# Genetic Profile in Genes Associated with Sports Injuries in Elite Endurance Athletes

#### Authors

David Varillas-Delgado<sup>1</sup>, Jorge Gutierrez-Hellín<sup>1</sup>, Antonio Maestro<sup>2</sup>

#### Affiliations

- 1 Faculty of Health Sciences, Universidad Francisco de Vitoria, Pozuelo de Alarcon, Spain
- 2 Faculty of Medicine, University of Oviedo, Oviedo, Spain

#### Key words

athletic performance, muscle performance, genes, injuries, single-nucleotide polymorphisms

accepted 03.08.2022 published online 29.09.2022

#### Bibliography

Int J Sports Med 2023; 44: 64–71 **DOI** 10.1055/a-1917-9212 **ISSN** 0172-4622 © 2022. Thieme. All rights reserved. Georg Thieme Verlag, Rüdigerstraße 14, 70469 Stuttgart, Germany

#### Correspondence

Dr. David Varillas-Delgado Universidad Francisco de Vitoria Faculty of Health Sciences Crta. Pozuelo-Majadahonda km1,800 28223 Pozuelo de Alarcon Spain Tel.: 917091400 david.varillas@ufv.es

#### ABSTRACT

Injuries are a complex trait that can stem from the interaction of several genes. The aim of this research was to examine the relationship between muscle performance-related genes and overuse injury risk in elite endurance athletes, and to examine the feasibility of determining a total genotype score that significantly correlates with injury. A cohort of 100 elite endurance athletes (50 male and 50 female) was selected. AMPD1 (rs17602729), ACE (rs4646994), ACTN3 (rs1815739), CKM (rs8111989) and MLCK ([rs2849757] and [rs2700352]) polymorphisms were genotyped by using real-time polymerase chain reaction (real time-PCR). Injury characteristics during the athletic season were classified following the Consensus Statement for injuries evaluation. The mean total genotype score (TGS) in non-injured athletes (68.263 ± 13.197 arbitrary units [a.u.]) was different from that of injured athletes (50.037 ± 17.293 a.u., p<0.001). The distribution of allelic frequencies in the AMPD1 polymorphism was also different between non-injured and injured athletes (p < 0.001). There was a TGS cut-off point (59.085 a.u.) to discriminate non-injured from injured athletes with an odds ratio of 7.400 (95% CI 2.548-21.495, p < 0.001). TGS analysis appears to correlate with elite endurance athletes at higher risk for injury. Further study may help to develop this as one potential tool to help predict injury risk in this population.

## Introduction

The increased ability to perform endurance exercise is strongly supported by enhanced mitochondrial function as suggested by increased mitochondrial gene expression, mitochondrial desoxyribonucleic acid (DNA), and mitochondrial enzyme activity [1]. Genetic and epigenetic factors may modulate adaptive responses to exercise through different mechanisms. Studies on genetic variants influencing aerobic endurance performance in elite athletes have shown that numerous types of genes may imply an improvement in endurance performance due to greater metabolic efficiency and decreased oxidative stress [2–4]. Although genotype is frequently associated with performance in sport, these same genes may also confer added risk for injury [5], and the implication of particular genes in a human phenotype like athletic performance or injury susceptibility requires an unbiased population data set [6].

Several genes have been related to muscle performance [7]. Adenosine monophosphate deaminase isoform 1 (*AMPD1*) c.34C>T (rs17602729) is an important regulator of skeletal muscle energy metabolism during exercise and leads to some related metabolic muscle diseases in subjects with the TT genotype [8]. This genotype causes metabolic myopathy with exercise-induced muscle symptoms such as early fatigue, cramps, and/or myalgia, which are related to the risk of injury [9] especially in football [10]. In angiotensin-converting enzyme (*ACE*) I/D (rs4646994), the I allele is associated with the resistance phenotype and the D allele with speed and power phenotypes [11]. The II genotype is associ-

ated in the susceptibility to developing muscle injuries among football players [12]. α-actinin-3 (ACTN3) polymorphism c.1729C>T (rs1815739) increased susceptibility to eccentric muscle damage [13]. The CC genotype (RR variant) could improve power performance in strength related physical exercise, while the beneficial influence of TT genotype (XX variant) is more related to endurance exercise performance [14]. Several studies have shown the involvement of the T allele in lesions, however the effect of heterozygosity for the ACTN3 T allele is not well documented or understood in human muscle performance [15]. Muscle-specific creatine kinase (CKM) plays a vital role in the energy homeostasis of muscle cells [7, 16]. The G allele of the c. \* 800A>G (rs8111989) polymorphism reduced activity of the skeletal muscle in endurance athletes [17, 18]. Myosin light chain kinase (MLCK) polymorphisms c.37885C>A (rs28497577) and c.49C>T (rs2700352) might alter regulatory light chain phosphorylation, thereby decreasing the ability to resist strain during voluntary muscle contractions, showing that the C allele of the c.37885 C>A (rs28497577) polymorphism [19] and T allele of the c.49C>T (rs2700352) polymorphism [20] could predispose an athlete to higher values of muscle damage during endurance competitions.

Hence, the aim of this study was to examine for the first time the relationship between muscle performance-related genes and the risk of overuse injuries in elite endurance athletes, and to examine the feasibility of determining a total genotype score that significantly correlates with injury.

### Materials and Methods

#### Participants

We used a longitudinal cohort design with a sample of 100 elite athletes (50 male and 50 female). The inclusion criteria were marked by the following cut-off points. In the male category: 3,000 meters personal best in 8:30 minutes; 10,000 meters in 29:30; the half-marathon in 1 hour 04 minutes; and marathon in 2 hours 13 minutes. In the female category the cut-off points were: 3,000 meters personal best in 9:30 minutes; 10,000 meters in 33:00 minutes; the half-marathon in 1 hour 13 minutes; and marathon in 2 hours 40 minutes. All the participants had to be top-level athletes listed in the Official State Gazette, which implies having competed at the international level and been a finalist in official competitions.

Written informed consent was obtained from all participants. The protocol of the study was approved by the Ethics Committee of the Francisco de Vitoria University (33/2019) and followed the Declaration of Helsinki for Human Research of 1964 (last modified in 2013).

Age, anthropometric characteristics, career experience, training, and competitions of the athletes are shown in ▶ **Table 1**.

#### Sample collection and genotyping

Genomic DNA was obtained from swab-extracted buccal mucosa samples, which were kept refrigerated until shipment to VIVOLabs, S.L., Madrid, Spain, where the extraction and genotyping were carried out.

The extraction of genomic DNA from the oral mucosa samples was carried out by automatic extraction in QIACube equipment (QIAGEN, VenIo, Netherlands). They had a DNA concentration of 25-40 ng/ml and were kept in solution in a volume of  $100 \mu \text{g/ml}$  at  $-20 \,^{\circ}\text{C}$  until genotyped.

All polymorphisms were genotyped using single nucleotide primer extension (SNPE) with the SNaPshot Multiplex Kit (Thermo Fisher Scientific, Waltham, MA, USA). The reaction result was performed by capillary electrophoresis fragment analysis in ABI3500 equipment (Applied Biosystems, Waltham, MA, USA) with bioinformatic analysis carried out by GeneMapper 5.0 software (Applied Biosystems).

#### Injury collection

The injuries were recorded prospectively throughout the 2019 season by clinical athletic staff according to the methodology included in the Consensus Statement for the evaluation of injuries in athletes [21]. Only those that prevented the athlete from participating in endurance race training or a competition the day after the incident (i. e., lost time injuries) were recorded. All traumatic injuries, defined as a condition caused by a single identifiable external transfer of energy, such as those caused by a fall or by contact with an obstacle or another athlete, were ruled out.

The questionnaire included all characteristics previously described by Timpka et al. [21] following baseline information, injury

▶ Table 1 Demographic characteristics of the elite endurance athletes. Data are presented as means (standard deviation).

	Elite endurance athletes (n = 100)	Male (n=50)	Female (n = 50)
Age (years)	25.67 (4.08)	24.78 (3.78)	26.56 (4.20)
Weight (kg)	58.28 (5.82)	62.33 (4.40)	54.23 (3.96)
Height (cm)	167.38 (6.78)	172.66 (4.62)	162.10 (3.82)
BMI (kg/m2)	17.37 (1.16)	18.04 (0.93)	16.71 (0.98)
Experience (years)	11.56 (3.39)	10.98 (3.80)	12.14 (2.83)
Training volume (km/week)	105.36 (16.25)	110.90 (15.96)	99.83 (9.97)
Training sessions (week)	10.03 (1.35)	10.24 (1.50)	9.82 (1.15)
Training volume (h/year)	1498.39 (417.11)	1570.34 (531.70)	1426.44 (260.45)
Year competitions	15.55 (6.74)	15.90 (8.24)	15.20 (4.86)

conditions, injury severity, body location and injury type, season, and possible cause of injury.

A clear definition of the injury was specified, and it was requested whether the injury was sustained during a training routine or competition. The injury rate per 1,000 hours of endurance running was calculated by numbers of injuries divided by the number of training hours in the year [21]. The onset of each injury was classified as sudden-onset when the disability developed during minutes or seconds, or as a gradual-onset injury when the disability developed during hours, days, or more.

The assessment of severity started on the day following the disability until full recovery was determined by expert medical opinion, and it was classified as slight (0–3 days), mild (4–7 days), moderate (8–28 days), or severe (>28 days) [21].

Body location of the injury was classified in the lower limb as hip/ groin, thigh, knee, lower leg, ankle, foot/toe, and Achilles tendon. The type of injury was classified as strain/muscle injuries, tendinosis/tendinopathies, bone injuries, sprain/ligament injuries, and nerve injuries. Concussions, traumatic fractures, dislocation/subluxations, contusions, skin lesions, and dental injuries were not included as injury type as they were related to traumatic injuries [21].

The time of the injury was classified by season as pre-season when the injury occurred in the period immediately before the start of a new season, or in-season when it occurred during the training period for a specific competition. Also, the time of injury was classified by summer, autumn, winter, and spring [21].

To determine the possible cause for the development of the injury, the questionnaire included a section to indicate if the injury coincided with an abrupt increase in training volume or intensity (i. e., excessive load), a change of training surface, a change of running shoes, aspects related to biomechanics (e. g., new orthotic insoles) or if the cause was unknown [21].

# Polygenic potential for muscle performance and injury risk

The combined influence of the six polymorphisms studied was calculated using a total genotype score (TGS) following the Williams and Folland procedure [22]. A genotype score (GS) of 2 or 1 was assigned to the "protective" genotype for musculoskeletal injuries, while a GS of 0 was assigned to the "worst" genotype [23]. The GS scores for the six polymorphisms are shown in ▶ **Table 2**. Then the GSs of all genotypes were added up, and finally the score was transformed to a 0–100 arbitrary units (a.u) scale to facilitate interpretation, namely the total genotype score (TGS), as follows:

TGS = (GSAMPD1 + GSACE + GSACTN3 + GSCKM + GSMLCKC37885A + GSMLCKC49T) × 100/12

As previously indicated [22], a TGS of 100 a.u. represents a "perfect" profile and a TGS of 0 a.u. should be the "worst" possible profile for muscle performance when all GSs have a score of 0 a.u. Finally, the TGS distribution between non-injured and injured was assessed. **Table 2** Genotypic distribution of polymorphisms studied among athletes in the non-injuries and injuries groups. Data are shown in n (%).

Gene	Geno-	Non-injury	Injury	P value	
symbol	type	(n=45)	(n=55)		
AMPD1 (r	s17602729)				
CC	2=CC	39 (86.7)	24 (43.6)		
СТ	1=CT	6 (13.3)	26 (47.3)	< 0.001	
TT	0=TT	0 (0.0)	5 (9.1)	-	
ACE (rs46	46994)			1	
DD	2 = DD	18 (40.0)	23 (41.8)		
ID	1=ID	16 (35.6)	19 (34.5)	0.983	
11	0 = 11	11 (24.4)	13 (23.6)		
ACTN3 (rs	1815739)	1	l		
СС	2=CC	14 (31.1)	25 (45.5)		
СТ	1=CT	23 (51.1)	26 (47.3)	0.161	
TT	0=TT	8 (17.8)	4 (7.3)		
CKM (rs81	111989)				
AA	2=AA	19 (42.2)	26 (47.3)		
GA	1=GA	14 (31.1)	18 (32.7)	0.218	
GG	0=GG	12 (26.7)	11 (20.0)		
MLCK (rs2	28497577)	•			
AA	2=AA	0 (0.0)	0 (0.0)		
CA	1=CA	22 (48.9)	18 (32.7)	0.101	
CC	0 = CC	23 (51.1)	37 (67.3)		
MLCK (rs2	2700352)				
TT	2=TT	2 (4.4)	3 (5.5)		
TC	1 = TC	21 (46.7)	25 (45.5)	0.971	
СС	0 = CC	22 (48.9)	27 (49.0)		

#### Statistical analysis

Statistical Package for the Social Sciences (SPSS), v.21.0 for Windows (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

Compliance of Hardy-Weinberg Equilibrium (HWE) in each SNP was tested using  $\chi 2$  tests. The probability of having an optimal endurance genotype for these polymorphisms between injured and non-injured was calculated using the  $\chi 2$  test with a fixed  $\alpha$  error of 0.05. The genotypic frequencies of all polymorphisms were compared between injured and non-injured, using a  $\chi 2$  test with a fixed  $\alpha$  error of 0.05.

The ability of the TGS to correctly distinguish potential injuries from non-injuries (0 = non-injury, 1 = injury) in the athletes was assessed using receiver operating characteristic (ROC) curves [24]. With that purpose, the area under the ROC curve (AUC) was calculated with confidence intervals of 95 % (95 % CI). Finally, a binary logistic regression model was used to study the relationship between the TGS and injuries in all athletes and both sexes.

Data on the incidence of injuries were transferred from the questionnaire to an ad hoc database designed for this investigation. For continuous variables, comparisons between groups (injury vs. noninjury) were made using a t-test analysis. The odds ratio (OR) and 95% CI of injury were calculated for the cut-off point by TGS in all genes presented.

# Results

All the polymorphisms analyzed met the HWE. The mean value of the TGS was lower in the athletes with injuries  $(50.037 \pm 17.293 \text{ a.u.})$  than in the non-injuries group  $(68.263 \pm 13.197 \text{ a.u.}, p < 0.001)$ . The distributions of frequency of injured and non-injured athletes according to their TGSs are represented in **Fig. 1**.

Fifty-five athletes (55.0%) reported at least one injury during a season (a total of 99 injuries, range 1–4 injuries/year). The overall incidence of injuries was 1.23 per 1,000 h/training. A total of 28 male (56%) and 27 female athletes (54%) were injured during the season, and 22 male (44%) and 23 female athletes (46%) were non-injured.

The genotypic distribution in the AMPD1 polymorphism in the non-injuries group showed a higher frequency in the optimal genotype (CC 86.7%) compared with the injuries group (CC 43.6%, p < 0.001). In any case, there were no statistically significant differences in the genotypic distribution between the non-injuries and injuries groups in the other polymorphisms (**Table 2**). Among the polymorphisms studied, differences were found only in the genotypic distribution between male and female athletes in the *ACTN3* polymorphism (p = 0.046) (**Table 3**), whereas for the other polymorphisms studied, no differences were found in the distribution of genotypes between the two genders.

ROC analysis showed significant discriminatory accuracy of the TGSs in the identification of injuries in athletes (AUC = 0.740, 95% CI 0.642–0.828, p < 0.001) with a sensitivity of 0.733 and a specificity of 0.309 (▶ **Fig. 2**) with a corresponding TGS value at this point of 59.085 a.u. Using binary logistic regression, athletes with a TGS lower than 59.085 a.u. presented an OR 7.400 (95% CI 2.548–21.495, p < 0.001) for sports injuries.

This discrimination was statistically significant both in male and female athletes, showing in females a cut-off point of TGS with a value of OR 8.197, 95% CI 1.954–19.421, p = 0.006, and in males an OR 7.925, 95% CI 2.042–20.214, p = 0.004.

No differences were shown in the conditions, severity, onset, season, and time of year in which the injuries occurred. However, differences were found in body location between injuries and non-injuries groups (p = 0.013), as in type of injury (p = 0.001) and in the possible causes of injury (p = 0.038) ( $\triangleright$  **Table 4**).

The percentage of the victories of the athletes in the national/ international competitions in which they participated in 2019 was analyzed, showing that athletes with a favorable TGS for muscular performance presented a higher percentage (23.24%) than the athletes with < 59.085 a.u. (14.23%) (p<0.001). This percentage of victories in male athletes was 22.86% in the > 59.085 a.u. group and 12.62% in the < 59.085 a.u. group (p<0.001), and in the female's group a percentage of victories in competitions of 23.60% in the athletes with > 59.085 a.u. compared to 15.71% in the <59.085 a.u. group (p=0.001).

# Discussion

The purpose of this research was to study the association between SNPs involved in muscle performance and injuries in elite endurance athletes. The main findings in this study are the correlation between TGS scores and injury rates, and the *AMPD1* polymorphism suggests some type of injury-related benefit to the C allele.



▶ **Fig. 1** Distribution of individuals according to their TGS in noninjured and injured athletes. \*The distribution is different from the distribution of injuries at p<0.001.

► **Table 3** Genotypic distribution of polymorphisms studied among male and female athletes. Data are shown in n (%).

Gene	Male athletes	Female athletes	P value	
symbol	(n=50)	(n = 50)		
AMPD1 (rs1	7602729)			
CC	32 (64.0)	31 (62.0)		
СТ	16 (32.0)	12 (24.0)	0.312	
TT	2 (4.0)	3 (6.0)		
ACE (rs4646	5994)			
DD	22 (44.0)	19 (38.0)	0.813	
ID	17 (34.0)	18 (36.0)		
II	11 (22.0)	13 (26.0)		
ACTN3 (rs1	815739)			
СС	22 (44.0)	17 (34.0)		
СТ	26 (52.0)	23 (46.0)	0.046	
TT	2 (4.0)	10 (20.0)		
CKM (rs811	1989)	L.		
AA	12 (24.0)	11 (22.0)		
GA	17 (34.0)	15 (30.0)	0.832	
GG	21 (42.0)	24 (48.0)		
MLCK (rs284	497577)			
AA	0 (0.0)	0 (0.0)		
CA	19 (38.0)	21 (42.0)	0.683	
CC	31 (62.0)	29 (58.0)		
MLCK (rs27	00352)			
TT	4 (8.0)	1 (2.0)		
TC	22 (44.0)	24 (48.0)	0.385	
СС	24 (48.0)	25 (50.0)	1	

Although excellent muscle performance in endurance sports is facilitated by an optimal polygenic profile in several key polymorphisms by muscle fibers, elasticity, and metabolism [23, 25–27], this analysis indicates that the influence of the five genes is strong enough to discriminate this profile and the risk of sports injuries. Previous research has improved the understanding of several genes and their expression influencing the response to physical training and predisposition to sports-related injuries [28]. All biological pro-



▶ Fig. 2 Receiver operating characteristic curve (ROC) summarizing the ability of the weighted total genotype score to distinguish potential injuries from non-injuries. AUC: area under the curve.

cesses associated with high sports performance, including energy metabolism, are influenced by genetics [29]. Identification of genetic markers associated with the regulation of energy metabolism in skeletal muscles can help sports physicians and coaches develop personalized strategies for talent selection for endurance, strength, and speed sports and to adapting the training protocols in function of the athletes' genetic profile. However, the multifactorial aspect of sport performances, including impact of genetics, epigenetics, environment (training, etc.), is important for personalized strategies for talent identification and for best expression, in safety, the potential of each athlete [30].

Subjects with the TT genotype in the c.34 C>T AMPD1 gene have diminished exercise capacity and cardiorespiratory responses to exercise [27, 31]. Moreover, carriers of the T allele have a limited training response of ventilatory phenotypes during maximal exercise [31–33] and a reduced submaximal aerobic capacity [33, 34]. This study shows that the AMPD1 gene is the most relevant for sports injuries in endurance athletes, suggesting that optimal muscle metabolism has an influence on injuries in professional athletes. These results are valuable for future studies to demonstrate a cause of muscle performance that could lead to knowledge on sports injuries.

Endurance running is one of the most popular physical activities in the world, but there is a prevalence of injuries ranging from 18.2–92.4%, depending on the type and definition of the injury [35]. In this research, 55 of the 100 endurance runners (55%) reported at least one injury during the athletic season with an OR that was 7.400 in those athletes in the TGS>59.085 a.u. group in comparison with those in the <59.085 a.u. group.

Although nowadays controversy exists, there is some evidence that genetic variation in the ACE gene might be associated with many heritable traits, including physical, physiological, and skill parameters and physical performance [36, 37], showing an increased frequency of the ACE I allele in elite endurance athletes [38–40], whereas the ACE D allele has been associated with elite sprint performance [38, 41]. The I allele may influence endurance performance through improvements in substrate delivery and the efficiency of skeletal muscle, with subsequent conservation of energy stores [7, 36, 39].

The ACTN3 gene is a highly conserved component of the contractile machinery in fast skeletal muscle fibers in mammals [7, 11]. The ACTN3 RR variant is nearly always present among elite power athletes, whereas the XX variant, associated with complete ACTN3 deficiency, is more prevalent among elite endurance athletes, such as marathon runners and cyclists [42–44]. Detailed analysis of the ACTN3 XX variant shows a reduced fast-fiber diameter, increased activity of multiple enzymes in the aerobic metabolic pathway, altered contractile properties, and enhanced recovery from fatigue [45]. Independent studies, however, failed to demonstrate a significant association between the XX variant and extreme endurance performance [46–48]. Finally, this study does not demonstrate the influence of the ACE and ACTN3 genes on the risk of injury in athletes.

Studies of *CKM* gene sequence variation are related to increased cardiorespiratory endurance as indexed by maximal oxygen uptake [49], peak performance, and less decline in force generation [50]. The G allele of c. \* 800 A > G polymorphism (rs8111989) contributes to individual running economy responses to endurance training [16–18], in this research showing non-allelic differences between injured and non-injured (▶ **Table 2**) by a higher frequency of the A allele in injured athletes (47.3 %) regarding non-injured athletes (42.2 %).

*MLCK* plays a critical role in the regulation of smooth muscle contraction [19]. Two polymorphisms in this gene are associated with post-exercise strength loss [19, 51, 52], showing that heterozygotes CA for c.37885 C>A might present higher exercise-induced muscle damage after an endurance competition than CC counterparts [20], data that do not coincide with those of our study because the injured CA athletes did not show a greater frequency of this genotype (32.7%) than non-injured athletes (48.9%) (**▶ Table 2**).

The likelihood of sustaining an injury in elite endurance athletes was higher in TGS < 59.085 a.u. group than in their > 59.085 a.u. counterparts. In this sense, the results found for body location show that athletes with TGS < 59.085 had a higher frequency of thigh injuries, being a predictor of injury using this gene pool, showing this TGS value association with higher risk of strain injury type and tendinosis/tendinopathies. This research completes and adds information to previous studies in which only the c.1729C>T ACTN3 polymorphism was studied and no association was found in the location and type of injury in a cohort of amateur marathon runners [53] and elite endurance runners [54]. The study of a single polymorphism with an injury phenotype should be avoided in the field of genetics in the future, and studies such as the one presented in this investigation using TGS should be implemented [30]. The design of specific programs to prevent muscle damage and injury in athletes using this genetic profile merits further investigation.

This model presents some limitations: 1) the sample size of the group of elite endurance athletes was limited because in Spain there are not enough elite endurance athletes with these charac-

		All		TGS a.u.	
			< 59.085	> 59.085	
Injury conditions	Training	39 (70.7)	20 (69.0)	19 (73.1)	0.727
	Competition	16 (29.3)	9 (31.0)	7 (26.9)	0.737
Mode of onset	Sudden	34 (61.8)	17 (58.6)	17 (65.4)	0.606
	Gradual	21 (38.3)	12 (41.4)	9 (34.6)	0.000
Injury severity	Minar	23 (41.8)	9 (31.0)	14 (53.8)	
	Moderate	23 (41.8)	14 (48.3)	9 (34.6)	0.221
	Severe	9 (16.4)	6 (20.7)	3 (11.6)	
Body location	Hip/groin	4 (7.3)	2 (6.9)	2 (7.7)	
	Thigh	12 (21.8)	10 (34.5) *	2 (7.7) *	
	Кпее	12 (21.8)	1 (3.4) *	11 (42.3) *	
	Lower leg	14 (25.5)	9 (31.0)	5 (19.2)	0.013
	Ankle	8 (14.5)	5 (17.2)	3 (11.5)	
	Foot/toe	4 (7.3)	2 (6.9)	2 (7.7)	
	Achilles tendon	1 (1.8)	0 (0.0)	1 (3.8)	
Injury type	Strain	27 (49.1)	22 (75.9) *	5 (19.2) *	
	Tendinosis/tendinopathies	13 (23.6)	11 (42.3) *	2 (6.9) *	
	Bone injury	6 (10.9)	1 (3.4)	5 (19.2)	0.001
	Sprains/ligament injury	7 (12.7)	3 (10.3)	4 (15.4)	
	Nerve injury	2 (3.6)	1 (3.4)	1 (3.8)	
Season .	Pre-season	22 (40.3)	11 (37.9)	11 (42.3)	0.741
	In-season	33 (59.7)	18 (62.1)	15 (57.7)	0.741
Time of year	Summer	13 (23.6)	8 (27.6)	5 (19.2)	
	Autumn	19 (34.5)	9 (31.0)	10 (38.5)	0.875
	Winter	14 (25.5)	7 (24.1)	7 (26.9)	0.075
	Spring	9 (16.4)	5 (17.2)	4 (15.4)	
Possible cause	Cumulative overload	19 (34.5)	14 (48.3) *	5 (19.2) *	
	Surface	16 (29.3)	12 (46.2) *	4 (13.8) *	
	Shoe	10 (18.2)	5 (17.2)	5 (19.2)	0.038
	Biomechanics	8 (14.5)	4 (13.8)	4 (15.4)	
	Unknown	2 (3.6)	2 (6.9)	0 (0.0)	

▶ Table 4 Injury factors for elite endurance athletes. Data are shown in n (%).

<sup>\*</sup> Different from expected at p < 0.05; TGS a.u.: Total Genotype Score (arbitrary units).

teristics, a representative sample is presented in the current study; 2) numerous genetic variants that have not been included in the model are likely to appear in the foreseeable future that can also explain individual variations in the potential for attaining elite endurance athletic performance and injuries; and 3) more longitudinal studies of large cohorts of elite athletes are necessary before the evidence linking the genetics and epidemiology of injuries can be established, and it is still premature to use genetic testing among elite long-distance runners to effectively predict the risk of injury.

This study is the first to demonstrate that injury incidence in elite endurance athletes was affected by the TGS in genes involved in muscle performance. TGS appears to correlate with elite endurance athletes at higher risk for injury. Further study may help to develop this as one potential tool to help predict injury risk in this population. In fact, the only gene with a positive association with muscle performance and protection against injuries was the *AMPD1*  polymorphism. Lastly, the current investigation raises the need to include epigenetics and environmental aspects in the analysis of the factors associated with elite athlete performance according to muscle metabolism, improving the understanding of the links between genetics and exercise performance [55].

# Acknowledgements

The authors wish to thank all the athletes for participating in this research study.

# Conflict of Interest

The authors declare that they have no conflict of interest.

# References

- Zanini G, De Gaetano A, Selleri V et al. Mitochondrial DNA and exercise: implications for health and injuries in sports. Cells 2021; 10: 2575. doi:10.3390/cells10102575
- [2] Al-Khelaifi F, Diboun I, Donati F et al. A pilot study comparing the metabolic profiles of elite-level athletes from different sporting disciplines. Sports Med Open 2018; 4: 2. doi:10.1186/s40798-017-0114-z
- [3] Lee EC, Fragala MS, Kavouras SA et al. Biomarkers in sports and exercise: tracking health, performance, and recovery in athletes. J Strength Cond Res 2017; 31: 2920–2937. doi:10.1519/ isc.00000000002122
- Petibois C, Cazorla G, Poortmans JR et al. Biochemical aspects of overtraining in endurance sports: a review. Sports Med 2002; 32: 867–878. doi:10.2165/00007256-200232130-00005
- [5] Baumert P, Lake MJ, Stewart CE et al. Genetic variation and exerciseinduced muscle damage: implications for athletic performance, injury and ageing. Eur J Appl Physiol 2016; 116: 1595–1625. doi:10.1007/ s00421-016-3411-1
- [6] Gibson WT. Core concepts in human genetics: understanding the complex phenotype of sport performance and susceptibility to sport injury. Med Sport Sci 2016; 61: 1–14. doi:10.1159/000445237
- [7] Lippi G, Longo UG, Maffulli N. Genetics and sports. Br Med Bull 2010; 93: 27–47. doi:10.1093/bmb/ldp007
- [8] Feng AF, Liu ZH, Zhou SL et al. Effects of AMPD1 gene C34T polymorphism on cardiac index, blood pressure and prognosis in patients with cardiovascular diseases: a meta-analysis. BMC Cardiovasc Disord 2017; 17: 174. doi:10.1186/s12872-017-0608-0
- [9] Gross M. Clinical heterogeneity and molecular mechanisms in inborn muscle AMP deaminase deficiency. J Inherit Metab Dis 1997; 20: 186–192. doi:10.1023/a:1005352605421
- [10] McCabe K, Collins C. Can genetics predict sports injury? The association of the genes GDF5, AMPD1, COL5A1 and IGF2 on soccer player injury occurrence. Sports (Basel) 2018; 6: 21. doi:10.3390/ sports6010021
- [11] Eynon N, Hanson ED, Lucia A et al. Genes for elite power and sprint performance: ACTN3 leads the way. Sports Med 2013; 43: 803–817. doi:10.1007/s40279-013-0059-4
- [12] Massidda M, Miyamoto-Mikami E, Kumagai H et al. Association between the ACE I/D polymorphism and muscle injuries in Italian and Japanese elite football players. J Sports Sci 2020; 38: 2423–2429. doi:1 0.1080/02640414.2020.1787683
- [13] Massidda M, Voisin S, Culigioni C et al. ACTN3 R577X polymorphism is associated with the incidence and severity of injuries in professional football players. Clin J Sport Med 2019; 29: 57–61. doi:10.1097/ jsm.00000000000487
- [14] Del Coso J, Hiam D, Houweling P et al. More than a 'speed gene': ACTN3 R577X genotype, trainability, muscle damage, and the risk for injuries. Eur J Appl Physiol 2019; 119: 49–60. doi:10.1007/s00421-018-4010-0
- [15] Garton FC, North KN. The effect of heterozygosity for the ACTN3 null allele on human muscle performance. Med Sci Sports Exerc 2016; 48: 509–520. doi:10.1249/mss.00000000000784
- [16] Eider J, Ahmetov II, Fedotovskaya ON et al. CKM gene polymorphism in Russian and Polish rowers. Genetika 2015; 51: 389–392. doi:10.7868/ s0016675815030029
- [17] Zhou DQ, Hu Y, Liu G et al. Muscle-specific creatine kinase gene polymorphism and running economy responses to an 18-week 5000-m training programme. Br J Sports Med 2006; 40: 988–991. doi:10.1136/bjsm.2006.029744
- [18] Fedotovskaia ON, Popov DV, Vinogradova OL et al. [Association of the muscle-specific creatine kinase (CKMM) gene polymorphism with physical performance of athletes]. Fiziol Cheloveka 2012; 38: 105–109

- [19] Del Coso J, Valero M, Lara B et al. Myosin light chain kinase (MLCK) gene influences exercise induced muscle damage during a competitive marathon. PLoS One 2016; 11: e0160053. doi:10.1371/journal. pone.0160053
- [20] Clarkson PM, Hoffman EP, Zambraski E et al. ACTN3 and MLCK genotype associations with exertional muscle damage. J Appl Physiol (1985) 2005; 99: 564–569. doi:10.1152/japplphysiol.00130.2005
- [21] Timpka T, Alonso JM, Jacobsson J et al. Injury and illness definitions and data collection procedures for use in epidemiological studies in athletics (track and field): consensus statement. Br J Sports Med 2014; 48: 483–490. doi:10.1136/bjsports-2013-093241
- [22] Williams AG, Folland JP. Similarity of polygenic profiles limits the potential for elite human physical performance. J Physiol 2008; 586: 113–121. doi:10.1113/jphysiol.2007.141887
- [23] Ruiz JR, Gomez-Gallego F, Santiago C et al. Is there an optimum endurance polygenic profile? J Physiol 2009; 587: 1527–1534. doi:10.1113/jphysiol.2008.166645
- [24] Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993; 39: 561–577
- [25] Muniesa CA, Gonzalez-Freire M, Santiago C et al. World-class performance in lightweight rowing: is it genetically influenced? A comparison with cyclists, runners and non-athletes. Br J Sports Med 2010; 44: 898–901. doi:10.1136/bjsm.2008.051680
- [26] Varillas Delgado D, Telleria Orriols JJ, Martin Saborido C. Livermetabolizing genes and their relationship to the performance of elite spanish male endurance athletes; a prospective transversal study. Sports Med Open 2019; 5: 50. doi:10.1186/s40798-019-0227-7
- [27] Varillas Delgado D, Tellería Orriols JJ, Monge Martín D et al. Genotype scores in energy and iron-metabolising genes are higher in elite endurance athletes than in nonathlete controls. Appl Physiol Nutr Metab 2020; 45: 1225–1231. doi:10.1139/apnm-2020-0174
- [28] Vlahovich N, Hughes DC, Griffiths LR et al. Genetic testing for exercise prescription and injury prevention: AIS-Athlome consortium-FIMS joint statement. BMC Genomics 2017; 18: 818. doi:10.1186/s12864-017-4185-5
- [29] Balberova OV, Bykov EV, Medvedev GV et al. Candidate genes of regulation of skeletal muscle energy metabolism in athletes. Genes (Basel) 2021; 12: 1682. doi:10.3390/genes12111682
- [30] Varillas-Delgado D, Del Coso J, Gutiérrez-Hellín J et al. Genetics and sports performance: the present and future in the identification of talent for sports based on DNA testing. Eur J Appl Physiol 2022; 122: 1811–1830. doi:10.1007/s00421-022-04945-z
- [31] Rico-Sanz J, Rankinen T, Joanisse DR et al. Associations between cardiorespiratory responses to exercise and the C34T AMPD1 gene polymorphism in the HERITAGE Family Study. Physiol Genomics 2003; 14: 161–166. doi:10.1152/physiolgenomics.00165.2002
- [32] Rubio JC, Martin MA, Rabadan M et al. Frequency of the C34T mutation of the AMPD1 gene in world-class endurance athletes: does this mutation impair performance? J Appl Physiol (1985) 2005; 98: 2108–2112. doi:10.1152/japplphysiol.01371.2004
- [33] Gineviciene V, Jakaitiene A, Pranculis A et al. AMPD1 rs17602729 is associated with physical performance of sprint and power in elite Lithuanian athletes. BMC Genet 2014; 15: 58. doi:10.1186/1471-2156-15-58
- [34] Lucia A, Martin MA, Esteve-Lanao J et al. C34T mutation of the AMPD1 gene in an elite white runner. BMJ Case Rep 2009; 2009: bcr07.2008.0535. doi:10.1136/bcr.07.2008.0535
- [35] Saragiotto BT, Yamato TP, Hespanhol Junior LC et al. What are the main risk factors for running-related injuries? Sports Med 2014; 44: 1153–1163. doi:10.1007/s40279-014-0194-6

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

- [36] Moran CN, Vassilopoulos C, Tsiokanos A et al. The associations of ACE polymorphisms with physical, physiological and skill parameters in adolescents. Eur J Hum Genet 2006; 14: 332–339. doi:10.1038/sj. ejhg.5201550
- [37] Scott RA, Moran C, Wilson RH et al. No association between angiotensin converting enzyme (ACE) gene variation and endurance athlete status in Kenyans. Comp Biochem Physiol A Mol Integr Physiol 2005; 141: 169–175. doi:10.1016/j.cbpb.2005.05.001
- [38] Myerson S, Hemingway H, Budget R et al. Human angiotensin I-converting enzyme gene and endurance performance. J Appl Physiol (1985) 1999; 87: 1313–1316. doi:10.1152/jappl.1999.87.4.1313
- [39] Nazarov IB, Woods DR, Montgomery HE et al. The angiotensin converting enzyme I/D polymorphism in Russian athletes. Eur J Hum Genet 2001; 9: 797–801. doi:10.1038/sj.ejhg.5200711
- [40] Amir O, Amir R, Yamin C et al. The ACE deletion allele is associated with Israeli elite endurance athletes. Exp Physiol 2007; 92: 881–886. doi:10.1113/expphysiol.2007.038711
- [41] Costa AM, Silva AJ, Garrido ND et al. Association between ACE D allele and elite short distance swimming. Eur J Appl Physiol 2009; 106: 785–790. doi:10.1007/s00421-009-1080-z
- [42] Yang N, MacArthur DG, Gulbin JP et al. ACTN3 genotype is associated with human elite athletic performance. Am J Hum Genet 2003; 73: 627–631. doi:10.1086/377590
- [43] Clos E, Pruna R, Lundblad M et al. ACTN3 single nucleotide polymorphism is associated with non-contact musculoskeletal soft-tissue injury incidence in elite professional football players. Knee Surg Sports Traumatol Arthrosc 2019; 27: 4055–4061. doi:10.1007/ s00167-019-05381-x
- [44] Shang X, Huang C, Chang Q et al. Association between the ACTN3 R577X polymorphism and female endurance athletes in China. Int J Sports Med 2010; 31: 913–916. doi:10.1055/s-0030-1265176
- [45] Broos S, Malisoux L, Theisen D et al. Role of alpha-actinin-3 in contractile properties of human single muscle fibers: a case series study in paraplegics. PLoS One 2012; 7: e49281. doi:10.1371/journal. pone.0049281

- [46] Lucia A, Gómez-Gallego F, Santiago C et al. ACTN3 genotype in professional endurance cyclists. Int J Sports Med 2006; 27: 880–884. doi:10.1055/s-2006-923862
- [47] Houweling PJ, Papadimitriou ID, Seto JT et al. Is evolutionary loss our gain? The role of ACTN3 p.Arg577Ter (R577X) genotype in athletic performance, ageing, and disease. Hum Mutat 2018; 39: 1774–1787. doi:10.1002/humu.23663
- [48] Baltazar-Martins G, Gutiérrez-Hellín J, Aguilar-Navarro M et al. Effect of ACTN3 genotype on sports performance, exercise-induced muscle damage, and injury epidemiology. Sports (Basel) 2020; 8: 99. doi:10.3390/sports8070099
- [49] Echegaray M, Rivera MA. Role of creatine kinase isoenzymes on muscular and cardiorespiratory endurance: genetic and molecular evidence. Sports Med 2001; 31: 919–934. doi:10.2165/00007256-200131130-00003
- [50] Bouchard C, Chagnon M, Thibault MC et al. Muscle genetic variants and relationship with performance and trainability. Med Sci Sports Exerc 1989; 21: 71–77. doi:10.1249/00005768-198902000-00013
- [51] Huang J, Gao N, Wang S et al. Genetic approaches to identify pathological limitations in aortic smooth muscle contraction. PLoS One 2018; 13: e0193769. doi:10.1371/journal.pone.0193769
- [52] Shen K, Ramirez B, Mapes B et al. Structure-function analysis of the non-muscle myosin light chain kinase (nmMLCK) isoform by NMR spectroscopy and molecular modeling: influence of MYLK variants. PLoS One 2015; 10: e0130515. doi:10.1371/journal.pone.0130515
- [53] Moreno V, Areces F, Ruiz-Vicente D et al. Influence of the ACTN3 R577X genotype on the injury epidemiology of marathon runners. PLoS One 2020; 15: e0227548. doi:10.1371/journal.pone.0227548
- [54] Gutiérrez-Hellín J, Baltazar-Martins G, Aguilar-Navarro M et al. Effect of ACTN3 R577X genotype on injury epidemiology in elite endurance runners. Genes (Basel) 2021; 12: 76. doi:10.3390/genes12010076
- [55] Joyner MJ. Genetic approaches for sports performance: how far away are we? Sports Med 2019; 49: 199–204. doi:10.1007/s40279-019-01164-z