile SNPs in selected Iranian male elite weightlifters and non-athletes.

Materials and Methods

Subjects

This cross-sectional qualitative-quantitative study was conducted in 2021. Due to the brilliance of the champions of Isfahan province in the Olympic and world competitions to select the subjects, necessary coordination was made by sending a written letter to the General Directorate of Sports and Youth of Isfahan province for the participation of the weightlifting champions of this province in this study. All elite weightlifting champions (including national league champions, national team champions, Asian, Olympic, world, and international champions, n = 31) aged 16 and over were announced to the researchers by the head coach of the provincial weightlifting team and were examined in this study. By examining the family relationship between the champions, due to the existence of a family relationship, one of them had the priority of obtaining a higher sports rank in the study of preservation, and the other person was excluded from the study. After completing the consent form and physical health measurement questionnaire, 30 volunteer available healthy non-athletes similar in age, height, and weight to elite weightlifters were selected as the non-athlete group.

Salivary sampling

For sampling, the subjects were asked to brush their teeth and abstain from eating, drinking, and chewing gum for one hour prior to sampling. Fresh saliva samples were collected in a 15 mL sterile Falcon. To extract from the DNA extraction kit, saliva from the biogenic company was used, which required at least 1 mL of foamless individual saliva. The samples were then labeled and transported in boxes containing ice packs to the laboratory and stored in the refrigerator at -20°C. The extracted DNA concentration was between 440 and 130 ng/µL. The absorption ratio of 260-280 nm of the samples was between 1.8 and 1.96 (Figure 1).

For sampling non-athletes, two staff teams were present at the Mahdieh Diagnostic Laboratory in Isfahan, located in Ahmadabad Square, for a specified period of time. Saliva samples were taken, and in selecting non-athletes, the homogeneity of the non-athlete group in terms of anthropometric demographic characteristics (age, height, and weight) with the athlete groups was considered as much as possible.

The Tetra-Arms method was used to study genetic diversity in the *ACTN3* gene. Fin and Rout primers are also a product if the person has an X allele whose product is 199 bp. Fin and Rout primers are also a product if the person has an R allele whose product is 241 bp. If a person is heterozygous, he will have both 199 and 241 bands. Of course, the non-specific 402 bp band, which is the product of Fin and Rout primers, should be observed in all samples. The ladder was also considered 100 bp (Figure 2).

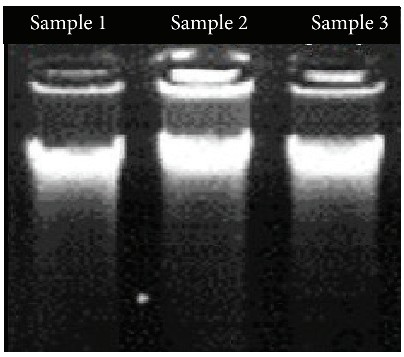

Figure 1. Results of the extraction of genomic DNA from human salivary cells on 1% agarose gel, including three samples (typical)

Data analysis

Descriptive statistical methods included frequency distribution, means, frequency percentages, standard deviations, and tables. The Shapiro-Wilk test was used to examine the normality of data. Moreover, an independent *t* test was employed to compare height and weight between groups, and the Mann-Whitney U test was utilized to compare the age between groups. Differences in the frequency and the evaluation of the association between qualitative variables were assessed by the chi-square test via SPSS software, version 20. The differences were considered statistically significant at $P<0.05$.

Results

The biographical characteristics of the two groups (age, height, and weight) are summarized in Table 1. Based on the results, there were no significant differences in age (Mann-Whitney U = 415.50, $P=0.609$), height (T = 1.661, $P=0.102$), and weight (T = 1.907, $P=0.061$) between the two groups.

The results of genotyping distribution revealed that the RR genotype was the most common in weightlifters (56.7%). Based on the results of the chi-square test, a significant difference was found in ACTN3 R577X genotyping distribution in weightlifters ($\chi^2=13.40$, $P=0.03$). The results of genotyping distribution represented that the RR genotype was the most common in the non-athlete group (40%). The Chi-square test results showed that there was no significant difference in ACTN3 R577X genotyping distribution in the non-athlete group ($\chi^2=0.800$, $P=0.670$). According to the results of the chi-square analysis, a significant difference was observed in the genotypic (R/X) SNP distribution of the *ACTN3* gene in weightlifters and non-athletes ($P=0.039$, $\chi^2=6.488$) (Table 2).

It can be inferred that there was a significant difference in the percentage of the frequency of R/X genotypes between athletes and non-athletes. The frequency of the

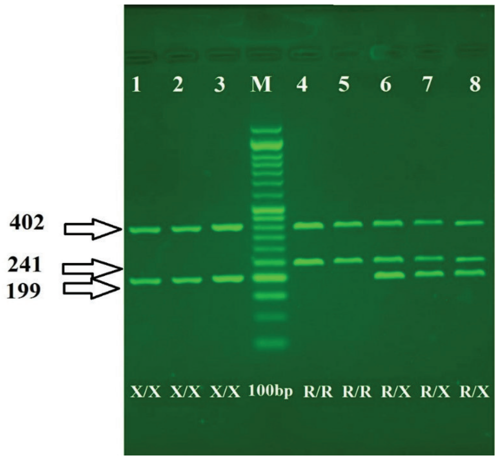

Figure 2. Representation of an agarose gel electrophoresis of PCR minor products of the *ACTN3* gene. *Note*. PCR: Polymerase chain reaction; ACTN3: α-actinin-3. Wells 1, 2, and 3 indicate genotype XX. Wells 4 and 5 represent the RR genotype, and Wells 6, 7, and 8 demonstrate R/X and M Ladder genotype (100 bp)

RR genotype in athletes was higher than in non-athletes, while the frequency of the XX genotype in athletes was lower than in non-athletes (Figure 3).

Discussion

The results of the chi-square analysis demonstrated that there was a significant difference in the genotype (R/X) SNP distribution of the *ACTN3* gene in weightlifters and non-athlete groups; the frequency of the RR genotype in athletes was higher than that in non-athletes, whereas the frequency of the XX genotype in athletes was lower than that in non-athletes.

In their survey about muscle work and its relationship with ACTN3 polymorphisms and with the improvement of explosive strength, Melián Ortiz et al reported that the RR variable obtained improvement results with regard to RX and XX variables in the long jump, Sargent test, and power jump (18), which is consistent with the results of the present study. Similarly, Fallah et al (19) compared the frequency of the R577X polymorphism of the *ACTN3* gene between the two groups of elite Iranian male judo-ka (n = 40) and the control group (120 healthy non-athlete males). They found that the frequency of the RR genotype of the *ACTN3* gene polymorphism in judo-ka subjects was significantly higher than in the control group, and the frequency of the R allele in the judo-ka group was higher than in the control group as well. Therefore, they concluded that ACTN3 R577X polymorphism is a genetic marker for the identification of judo-susceptible individuals in the Iranian population. The presence of ACTN3 has a beneficial effect on skeletal muscle function in the production of high-velocity force contractions and provides an evolutionary advantage due to the increase in sprint function. ACTN3 deficiency affects muscles; sprint and strength activities rely on fast muscle fibers. The speed and strength of muscle contraction decrease in the absence of ACTN3 (20). Azarakhsh reported that the expression of the ACTN3 of muscles in the exercise group, compared to both control and non-exercise groups, increased significantly (21).

In contrast, the results of the present study contradict those of Abubakri et al (22) and Khaledi et al (16). Abubakri et al also studied the frequency of the rs1815739 polymorphisms of the *ACTN3* gene in 30 professional football players of the Iranian premier league and the control group, including 100 healthy non-athlete men. The results of the chi-square test showed a significant difference in the frequency of the XX genotype in *ACTN3* gene polymorphism between football players and the control group, but there was no significant difference in the frequency of the RR genotype between football players and the control group. Accordingly, they concluded that ACTN3 polymorphism is a genetic marker for football and the identification of talented individuals in this field in the Iranian population (22). Khaledi et al also

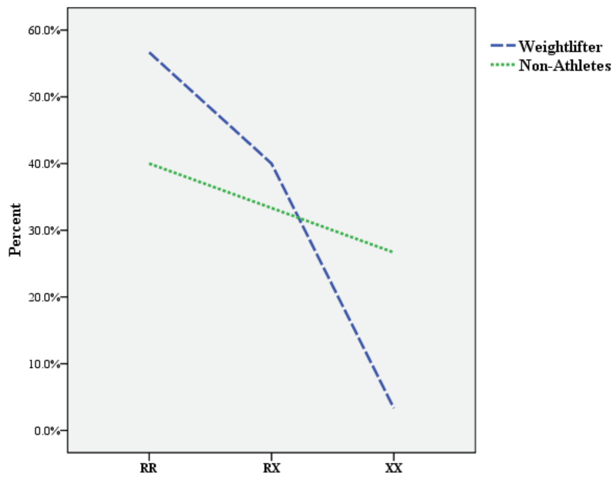

Figure 3. Comparison and frequency of the R577X polymorphic genotype of the *ACTN3* gene in the two groups of weightlifters and non-athletes. *Note*. ACTN3: α-actinin-3

Table 1. The characteristics of age, height, and weight in two groups

Factor	Group	N	Mean ± SD	Result of normality (Shapiro-Wilk)	Comparison of two groups	*P* value
Age	Elite weightlifters	30	21.77 ± 7.11	No	Mann-Whitney U = 415.50	0.609
	Non-athletes	30	28.45 ± 5.71			
Height	Elite weightlifters	30	179.87 ± 6.98	Yes	Independent T-test = 1.661	0.102
	Non-athletes	30	176.57 ± 8.34			
Weight	Elite weightlifters	30	98.87 ± 22.73	Yes	Independent T-test = 1.907	0.061
	Non-athletes	30	88.47 ± 8.05			

Note. SD: Standard deviation.

Table 2. Comparison and frequency of the R577X polymorphic genotype of the *ACTN3* gene in the two groups of weightlifters and non-athletes

Group	Frequency of R577X genotype of ACTN3 gene			Within group comparison	Between group comparison	
	X/X	R/X	R/R		χ^2	*P* value
Weightlifter	1 (3.3%)	12 (40%)	17 (56.7%)	$\chi^2 = 13.40$, $P = 0.03$	6.488	0.039
Non-athletes	8 (26.7%)	10 (33.3%)	12 (40%)	$\chi^2 = 0.800$, $P = 0.67$		

Note. ACTN3: α-actinin-3.

examined the frequency of polymorphism relating to physical function and found genetic predisposition in the Iranian population and elite athletes, and the results of the Chi-square test represented no significant difference in the frequency of the genotypes of five genes between groups (16).

The *ACTN3* gene may play a significant role in explaining the aspects of athletic performance (23). Due to discrepancies in the results of their research with those of the present study, it is possible to differentiate between the subjects (elite weightlifters versus other athletes), different climates, and different methods of DNA extraction (saliva versus bloody samples). Therefore, future studies are necessary to study the types of *ACTN3* gene polymorphisms in athletes of different disciplines as closely as possible.

In general, strength and maximum speed are important factors in determining the results of weightlifters during competitions, and the relationship between muscle strength and some genes has been proven. The ability of skeletal muscles to exert force at high speeds is a highly important factor in the success of athletes in strength and speed performance, which is influenced by genetic factors. In fact, a person's chances of becoming a particular power athlete decrease without a good genetic background (17). The *ACTN3* gene, which encodes an actin-binding protein, has been found to be associated with strong athletic performance (24). This gene is located on chromosome 11q13-q14 and encodes the isoform of skeletal muscle protein, which is the main structure of the Z-line involved in the binding of thin filaments containing muscle actin. The mentioned gene is responsible for the production of alpha-actin-3. The alpha-actin-3 protein in fast-twitch fibers enables them to produce large amounts of force at high velocities.

Although environmental factors such as lifestyle, diet, training, and stress can influence athletic performance, our data demonstrated the importance of genetic study in sports aimed at developing personalized training and achieving the best possible athletic excellence (25). Considering that in the present study, the frequency of the RR genotype in elite weightlifters was higher than in the control group, and that of the XX genotype in elite weightlifters was lower than in the control group, people with the RR genotype in strength and power sports such as weightlifting would be more successful. However, more research is needed in this area, especially on other polymorphisms in power athletes.

Conclusion

Polymorphisms are specific regions of DNA that can vary between individuals and may contribute to high levels of performance. The expression of ACTN3 is limited to fast muscle fibers responsible for generating force at a high velocity so that the SNPs of the *ACTN* gene (having XX, RX, and RR genotypes) are related to athletic performance. The results of the present study confirmed the superiority of the homozygous RR genotype related to the *ACTN3* gene of selected Iranian elite male weightlifters versus non-athletes. Therefore, it seems that the R/R genotype of the *ACTN3* gene polymorphism is probably considered a genetic marker for the identification of weightlifting individuals in the selected Iranian population.

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Authors' Contribution

Conceptualization: Hamidreza Taji, Mohammadreza Batavani.
Formal analysis: Mohammadreza Batavani.
Funding acquisition: Hamidreza Taji.
Investigation: Hamidreza Taji, Mohammadreza Batavani.
Methodology: Mohammadreza Batavani, Mohsen Ghofrani.
Project administration: Mohammadreza Batavani, Mohsen Ghofrani, Omid Mohammaddoost.
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Supervision: Mohammadreza Batavani, Mohsen Ghofrani.
Validation: Mohammadreza Batavani, Mohsen Ghofrani.
Writing–original draft: Hamidreza Taji, Mohammadreza Batavani, Mohsen Ghofrani.
Writing–review & editing: Hamidreza Taji, Mohammadreza Batavani, Mohsen Ghofrani, Omid Mohammaddoost.

Competing Interests

The authors declare that they have no conflict of interests to disclose.

Ethical Approval

This research was granted by the Ethics Committee of the University of Isfahan (Date 2022-01-26 No: IR.UI.REC.1400.112).

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