Association between ACTN3 R577X genotype and risk of non-contact injury in trained athletes: A systematic review

Zouhal, H., Coso, J. D., Jayavel, A., Tourny, C., Ravé, G., Jebabli, N., Clark, C. C. T., Barthélémy, B., Hackney, A. C. & Abderrahman, A. B

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lack of protein expression. In contrast, homozygous individuals for the R allele (RR genotype) or heterozygote individuals (RX genotype) express α-actinin-3, although recently it has been found that the capacity to express α-actinin-3 is dose-dependent for RR *vs*. RX individuals.[3] The deficiency of α-actinin-3 does not entail any disease or clinical condition but has been demonstrated to produce some potentially negative phenotypes in humans, such as lower muscle strength,[4,5] reduced muscle volume,[6,7] impaired capacity to tolerate the strain produced by explosive muscular actions,[8] and/or an association with decreased bone mineral density.[9]

In athletes, several investigations have confirmed that α-actinin-3 deficiency due to the *ACTN3* XX genotype may negatively influence sprint and power performance.[10] Lower performance in sport disciplines requiring near-to-maximal production of strength or power has been associated with decreased capacity of the ability of muscle fibers to produce powerful contractions in the absence of α-actinin-3.[7] This notion is supported by several investigations that found a higher occurrence of the *ACTN3* RR variant in elite sprint/power athletes when compared with nonathletes.[1] In contrast, a higher-than-expected frequency of the *ACTN3* XX genotype has been found in some groups of elite endurance athletes,[9,11] although the overrepresentation of the XX genotype has not been replicated in other cohorts of elite athletes.[12,13] Collectively, the information obtained from studies investigating the frequency of the different *ACTN3* genotypes in cohorts of elite athletes points toward a negative influence of the XX genotype on elite sprint/power-based exercise (hence, a positive influence of the R allele on this type of exercise) with little or no effect of the XX genotype on endurance-based exercise.

In addition to performance, the absence of α-actinin-3 has been linked to a higher probability of injury[14] in several sport and exercise scenarios. For example, soccer (football) players with the *ACTN3* XX genotype suffered a higher incidence of non-contact muscle injuries when compared to players with the RR or the RX genotype.[15,16] A higher probability of muscle-type injuries was also found in XX compared to RR runners.[17] On the other hand, at least 1 investigation has reported that athletes with the RR or the RX genotype had an increased risk of muscle injury when practicing various sport activities.[18] Additionally, there is an increase in passive hamstring stiffness in R-allele individuals compared to XX counterparts,[19] although the link between muscle stiffness and increased muscle injury risk has not yet been established.[14,20] The most consistent specific findings posit a link between the XX genotype and ankle injuries[21–23] and higher levels of exercise-induced muscle damage.[24–26] These associations suggest a negative impact of α-actinin-3 deficiency on muscle and ligament capacity to endure the forces generated during exercise and point toward an increased susceptibility to contraction-induced damage.[8,9] Despite the extant literature indicating an influence of the *ACTN3* genotype on exercise-related non-contact injuries, there is no previous investigation, to our knowledge, that has systematically reviewed the influence of the *ACTN3* R577X polymorphism on non-contact injuries. Hence, the aim of this investigation was to review evidence systematically concerning the link between the *ACTN3* R577X polymorphism and the rates and severity of non-contact injuries and exercise-induced muscle damage in athletes and individuals enrolled in exercise-training programs.

2. Methods

2.1. Eligibility criteria

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[27] Only studies that examined the link between the *ACTN3* R577X polymorphism and the rates or severity of non-contact injuries in individuals who were at any level/sex/age and currently participating in a sport or exercise-training program were included. The following inclusion criteria were established for studies of this topic (Table 1). The studies (1) were available in peer-reviewed journals; (2) involved participants aged 14 years; (3) included trained athletes (frequency of training 5 times/week) and individuals enrolled in exercise training programs (i.e., nonathletes); (4) used validated methods for the characterization of injury epidemiology (e.g., injury severity, occurrence and incidence); (5) obtained epidemiological data about non-contact injuries developed during sport competitions and exercise practice; and (6) full text available in English. Studies were excluded if they (1) did not contain an experiment study design with the original data, such as books, systematic or narrative reviews, case studies, or opinion

Table 1
Inclusion criteria according to the Population, Intervention, Comparison, Outcomes and Study (PICOS) approach.

Criteria	Inclusion	Exclusion
1	Original article published in peer-reviewed journals	Books, reviews, case studies, opinion pieces, non-peer-reviewed journals
2	Involved professional, amateur, or recreational athletes or individuals practicing exercise in regular programs	Untrained individuals
3	Participants 14 years old, described as young and/or senior athletes	Participants <14 years old, described as child athletes
4	Analyzed the effects of the *ACTN3* R577X polymorphism on the rates or severity of non-contact injuries	A gene polymorphism other than *ACTN3* R577X analyzed
5	Used quantification of injuries (e.g., injury severity, occurrence, and incidence)	Rates or severity of non-contact injuries not reported
6	Full text available in English	Full text not accessible in English

pieces; (2) did not meet the minimum requirements to classify the sample as exercisers (frequency of training <3 times/week); or (3) were not written in English.

2.2. Literature search strategy

Literature searches were conducted in 3 electronic databases including PubMed, Web of Science, and SPORTDiscus, from inception until November 2020. No year restriction was used for the search strategy, in an effort to obtain all studies on the topic. The following key terms (and synonyms searched for by the MeSH database) were included and combined using the operators "AND", "OR", "NOT": ((Alpha-actinin-3 OR ACTN3) OR ("ACTN3 gene" OR "ACTN3 R577X polymorphism" OR "ACTN3 R577X" OR "ACTN3 R577R" OR "ACTN3 577XX genotype*" OR "ACTN3 577RR genotype*" OR "ACTN3 577RX genotype*") AND ("injuries" OR "injury" OR "muscle injury*" OR "non-contact injury" OR "strain" OR "soreness*" OR "sprain*" OR "contusion" OR "fracture" OR "dislocation" OR "concussion") AND (correlation study OR association OR relationship)). The references used in the identified studies were searched as well, in order to identify additional relevant research papers. The search for published studies was independently performed by 2 authors (AJ and ABA), and disagreements were resolved through discussion.

2.3. Study selection

The screening and study selection were performed by 2 authors based on the above-mentioned inclusion and exclusion criteria. If the title and abstract of the article showed any potential relevance in terms of assessing the influence of the *ACTN3* genotype on the rates and severity of non-contact injuries, the full text was examined. A third author (HZ) was consulted if the 2 investigators (AJ and ABA) responsible for the study selection were not able to reach an agreement on the inclusion of an article. Fig. 1 depicts the details of the study-selection methodology.

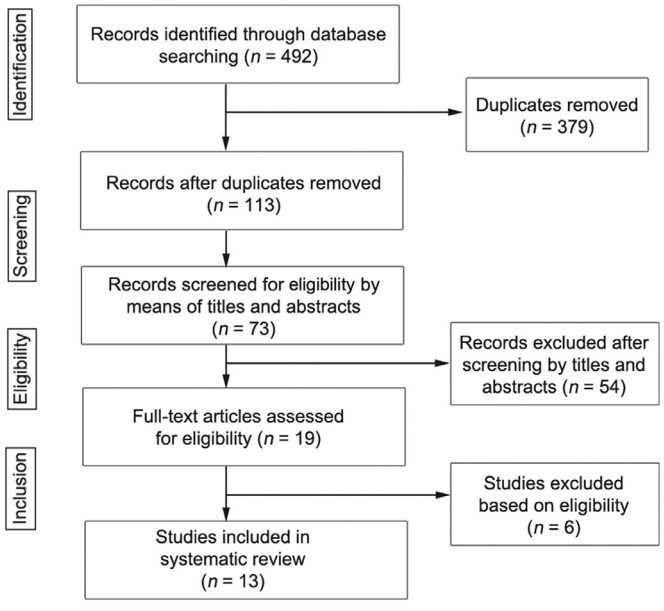

Fig. 1. Selection of research articles ($n = 13$) included in this systematic review using the recommendations in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.[26]

2.3.1. Data extraction

Once the inclusion/exclusion criteria were applied, data extraction was conducted to collect information about participants, intervention, comparisons, outcomes, and study design (PICOS) following PRISMA methodology. The following relevant data from each study were extracted: study details (author, year of publication, country, and duration of follow-up), study population (sample size, age, and participants' sex), sport or main exercise activity performed by the participants, the method used to determine the genotyping of *ACTN3* R577X (rs1815739), injury definition, workload (external and internal load parameters), and measures of association (i.e., relative risk (RR) or odds ratio (OR). Where possible, these associations were extracted directly from the original article. For articles in which this information was not present, associations were calculated using raw data, if available. Load parameters were classified as external or internal based on the International Olympic Committee consensus statement on load in sport and risk of injury.[28]

2.3.2. Quality assessment

The methodological quality of the included studies was assessed using the Physiotherapy Evidence Database (PEDro) scale.[29] A cut-off point of 6 points in the PEDro scale was used to discriminate high-quality studies, and only studies with scores above this threshold were considered for the systematic review.[26] Two authors (AJ and ABA) independently assigned a score to each study using the PEDro scale, and disagreements were resolved by consulting a third researcher (HZ).

3. Results

3.1. Study selection and description of the included studies

Our initial search identified 492 records (Fig. 1), and after the screening of titles, abstracts, and full texts, respectively, 13 studies were included in our final analysis. Accordingly, the characteristics of the participants, the follow-up periods, the injury definitions, and the methods used to determine the genotyping of *ACTN3* R577X (rs1815739) polymorphism are shown in Table 2. These studies were performed in 6 different countries (Spain, Japan, Brazil, China, the Republic of Korea, and Italy), involving a total participant pool of 1093 participants, where 20 represents the lowest number and 257 the highest number of participants within the studies. Two studies involved only women, 5 studies involved only men, and 6 studies involved both men and women. All the studies included were classified as high-quality studies (6 in the PEDro scale score) (Table 3).

Table 2
Characteristics of the studies that examined the association between the *ACTN3* R577X polymorphism and the rates and severity of non-contact injuries in highly trained athletes and in individuals enrolled in exercise training programs.

Study	Country	Population/sample size/sex/age (year, mean ± SD or range)/sport and level of practice	Follow-up	Injury data collection	Genetic testing
Pimenta et al. (2012)[33]	Brazil	37 male professional soccer players Age: 24.8 ± 1.7	Before and after eccentric exercise	Exercise-induced muscle damage (eccentric exercise) Post-exercise serum CK and α-actin immediately after (post), 2- and 4-h post-eccentric exercise	Extraction of genomic DNA from the peripheral blood samples. A DNA fragment carrying the exon 16 from the *ACTN3* gene was amplified from the genomic DNA, and the following initiators were used: 5'-CTGTTGCCTGTGGTAAGTGGG-3'; reverse, 5'-TGGTCACAGTATGCAGGAGGG-3', correlated to the adjacent intronic sequences
Iwao-Koizumi et al. (2014)[18]	Japan	99 female sports students Average age: 19.7 Sports: football, softball, basketball, and badminton Level of training: future professional athletes	–	A questionnaire was developed and filled out by each participant. The questionnaire asked whether the participant had suffered severe injuries during sports activity in the past	DNA was collected from a few drops of saliva on water-soluble paper (Mishima Dissolve Paper, 60 MDP; Nippon Paper Papylia Co., Ltd., Tokyo, Japan), Genotyping using TaqMan assay (TaqMan®SNP Genotyping Assays; Life Technologies, Carlsbad, CA, USA)
Kim et al. (2014)[21]	the Republic of Korea	97 elite ballet dancers and 203 normal female adults Age: 23.1 ± 1.3	–	Structured injury questionnaire and testing for injury risks on the joints	MGB TaqMan® SNP Genotyping assay method was used to analyze *ACTN3* R577X polymorphism (rs1815739) from the extracted DNA
Shang et al. (2015)[22]	China	142 males from a Chinese army infantry division Age: 21.0 ± 0.2	Past 1 year	Non-contact ankle sprains were diagnosed by an experienced clinician according to the standard validated criteria	DNA was extracted from blood cells by standard procedures by the kit manufacturer (Promega, Madison, WI, USA). Genotyping was done using a TaqMan allele discrimination assay that used the 5' nuclease activity of Taq polymerase
Belli et al. (2017)[26]	Brazil	20 men and women athletes Age: 40.5 ± 1.2	Before and after an official 37.1 km adventure race (22.1 km mountain biking, 10.9 km trekking, 4.1 km water trekking, 30 m rope course, and orienteering)	Post-exercise muscle damage	Genomic DNA was isolated from the buffy coat of centrifuged whole blood, using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Genotyping of *ACTN3* R577X (rs1815739) polymorphism was conducted using a TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA)
Del Coso et al. (2017)[24]	Spain	23 experienced men and women triathletes Age: 36.4 ± 5.2	Before and after the race	The changes in serum CK (CK−MM isoform) were measured in the blood samples, and muscle pain was measured with a visual analogue scale (0−10 cm)	DNA was isolated from the whole blood obtained before the race (QIAamp® DNA Blood Mini Kit, QIAGEN, The Netherlands) according to the manufacturer's protocol. Genotyping was performed using a TaqMan® SNP genotyping assay (Life Technologies™)
Del Coso et al. (2017)[25]	Spain	71 healthy, experienced (3 years) marathon runners, men and women Age: 42.7 ± 8.9	Before and after the race	Post-exercise muscle damage; post-exercise serum CK	Genomic DNA was isolated from the whole blood obtained before the race (QIAamp® DNA Blood Mini Kit) according to the manufacturer's protocol Genotyping was performed using a TaqMan® SNP genotyping assay (Life Technologies™)

(*continued on next page*)

Table 2 (Continued)

Study	Country	Population/sample size/sex/age (year, mean ± SD or range)/sport and level of practice	Follow-up	Injury data collection	Genetic testing
Massidda et al. (2019)[16]	Italy	257 male professional soccer players Age: 19.4 ± 5.2	1−5 years follow-up	Physical complaint occurring during practice that prevented a player from participating in training or match play for at least 1 day after the day of the onset. Ultrasound and magnetic resonance imaging scans were used to morphologically classify the injuries	DNA was extracted from a buccal swab and a commercially available kit (QIAGEN, Hilden, Germany) was used Concentration of extracted DNA was determined by fluorometric method (through Qubit by Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA)
Miyamoto et al (2018)[42]	Japan	76 male sports university students Age: 21.2 ± 2.8 Sport: multiple sports Level of training: regular training/recreationally active	–	Past hamstring-muscle strain injury	DNA was extracted from saliva and collected with a DNA self-collection kit (Oragene, DNA Genotek, Ontario, Canada) according to the manufacturer's protocol DNA was quantified using a spectrophotometer (Eppendorf Bio Photometer Plus, Eppendorf, Tokyo, Japan)
Del Coso et al. (2017)[31]	Spain	67 healthy and experienced marathon runners (male, all Spanish Caucasians) Low CK, *n* = 36; age = 43.0 ± 8.1 High CK, *n* = 31; age = 41.4 ± 9.4	Immediately after the race	Post-exercise muscle damage	A DNA fragment carrying the exon 16 from the ACTN3 gene was amplified from the genomic DNA and the following initiators were used: 5-CTGTTGCCTGTGGTAAGTGGG-3; reverse, 5-TGGTCACAGTATGCAGGAGGG-3, correlated to the adjacent intronic sequences
Clos et al. (2019)[15]	Spain	43 male professional soccer players Age: 27.5 ± 1.2	7 years follow-up	Injury rate, injury severity, and injury recovery times were established Injury severity was established according to the days a player needed to be absent from training and competition: mild, 1−15 days; moderate, 16−30 days; severe, more than 30 days	Genomic DNA isolation was performed using a QIAmp DNA Blood Minikit (Qiagen) DNA quantity was measured with a Nano-Drop ND-1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA)
Del Coso et al. (2020)[34]	Spain	22 experienced men and women triathletes Age: 35.4 ± 4.3 Low CK, *n* = 10 High CK, *n* = 12	Before and after the race	Post-exercise muscle damage	A DNA fragment carrying the exon 16 from the *ACTN3* gene was amplified from the genomic DNA and the following initiators were used: 5'-CTGTTGCCTGTGGTAAGTGGG-3'; reverse, 5'-TGGTCACAGTATGCAGGAGGG-3', correlated to the adjacent intronic sequences
Moreno et al. (2020)[17]	Spain	139 (XX: 32, RX: 67, RR: 40) Healthy marathon runners, men and women Age: 41.3 ± 10.2	1 year preceding to marathon	Physical complaints/visible damage to any part of lower limb assessed by qualified medical/healthcare practitioner	DNA was isolated using an organic-based DNA extraction method adapted to Amicon1 Ultra 0.5-mL columns, including a final concentration step to 50 μL

Abbreviations: CK = creatine-kinase; CK-MM = CK skeletal muscle.