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The relationship between ACE I/D gene polymorphism rs4646994 and incidence and severity of sport injuries in Iranian male elite weightlifters

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ABSTRACT

Introduction: Despite careful control of environmental factors, there is a wide variety of interpersonal muscle-induced muscle damage. Genetic changes can also be important in determining susceptibility to injury. Weightlifting is one of the most successful Iranian sports teams in Olympics; so, the aim of this study was the investigation of the relationship between ACE I/D gene polymorphism rs4646994 and incidence and severity of sport injuries in Iranian male elite weightlifters.

Materials and Methods: The study was cross-sectional research that conducted in 2022. The statistical population consisted of male elite weightlifters from Isfahan consisting of 31 athletes who all were investigated as sample. A questionnaire was used to collect the injuries data. Saliva samples were taken from all subjects and DNA was extracted and genotyped by using real-time polymerase chain reaction (real time-PCR). Chi-square and regression tests were used to analyze the data. Significance level was also considered at $P < 0.05$.

Results: There was a significant difference in the incidence of injuries in different genotypes. ID genotype (59.4%) had the highest incidence of injury in compare with DD (23.2%) and II (17.4%) genotypes ($\chi^2 = 21.47$, $P = 0.001$). Also, there was a significant difference in the severity of injuries in different genotypes ($\chi^2 = 15.59$, $P = 0.049$); but there was no relationship between genotype and incidence and the severity of sports injuries in selected Iranian elite male weightlifters ($P = 0.715$).

Conclusion: The results showed differences in the incidence and severity of sport injuries in different genotypes, hence it would be important in the prediction injury risk in weightlifters.

Keywords: Sports Genomics, Sport Injuries, Weightlifters, Elite

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Introduction

It is a rule of thumb that eight to ten years and 10,000 training hours are necessary to become an expert in either music or sports (1). Achieving athletic success is associated with the occurrence of sports injuries that may even lead to the termination of an

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athlete in some circumstances (2). In addition to threatening the athlete's health and reducing her efficiency, these injuries can lead to significant financial costs for the individuals and their teams, especially for the clubs (3).

Despite careful management of environmental conditions, exercise-induced muscle damage (EIMD) and injuries vary widely across individuals (4). In an elite team, for example, when athletes are often subjected to the same training and diet, there are players whose injury rate and severity are more significant than others who are seldom hurt (5). This interpersonal variety cannot be anticipated solely on an individual's age, ethnicity, body composition, or fitness level, suggesting that additional variables such as genetic variation may play a role in predicting injury vulnerability. The polymorphic or single-nucleotide polymorphism (SNP) profile of each person represents his or her chromosomal pair in the DNA sequence, the form of which varies from individual to individual or biological species in one nucleotide (6). In the other words, the kind of SNP arrangement provides distinct metabolic, structural-muscular, and personality aspects in one person compared to another, which might be proof of why certain people have different responses to particular stimuli, such as exercise, from a structural-muscular standpoint (7). The ACE gene, which is found on chromosome 17, gives instructions for creating an angiotensin-converting enzyme protein. It is one of the genes that is changed and linked to exercise capacity, which helps manage blood pressure and influences skeletal muscle performance (8).

The ACE I / D single nucleotide polymorphisms have three genotypes include of DD, II (homozygous), and ID (heterozygous) that change the activity of this gene. The DD genotype has the most significant amount of angiotensin-converting enzyme. The most significant fraction of quickly contracting muscle fibers and the highest rate of velocity are assumed to be associated with the DD pattern. The D allele is thought to boost muscular strength by allowing ACE to drive cell proliferation. As a result, the D allele may be advantageous to strong athletes (such as weightlifters) (9). Polymorphisms in the ACE I/D gene have recently been linked to vulnerability to inflammation and post-exercise muscle injury (10). Through the inflammatory response, differences in ACE activity may alter EIMD sensitivity. These data imply that the ACE I / D polymorph's D allele is linked to a lower risk of muscular injury. As a result, a genotypic link with vulnerability to exercise-induced muscle injury might explain the relationship between ACE I / D polymorphism and elite athlete status (4). Yamin et al. (2007), for example, looked at the circulating levels of Creatine Kinase (CK), a marker for EIMD, in post-exercise ACE genotypes and found that homozygotes II had the most excellent CK response (11). After vigorous exercise, however, DD homozygotes showed the lowest plasma CK activity. Individuals with EIMD who had one or two copies of the D allele, particularly, exhibited smaller rises and CK peak levels than those with genotype II. Furthermore, following triathlons (12) and marathons, a correlation between the D allele with a decreased CK response has been narrated (13). On the other hand, Heled et al. (2007), observed no relation between the ACE I / D polymorphism and CK response (14).

However, heavyweight athletes rely on strength or power much more than other athletes and Ulucan&Göle (2014) have stated that the D allele is an advantage for power strands in which explosive speed is important (15). A review of past research shows that although much research has been done to identify ACE I / D gene polymorphism in elite athletes; however, none of them has been specifically and extensively related to the injuries of power / strength sports such as weightlifting and it can be noted that none of these researches have been done specifically on Iranian

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weightlifters. Weightlifting is a kind of strength and power sport in which the basic structure is defined by high-intensity; frequent endurance exercises (16). Investigating the incidence of injuries and investigating its relationship with the kind of genes related to performance can be a step towards a more accurate information technology in weightlifting in order to decreasethe incidence ofsport injuries and its economic costs. There is a hypothesis that different ACE (I / D) genotypes can be associated with different infection to muscle injuries in elite weightlifters, so the question is whether the incidence of sports injuries in weightlifting has a genetic basis? And is there the relation between the ACE I/D gene polymorphism and the incidence and severity of muscle injuries among elite Iranian weightlifters? So the aim of this research was to investigate of the relation between ACE I/D genotypes and severity and incidence of muscle injury in elite male Iranian weightlifters. It is hope that the study's findings start the line for the further researches into genetic risk factor into the incidence and severity of sports injuries among elite Iranian male weightlifters.

Materials and Methods

Subjects

The study was cross-sectional research in type of Ex-Post Facto that conducted in 2022. The statistical population of the present study consisted of all elite male weightlifters (competitors in the Iranian weightlifting league, and international, Asian, world, and Olympic champions), above 18 years of agefrom Isfahan province (to consider the climate principle). According to the correspondence and inquiry from the Isfahan Sports province, they were 31 participants who were selected purposefully. Meanwhile, one of them was rejected from the study due to the existence of a family relationship.

In addition to the lack of family relationships, others criteria were considered as the inclusion criteria for this study such as the gender and the lack of specific disease history based on the Physical Activity Readiness Assessment Questionnaire (PAR-Q). Also, the samples of the non-athletes group that did not have any regular exercise during their life were selected from male volunteers referring to Mahdieh (AJ) diagnostic laboratory, which were include of 49 healthy volunteers' people. They had similar height, weight and age and selected to compare genotype with weightlifters. Demographic variables of samples and comparison of them are described in Table 1 After obtaining the necessary permits, health experts, laboratory science experts, and researchers went to the training camp area of elite weightlifting champions in three cities of Isfahan, Sedeh Lenjan, and Najaf Abad. After completing the consent form, the sports injury questionnaire of Fuller et al. (2006) (17) was used which it was reviewed by five expert weightlifters and finally was approved. Also, its Cronbach's alpha reliability coefficient was calculated to be 0.87.

Table1. The **c**omparison of biographic characteristics between weightlifters and non-athletes.

	Groups		
Variable	Weightlifters	Non-athletes	**P value**
Height (cm)	179.87 ± 6.98	177.59 ± 6.15	0.144
Weight (kg)	96.87 ± 2.27	89.93 ± 7.25	0.063
Age (year)	21.77 ± 7.11	22.41 ± 2.63	0.585

Data are shown as mean ± SD.

This questionnaire included different sections such as individual characteristics, time of injury, type of injury, injured area, and causes of injury. In this questionnaire, the severity of injuries is divided into four degrees based on the duration of absence days: low (1-3 days), mild (4-7 days), moderate (8-28 days), and severe (> 28

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days). Saliva sampling was conducted to perform sampling by health protocols in corona conditions. Before sampling, each subject was asked to brush tooth and avoid eating, drinking, smoking, and chewing gum for one hour. Then, at least 3 ml of Samples of saliva without foam was collected in a sterile 15 ml Falcon and kept at -20 Celsius degrees. This research was granted by the Ethics Committee of University of Isfahan (Date 2022-01-26 No: IR.UI.REC.1400.112

DNA Isolation

To extract the DNA, after melting the saliva sample, 1.5 ml of it was placed on ice and 0.5 ml of cold PBS was added to mix well. The microtube containing the sample was centrifuged at 4°C at 8000 rpm for 10 minutes and the supernatant liquid was discarded and thoroughly mixed with pipette. To the resulting mixture, 50 µl of buffer was added and vortexed for 30 seconds. 5 µl of proteinase K was added to each microtube and mixed thoroughly. The resulting mixture was incubated at 55 °C for one to two hours and vortexed for 30 seconds to obtain a milky mixture. The microtubes were centrifuged at 10,000 rpm for 4 minutes at 4 °C. The supernatant was transferred to a new 2 ml microtube and placed on ice; Then 1 ml of cold isopropanol was added to each microtube. Each microtube was gently rubbed ten times until a white strand of DNA was observed. The sample was centrifuged at 10,000 rpm for 4 minutes at 4 °C. The supernatant was discarded. The resulting DNA precipitate was washed with 500 µl of 70% ethanol. Ethanol was completely removed and the DNA precipitate was incubated for 5 to 10 minutes at room temperature to dry; The DNA was then dissolved in the desired amount of water or TE buffer (18).

The Program for PCR of ACE Gene

The initial denaturing temperature of 94°C for 5 minutes and then 35 cycles respectively with denaturing temperature of 94°C for 30 seconds and annealing temperature of 56°C for both pairs of primers for 45 seconds and an elongation temperature of 72 °C for 1 min, respectively were applied. The final elongation step was done at 72 °C for 7 minutes. The forward (GCCCTGCAAGGTGTCTGCAGCATGT) and reverse (GGATGGCTCTCCCCGCCTT) primers of the study was previously determined (29).

Electrophoresis of PCR Product on Agarose Gel

The PCR product was transferred to the gel wells using a sampler. The electrophoresis buffer is then poured into the electrophoresis tank so that the buffer completely covers the gel. The power supply was then connected to the electrophoresis tank and connected to the power supply at a voltage of 120 for 60 minutes. Based on the length of PCR products, the genotype of each sample was determined. Since the polymorphism 4646994 rs of the ACE (I / D) gene is deleted and added, genetic diversity can be determined by examining the length of the product by designing a primer for both sides of the genetic diversity. The primers were designed so that the PCR product would have a length of 319 nucleotides if there was a D allele and a length of 597 nucleotides if it was an allele I. Figure 2 shows an example of three homozygous individuals for alleles I and D and one heterozygous individual (Figure 1).
To ensure the accuracy of the results, a sample of each genotype was randomly selected and amplified by external primers, Tetra-primer ARMS PCR method, and after observing the appropriate band on the gel along with the external return primer; it was sent to Kosar Technology Company and approved.

Statistical Analyses

To analyze the data, descriptive statistics including frequency distribution, frequency

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percentage, mean and standard deviation as well as inferential statistics including the Chi-square test (used to compare the size of any discrepancies between the expected results and the actual results) and logistic regression (to check the relationship between the type of genotype and incidence and severity of injury) was done. The significance level was also considered at P < 0. 05.

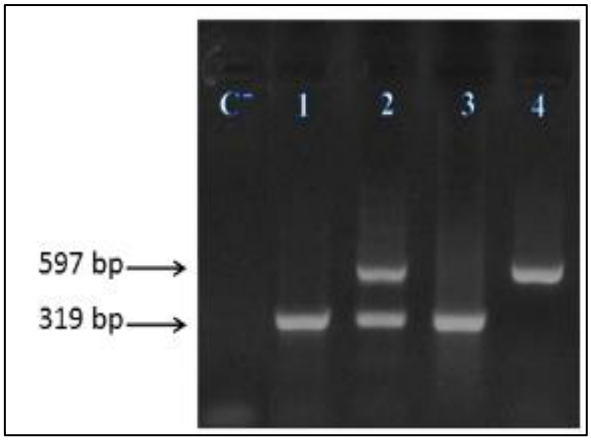

Figure 2. Genotyping results of a sample of three homozygous individuals for alleles D (lanes 1 and 3) and I (lane 4) and one heterozygous ID (lane 2).

Results

The results showed that the distribution of different genotypes of ACE rs 4646994 markers in selected elite male weightliftersand non-athletes were not significantly different (Table 2). Also, the results of the comparison of ACE (I/D) genotypes distribution in selected elite male weightlifters with non-athletes did not show any significant difference (χ^2 = 2.715, P = 0.257).

Table 2. Comparison of ACE I/D genotypes distributions in selected elite male weightlifters and non-athletes

Groups	Genotypes (I/D)	Frequency (N)	Percent (%)	χ^2 test (Within Group)
Weightlifters	DD	9	30.00	χ^2= 1.400, P =0.497
	DI	13	43.3	
	II	8	26.70	
Non-athletes	DD	19	38.8	χ^2= 10.571, P =0.005
	DI	24	49	
	II	6	12.2	

II: Insertion / Insertion, ID: Insertion / Deletion and DD: Deletion/ Deletion.

As depicted in Table 3, the incidence of sport injuries in different ACE I/D genotypes in Iranian elite male weightlifters was significantly different (χ^2 = 21.478, P = 0.001). The incidence of sport injury per each person in different genotypic categories was significant (Table 4). The results of ANOVA test showed a significant difference in the incidence of sport injury per each person in different ACE I/D genotype categories (P = 0.001, F = 169.1).

Table 3. Comparison of the incidence of sport injuries that subdivided in different ACE I/D genotypes classifications.

Genotype	Observed N	Percent	Expected N	Residual	DF	Chi-Square	P value
DD	16	23.18	23.00	-7.0			
DI	41	59.42	23.00	18.0	2	21.478	0.001
II	12	17.39	23.00	-11.0			
Total	69						

II: Insertion / Insertion, ID: Insertion / Deletion and DD: Deletion/ Deletion.

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Table 4. Comparison of incidence of sport injuries per each person in different ACE I/D genotype classifications

Genotype	Sample size	Number of injuries	Number of injuries per person	ANOVA	P value
DD	9	16	1.8		
DI	13	41	3.15	F = 169.1	P = 0.001*
II	8	12	1.5		
Total	30	69	2.3		

II: Insertion / Insertion, ID: Insertion / Deletion and DD: Deletion/ Deletion.

Table 5 shows the severity of sport injuries in different types of ACE I/D genotypes in elite male weightlifters. The results of statistical analyze showed that the severity of sport injuries in different types of ACE I/D genotypes in elite male weightlifters were significantly different (χ^2 = 15.592, P = 0.049) (Table 5).

Table 5. Comparison of severity of sport injuries in different ACE I/D genotype classifications.

	Intensity					
Genotype	0	Up to 3 days	Up to 7 days	Up to 28 days	More than 28 days	Total
DD	0	0	3	7	6	16
DI	0	6	5	23	7	41
II	1	0	2	3	6	12
Total	1	6	10	33	19	69

II: Insertion / Insertion, ID: Insertion / Deletion and DD: Deletion/ Deletion.

In the present study, the relationship between the distributions of ACE gene I/D polymorphisms rs4646994 with the incidence of sport injuries in Iranian elite male weightlifters investigated. The results of regression showed that the variable in the entered model was not able to predict the changes of the dependent variable of sport' injuries (P = 0.234, OR = 2.901). Also, in the study of relation between the severity of sport injuries and distributions of ACE gene I/D polymorphisms in selected Iranian elite male weightlifters, the value of sig for the gene variable was more than 0.05, which indicates that this parameter was not significant. Therefore, the severity of sport injury was not related to ACE I/D genotypes in selected Iranian elite male weightlifters.

Discussion

The ACE ID genotype was the most common in the weightlifter group (43.3%). There was no significant difference in the distribution of the ACE gene I / D polymorphism genotype in the weightlifter group vs. non-athletes. Eider et al., and Gineviciene et al., in the study of Lithuanian weightlifters reached to the similar approaches (19, 9). But these results were in inconsistent with the results of Pimjan et al., on 117 Thai male and female adolescent weightlifters (20). Exercise performance is a very complex phenotypic trait, which affected by many other traits, such as distribution of muscle fibers, aerobic and anaerobic strength and capacity, and physical abilities. Some of other characteristics related to physical function are: body composition, aerobic strength and muscular strength.

Also skills would be required to reach to the elite performance. For this reason, it is possible that the effect of the gene on the identification of elite people from non-athletes has decreased. Nazarov et al., narrated that in addition to the fact that physical strength will play an essential role in the success of weightlifters, success in weightlifting also depends on the body's (athletic) throwing abilities (explosive power) in performing fast movements (21). The D allele associates with strength as a beneficial factor for athletes undergoing heavy resistance training (such as power lifters), but is not necessary to improve

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speed in athletes (19). The difference in results with Pimjan et al., may be due to differences in the subjects' gender, age and ethnic background. The subjects of the study of Pimjan et al., were at a young age and both sexes (20), while the subjects of the present study were Iranian adult men.

Also, no difference was observed between the genotype distribution of selected elite male weightlifters and non-athletes. In interpreting this result, it should be noted that the selected Iranian weightlifting community, which belongs to the Caucasian race, probably has potential genetics in performing endurance and power / strength abilities (22). Considering the championships experiences of the Iranian athlete community in the long years of weightlifting in the Olympic and world events, there is also the hypothesis that the Iranian community is located in the Caucasus region; in general, it has a favorable genotype for success in the field of weightlifting. It is suggested that this genotype in the population of countries or regions that are not successful in weightlifting be compared with the elite champions of this field. Different living conditions between Asians and Caucasians may contribute to different genetic effects (5). Due to the lack of genotype differences between elite weightlifters and non-athletes, the results of the present study can be helpful in diagnosing predisposed individuals or exposed to sports injuries via a genetic aspect, preventing injuries and conducting future additional research.

The results of the present study showed that athletes carrying ID genotype (average: 3.15 injuries per person) had the highest incidence of sports injuries compared to carriers of DD genotypes (average: 1.8 injuries per person) and II (1.5 injuries per person). This result is consistent with the findings of Massidda et al., on the incidence of injury in the ACE genotype of the Italian soccer players and inconsistent with their findings in the group of Japanese soccer players (5). Massidda et al., reported that the incidence of injury in Italian football players carrying the ID genotype (43.5%) is higher than whose they were carriers of the DD (2.42%) and II (3.14) genotypes, but they also narrated that the incidence of sport injury in Japanese football players that carrying the genotype II (54.1%) was more than whose they were carriers of ID (29.7%) ID and DD (16.2) genotypes (5). Sierra et al., also investigated the association between ACE-related polymorphisms and inflammation and muscle injury in Brazilian male runners after a marathon match (23). It’s reported that the incidence of sport injury in carrier’s II genotype is more than carriers of DD genotype. It is shown that the polymorphism of ACE I/D were associated with a variety of exercise-related phenotypes, including muscle strength (24), muscle metabolism and muscle mass (25), heart response to exercise (26), differences in skeletal muscle fiber distribution, composition and capillary (27), and fatigue resistance in response to exercise (28). On the other hand, success in weightlifting depends on the body's (muscular) throwing abilities (explosive power as well as fast movement). Weightlifting is a discipline that relies on muscle strength and power, the main structure of which is characterized by high intensity and frequent performance of endurance movements. Mousavi et al., reported the most common genotype of elite weightlifters as ID (as a strength / endurance polymorphism) (29). This genotype has the highest rate of injury in the present study, which shows that elite weightlifters with ID genotype, in addition to using their genetic advantage to succeed in weightlifting, are also to be more at risk of sport injury, likely.

In the grouping of muscle injuries according to the severity, the results showed that there is a significant difference in the severity of muscle injuries of Iranian selected elite male weightlifters in ACE I/D genotypes. In Massida et al., survey, the ratio of severe damage in DD genotype compared to ID and II was lower in both Japanese and Italian groups (5). Some

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reports suggest that in the ACE ID polymorphism, D allele is associated with a protective effect against the occurrence and exacerbation of muscle injury (5). Different levels of circulating CK have been observed between different post-workout ACE genotypes, suggesting that the D allele may have a protective effect against post-workout muscle damage. The result of the study by Yamin et al., also partially supports this finding (11). Since the allele D polymorphism of ACES I/D in comparison of I allele is associated with higher serum and tissue ACE activity, higher levels of ACE activity may be involved in protection against muscle injury. In this regard, ACE inhibition has been shown to increase muscle damage in the model of rabbit muscle overuse due to electrical stimulation (30). ACE has a key role in rennin angiotensin system (RAS) and tissue kallikrein-kinin system (TKKS). In RAS, ACE catalyzes the conversion of angiotensin I to angiotensin II and in TKKS, ACE catalyzes the breakdown of bradykinin into inactive biological components. Thus, higher ACE activity is associate with the D allele of the ACE I / D polymorphism, leads to its greater production of angiotensin II and reduces the half-life of bradykinin. It is known that angiotensin II and bradykinin, both of which are involved in inflammatory processes following muscle injury (4).

Results showed that the relationship between ACE I/D genotype incidence and severity of sport injury in selected Iranian male elite weightlifters not found. Contrary to these results, Massida et al., found the relation between the ACE D allele and muscle injury between the two groups of athletes (Italian and Japanese), while the relationship between the Japanese football players was more highlighted than the Italian group in their research (5). Also, Sierra et al., reported the relationship between polymorphisms associated with ACE and muscle injury in Brazilian male runners after a marathon match (23). The purpose of their study was to investigate the role of polymorphism ACE I/D to indirect muscle injury in elite football players of two different ethnicities. For the first time they found the relationship between polymorphisms of ACE I/D and the incidence of muscle injury for carriers of D allele genotype (ID + DD); while in injured group were less than not injured people. The results of their study showed that blood markers of muscle injury were lower in runners with DD genotype compared with genotype II, which suggests that ACE DD genotype reduces inflammation sensitivity and muscle injury after exercise. The low inflammatory response and muscle injury in carriers of D allele in their research showed that may be the relationship between polymorphisms of ACE ID and muscle injury in elite soccer players.

Numerous factors may play a role in creating this difference between ethnicities. Different genetic backgrounds may have different results. Different living conditions between Asians and Caucasians may contribute to different genetic effects. Interaction with other polymorphisms of susceptible genes in different ethnicities may also be an influential factor. It is important to emphasize that several genetic variants (multi-gene nature) influence sport-related phenotypes (31). Most genetic studies of athletic performance have evaluated the effect of only one type of gene on performance (32). Muscle injury is a complex multifactorial trait that is influenced by genetic factors, including some of SNPs, environmental factors, and the interactions between them. In the present study, we focused on a specific polymorphism that it could have some effect on the risk factor for muscle injury in weightlifters.

It is reasonably that the effect of common genetic species on performance is slight, but the combination of several polymorphisms would be more effective, likely. Because the requirement of athletic performance is multi-dimensional, the effect of one type of gene seems to be small (33). According to Fluck et al., an important

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weakness of the single gene approach in sports science is that the candidate gene may only havelittle contribution on sport performance (34) or may reduce the performance (35). The regulation of any physiological system involved in athletic performance and training responses depends on a complex network of interconnected genes. Therefore, it is assumed that the distribution of Athletic Performance Responsible Genes depends on the combined function of different genes, hence some different polymorphisms consider for athletic performance and training adaptations.

On the other hand, the limitation of the present study about the number of subjects for evaluating sport injuries can be considered as another reason for no relationship between the ACE I/D genotype and the number of sport injuries. In addition, one of the possible reasons for the lack of correlation between injury severities in the present study is the different levels of the subjects' championships, probably. They were in different levels; from the national championship level to the Olympic championship. In addition, the duration of training exposure of these champions was different. (from 3- 6 training sessions per week and also from 1.5 - 4 hours for their exercise training session).

It can also be said that muscle injuries are a heterogeneous group of multifactorial disorders that appear with differences in type, location, severity and size that make them difficult to define and classify (2). Likely, by increasing the number of subjects or a meta-analysis to analyze the data to homogenize the number of injuries by classifying championship levels or categorizing the number of injuries per specific training unit per genotype (e.g., duration in exposure to exercise or competition to subjects) communication is more evident in genotypic groups.

Conclusion

The research showed some extent that the Iranian society, especially Isfahan province, which belongs to the Caucasian race, has potential genetics in performing strength and endurance sports. The results of the present study showed the superiority of the ID genotype of selected elite Iranian male weightlifters and a no significantly difference was observed between all three genotypes. In the study of weightlifters, this study showed that among the selected elite weightlifters, higher rate of muscle injury was in ACE ID genotype carriers. As a result, our findings suggest that the ACE ID genotype is likely to be one of the genetic variants that could be different with a higher risk factor for muscle injury in terms of incidence and severity among selected elite weightlifters; however, it seems that more studies are needed to investigate the relationship between the incidence and severity of injuries with more subjects.

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Authors' Contributions

FZ and MB carried out tests and collected the data. FZ, MB and VZ designed the study and analyzed the data. FZ and MB wrote and revised the manuscript. All authors read and approved the final version of the manuscript.

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