Influence of ACTN3 R577X on injury risk: systematic review

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Summary

Regular physical activity is recommended as part of a healthy lifestyle to reduce the risk of disease and improve quality of life. However, sport can also increase the risk of tendon, muscle, and bone injuries. Among the risk factors that can predispose the human body to suffer this type of injuries, genetics, and in particular, the presence of single nucleotide polymorphisms (SNPs), can play a key role. However, studies analyzing the risk of injury associated with the genetic component are currently scarce and in many cases contradictory. In this regard, the ACTN3 gene, coding for the α-actin-3 protein, is one of the most studied genetic markers. The aim of this systematic review was to analyze and synthesize the state of knowledge on the relationship between the ACTN3 R577X polymorphism and the risk of injury in the sports practice. Therefore, an exhaustive review of all works published up to 28th January 2020 that analyzed the relationship between the ACTN3 R577X polymorphism and the risk of injury was carried out using the PubMed database. Eleven articles that met the inclusion criteria were selected. Although the number of studies analyzed is relatively low, it seems that carriers of the XX genotype may have a higher tendency to suffer lesions compared to the RX and RR genotypes. This increased risk of injury appears to be associated with α-actin-3 protein deficiency. These results can be useful in developing prevention programs to reduce the risk and severity of sports injuries.

Key words:
Polymorphism. Genotype. Athletic injuries. Physical exercise. Injury. Muscle damage

Influencia de ACTN3 R577X sobre el riesgo de lesión

Resumen

La práctica de actividad física regular se encuentra dentro de las recomendaciones para seguir un estilo de vida saludable con el fin de reducir el riesgo de enfermedades y mejorar la calidad de vida. Sin embargo, el deporte también puede aumentar el riesgo de sufrir lesiones tendinosas, musculares u óseas. Entre los factores de riesgo que pueden predisponer al cuerpo humano a sufrir lesiones de este tipo se encuentra el componente genético y, en particular, la presencia de polimorfismos de un solo nucleótido (SNPs). Sin embargo, actualmente los estudios que se han llevado a cabo sobre el riesgo de lesión asociado al componente genético son escasos y en muchos casos contradictorios. En este sentido, el gen ACTN3 que codifica para la proteína α-actina-3 es uno de los marcadores genéticos más estudiados. El propósito de la presente revisión sistemática fue analizar y sintetizar la información existente sobre la relación entre el polimorfismo ACTN3 R577X y el riesgo de lesión muscular en la práctica deportiva. Para ello, se realizó una revisión exhaustiva de todos los artículos publicados hasta el 28 de enero de 2020 que analizaban la relación entre el polimorfismo ACTN3 R577X y el riesgo de lesión, utilizando la base de datos PubMed. Se seleccionaron 11 artículos que cumplían con los criterios de inclusión. Aunque el número de estudios analizados es relativamente bajo, parece que los portadores del genotipo XX pueden presentar una mayor tendencia a sufrir lesiones en comparación con los genotipos RX y RR. Este mayor riesgo de lesión parece estar asociado a la deficiencia de la proteína α–actina-3. Estos resultados pueden ser de utilidad a la hora de elaborar programas de prevención de cara a disminuir el riesgo de las lesiones deportivas y su gravedad.

Palabras clave:
Polimorfismo. Genotipo. Lesión. Daño muscular. Ejercicio físico. Lesiones deportivas

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Introduction

An injury is damage to some part of the human body caused by a blow or fall. If the injury is caused when doing sport or exercise, it can be of two types: acute injuries which occur suddenly, such as ankle sprain, produced mainly during middle age and one of the most common injuries to the joints of the musculoskeletal system during physical activity, and chronic injuries that occur with doing exercise over time[1,2]. Several risk factors can predispose the human body to tendon, muscle and bone injuries; of these, the importance attached to the genetic component has grown in recent years and this factor has been studied with the aim of developing prevention programmes and reducing the risk of injuries and their severity[3-5]. Within this intrinsic factor, the presence of single nucleotide genetic polymorphisms (SNPs) can cause predisposition to injury[6,7].

SNPs are single-base alterations in DNA sequence and are present in all individuals. Some studies seem to indicate that SNPs can predispose the human body to behave differently in response to the same injury. Of the SNPs related to muscle injury, the R577X (rs1815739) of the ACTN3 gene is one of the most studied[6,7].

ACTN3 is the gene that encodes for a muscle protein, α-actinin-3 (a sarcomeric actinin-binding protein with a fundamental role in muscle structure and metabolism), which is expressed in muscle fibres, especially in type II or fast-twitch fibres[8-10].

The ACTN3 R577X polymorphism consists of a transition from cytosine (R allele, the ancestral allele) to thymine (X allele), resulting in a premature termination codon, which is associated with the absence of α-actinin-3 expression in individuals with the XX genotype. Some studies seem to indicate that individuals with the XX genotype present a reduction in muscle mass associated with a greater tendency to injury, meaning that interindividual differences in vulnerability to exercise-induced damage may be, in part, genetically determined by ACTN3 R577X[10-13]. However, studies conducted on the risk of injury associated with the genetic component are scarce and, in many cases, contradictory. Therefore, in this review we have compiled all those studies that have analysed the relationship between the ACTN3 R577X polymorphism and the risk of injury with the aim of providing an overview of the findings to date and elucidating any possible relationship which may exist. In this way, individuals with a greater predisposition to injury could be identified and personalised prevention programmes and treatments for individual athletes could be developed in the future.

Objectives

The main objective of this systematic review was to analyse and summarise the information from the studies available to date on the association between the different genotypes of the ACTN3 R577X polymorphism and the risk of injury in sport.

Material and method

A comprehensive review of all published articles related to the ACTN3 R577X polymorphism and risk of injury until 28 January 2020 was conducted using the PubMed database. The search criteria used were as follows: ((((((((((“rs1815739”[All Fields] AND “injurie”[All Fields] OR “injuried”[All Fields]) OR “injuries”[MeSH Subheading]) OR “injuries”[All Fields]) OR “wounds and injuries”[MeSH Terms]) OR (“wounds”[All Fields] AND “injuries”[All Fields])) OR “wounds and injuries”[All Fields]) OR “injurious”[All Fields]) OR “injury s”[All Fields]) OR “injuryed”[All Fields]) OR “injurys”[All Fields]) OR “injury”[All Fields])) OR (“R577X”[All Fields] AND ((((((((((“injurie”[All Fields] OR “injuried”[All Fields]) OR “injuries”[MeSH Subheading]) OR “injuries”[All Fields]) OR “wounds and injuries”[MeSH Terms]) OR (“wounds”[All Fields] AND “injuries”[All Fields])) OR “wounds and injuries”[All Fields]) OR “injurious”[All Fields]) OR “injury s”[All Fields]) OR “injuryed”[All Fields]) OR “injurys”[All Fields]) OR “injury”[All Fields]))) OR (“rs1815739”[All Fields] AND ((((“damage”[All Fields] OR “damaged”[All Fields]) OR “damages”[All Fields]) OR “damaging”[All Fields])))) OR (“R577X”[All Fields] AND ((((“damage”[All Fields] OR “damaged”[All Fields]) OR “damages”[All Fields]) OR “damaging”[All Fields])))) OR (“rs1815739”[All Fields] AND (((((“ruptur”[All Fields] OR “rupture”[MeSH Terms]) OR “rupture”[All Fields]) OR “ruptured”[All Fields]) OR “ruptures”[All Fields]) OR “rupturing”[All Fields])))) OR (“R577X”[All Fields] AND (((((“ruptur”[All Fields] OR “rupture”[MeSH Terms]) OR “rupture”[All Fields]) OR “ruptured”[All Fields]) OR “ruptures”[All Fields]) OR “rupturing”[All Fields])))) OR (“rs1815739”[All Fields] AND ((((“lesion”[All Fields] OR “lesion s”[All Fields]) OR “lesional”[All Fields]) OR “lesions”[All Fields])))) OR (“R577X”[All Fields] AND ((((“lesion”[All Fields] OR “lesion s”[All Fields]) OR “lesional”[All Fields]) OR “lesions”[All Fields])).

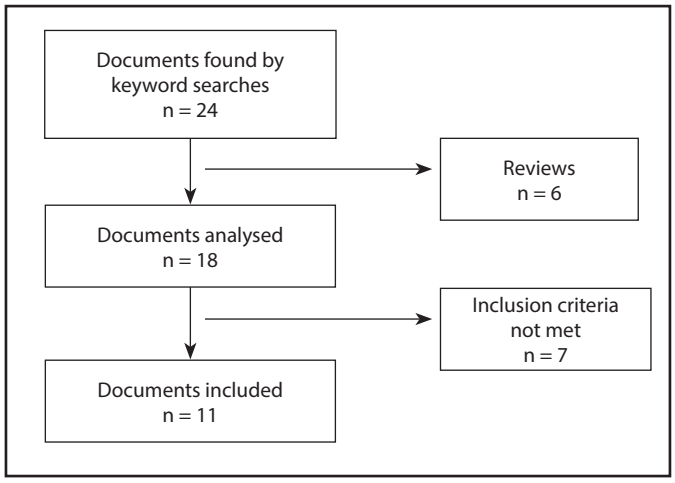
The inclusion criteria for the study were: studies carried out on healthy individuals without ruling anybody out for reasons of sex, ethnicity, age or country. We included only those studies that analysed the ACTN3 R577X polymorphism individually and not as part of a score, as a haplotype or in genome-wide association studies (GWAS).

Results and discussion

Figure 1 shows the flowchart of the search details and selection process used in the systematic review. After the initial search applying the aforementioned criteria, 24 articles were found. Subsequent analysis excluded 6 publications because they were reviews and another 7 for not meeting the inclusion criteria defined. After the whole process, 11 papers were included in the final analysis (Table 1).

Of the 11 documents included, studies were found that addressed the influence of the different genotypes (RR, RX, XX) of the ACTN3 R577X polymorphism on the risk of injury in patients both before and after doing different types of exercise or sports (football, marathon, half-ironman). Specifically, they analysed both the incidence of injuries and the levels of hormones associated with these injuries (creatine

Figure 1. Flowchart. Search and selection details for the systematic review process.



kinase (CK), myoglobin (Mb), cortisol or testosterone) in the different genotypes of the athletes.

In the 11 studies, the association of the participants' genotype with having a higher risk of injury or protecting them against the incidence of injuries was analysed. In their study on the role of the ACTN3 R577X polymorphism in the response to exercise-induced muscle damage, the researcher Barbara Vicent and her team14 concluded that the XX genotype was associated with an increased risk of injury compared to the RR genotype. In the study, a blood sample was taken from 19 healthy young men before and 6, 24 and 48 hours after performing an eccentric exercise that consisted of 4 sets of 20 knee extensions on both legs. A significant increase in CK levels was observed in the entire sample, but this increase was significantly greater in individuals with the XX genotype compared to those with RR. CK is an enzyme that participates in cellular energy metabolism and is composed of three isoenzymes distributed in skeletal muscle, cardiac muscle and the brain. CK is normally found

Table 1. Summary of studies on the association of the different ACTN3 R577X genotypes and the risk of injury.

Study	Aim	Characteristics of the sample	Results	Conclusion
Clarkson PM, *et al.* 2005	To study variations in genes coding two myofibrillar proteins (ACTN3 and MLCK) and their response to muscle damage produced by exercise.	208 healthy subjects. Male and female. USA.	The subjects with the XX genotype had a lower baseline CK and Mb compared to RX group but no different from that of the RR group. However, there was no association between ACTN3 XX and increased CK in response to eccentric exercise.	The subjects with the XX genotype showed a lower risk of injury than the RX subjects.
Vicent B, *et al.* 2010	To investigate the possible role of the ACTN3 R577X polymorphism in the response to muscle damage and recovery after eccentric exercise.	19 young healthy subjects. Male. Belgium.	After eccentric exercise, the participants with the XX genotype had greater CK activity. Participants with the RR genotype showed higher repair responses compared to those with the XX genotype.	The XX genotype was associated with an increased risk of injury compared to the RR genotype.
Venckunas T, *et al.* 2012	To compare the impact of and recovery from exercise-induced muscle damage between the different genotypes of the ACTN3 R577X polymorphism.	18 young healthy subjects. Male. Country not specified.	There were no significant differences between the different genotypes of the ACTN3 R577X polymorphism.	There was no association between the different genotypes and the risk of injury.
Shang X, *et al.* 2015	To investigate if the ACTN3 R577X polymorphism is associated with non-contact acute ankle sprains.	142 subjects with non-contact acute ankle sprain and a control group of 280 without ankle sprain. Male. China.	The XX genotype was significantly greater among participants with ankle sprain compared to the RX and RR genotypes.	Participants with the RR genotype had less risk of ankle sprains than those with the XX and RX genotypes.
Qi B, *et al.* 2016	To study the association between the ACTN3 R577X polymorphism and the incidence of non-acute ankle sprain in a Han Chinese population.	100 patients with ankle sprain and a healthy control group of 100 with no history of ankle injuries. Male and female. China.	The frequency of the RR genotype in ankle sprain was significantly lower than in the control group.	The RR genotype protected against ankle sprain in the Han Chinese population.
Del Coso J, *et al.* 2017a	To investigate the influence of the ACTN3 R577X polymorphism on the level of exercise-induced muscle damage during an official half-ironman race.	23 experienced athletes. Male and female. Spain.	At the end of the half-ironman, the athletes with the XX and RX genotypes had higher concentrations of CK and Mb than those with the RR genotype.	Athletes with the XX and RX genotypes had a higher risk of muscle injury during a half-ironman race than those with RR.

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Table 1. Summary of studies on the association of the different ACTN3 R577X genotypes and the risk of injury *(continuation)*.

Study	Aim	Characteristics of the sample	Results	Conclusion
Del Coso J, *et al.* 2017b	To determine the influence of the ACTN3 R577X polymorphism on exercise-induced muscle damage during a marathon.	71 runners. Male and female. Country not specified.	Mb and CK levels were higher in marathoners with the XX and RX genotypes than in those with the RR genotype.	Marathoners with the XX and RX genotypes had higher exercise-induced muscle injury marker values than marathoners with the RR genotype.
Moreno V, *et al.* 2017	To determine the influence of the genotypes of the ACTN3 R577X polymorphism on marathoner injury incidence during the year prior to participation in a competitive marathon race.	139 amateur marathoners. Male and female. Spain.	The likelihood of suffering a muscle injury during the year prior to the competition was twice as high in runners with the XX genotype compared to those with RR and RX.	The XX genotype showed a greater incidence of injuries than the RX and RR genotypes.
Miyamoto N, *et al.* 2018	To study if the ACTN3 R577X polymorphism may have an influence on muscle stiffness.	76 university students. Male. Japan.	Greater hamstring muscle stiffness was detected in participants with the RR and RX genotypes than in those with the XX genotype. However, the frequency of injury episodes among the 3 genotypes was the same.	The RR and RX genotypes were associated with increased muscle stiffness, although they do not appear to be linked to more episodes of injury.
Massidda M, *et al.* 2019	To investigate the association between the ACTN3 R577X polymorphism and indirect muscle disorders/injuries in professional football players.	257 professional football players. Male. Italy.	The players with the XX and RX genotypes were 2.66 and 1.63 more likely to be injured than players with the RR genotype, respectively.	The X allele showed a greater risk of injury compared to the RR/RX genotypes.
Coelho DB, *et al.* 2019	To assess muscle damage indicators and hormonal responses after football matches and their relationship to the expression of the ACTN3 R577X polymorphism (XX vs RR/RX).	0 U16 football players. Male. Brazil.	The concentration of CK, testosterone and cortisol after the game was higher in players with the RR/RX genotype compared to players with the XX genotype.	The RR and RX genotypes were associated with an increased risk of injury compared to the XX genotype.

CK: creatine kinase; MLCK: myosin light-chain kinase.

in blood at very low levels and comes mainly from skeletal muscle. In the event of an injury or muscle damage, significant amounts of CK are released into the blood, its concentration being proportional to the intensity, severity and duration of the damage. The peak in plasma is reached 24 hours after finishing exercise and the presence of CK may remain high for 48-72 hours[15,16]. In the same study, the subjects were asked to rate their perception of muscle pain after repeating the sets of eccentric muscle contractions using an analogue visual scale (EVA scale) and the individuals with the RR genotype indicated lower levels of muscle pain.

In two studies carried out on Chinese and Han Chinese populations of both sexes[17,18], clinical questionnaires, anthropometric measurements and analytical tests were used to analyse the possible association between the different genotypes and ankle sprain (the most frequent injury in musculoskeletal joints). No significant differences in age, sex, height, weight or overall health were found between the clinical questionnaires and physical examinations. However, when comparing the distribution of the 3 possible genotypes of the ACTN3 R577X polymorphism among the group of participants with ankle sprain and a control group (healthy), it was observed that healthy people manifested a greater presence of the RR genotype while the XX genotype prevailed among the people with ankle sprain. These differences in genomic DNA led the researchers to the conclusion that the participants with the RR genotype were less at risk of suffering ankle sprain than those with XX and that RR was a protective genotype.

In the studies carried out by Del Coso, J., *et al*. in 2017[19,20] based on endurance sports, a half-ironman race and a marathon, a greater risk of injury was observed in individuals with the XX and RX genotypes after analysing two indicators of injury risk, CK and Mb, in 23 and 71 participants, respectively. Mb is a protein composed of a polypeptide chain present in all striated muscle fibres, the highest concentrations being in cardiac and skeletal muscle. It is not found in smooth muscle. Its main function is to transport and store oxygen in the muscle for energy and it also helps muscles to contract. Like CK, in the event of an injury, its blood concentration increases in response to high oxygen demand and hypoxia, and the destruction of muscle tissue[21,22]. Two groups were created in both studies, one with individuals with the RR

genotype and the other with individuals with the XX and RX genotypes, the latter two showing very similar phenotypic responses. Blood samples were collected both before and after the race. Before the race, the test showed higher concentrations of serum CK and Mb in the XX+RX group compared to the RR group. After the race, the increase in CK and Mb concentrations was more pronounced in the XX+RX group than in the RR genotype group. Therefore, in both studies it was concluded that marathon and half-ironman athletes with the XX+RX genotypes of the ACTN3 R577X polymorphism showed higher levels of exercise-induced muscle damage than their RR counterparts.

The same result was obtained in another study published that same year[23] in which, through a cross-sectional study, the association between the genotypes of the ACTN3 R577X polymorphism and the risk of sports-related injury in a group of 139 Spanish female and male marathon runners was analysed. To do this, the participants completed a questionnaire about the injuries they had suffered during the year prior to competing in a race and a DNA swab test was performed on them. Those individuals with the XX genotype were seen to have had twice as many injury episodes during the year prior to the marathon compared to runners with the RR and RX genotypes. It was also observed that the most common cause of injury was an excessive training load. This result may suggest that the fact that participants with the XX genotype have an α-actinin-3 deficit in their fast-twitch muscle fibres causes decreased muscle function, exposing them to a greater risk of injury.

However, other studies have obtained results contrary to those mentioned above, observing an increased risk of injury in individuals with the RR genotype. In the Clarkson *et al*. study, the blood samples of 208 subjects were analysed before and after performing an eccentric elbow flexion exercise in two sessions of 25 repetitions each[24]. Contrary to expectations, there was no association between the XX genotype and a higher blood concentration of CK and Mb in response to eccentric exercise. The individuals with the XX genotype showed lower CK and Mb concentrations in the blood in response to eccentric exercise compared to the RX genotype and did not differ from the RR genotype.

A subsequent study[25] sought to study the association of the ACTN3 R577X polymorphism with muscle stiffness and the risk of muscle strain injury. To this end, the medical histories and different measurements of 76 university students were analysed. It was one of the first studies to ask whether there was an association between genotypes and hamstring strain injury. At the time, it was known that muscle stiffness mainly affected joint flexibility and was related to muscle strain injury. At the end of the study, it was concluded that the RR and RX genotypes were associated with greater muscle stiffness and, therefore, had a greater risk of injury compared to the XX group.

In another study conducted on male football players[26], the relationship between the ACTN3 R577X polymorphism and muscle injury after a football match was examined. Muscle damage was assessed using muscle microtrauma and hormonal stress markers, analysing hormonal indicators such as cortisol, testosterone and CK. Cortisol is a steroid hormone produced by the adrenal gland which has an effect on virtually every organ and tissue in the body. Faced with a stressful situation such as muscle damage, the level of cortisol in the blood increases. If this increase is protracted, it leads to a greater need for glucose, which the body obtains from the amino acids in the muscle, which can lead to the loss of muscle mass[27,28]. Meanwhile, testosterone is a hormone produced by the adrenal glands which influences the development of the sexual organs, bone maintenance and increases in muscle mass. When muscle damage occurs after intense exercise, the concentration of testosterone increases[27-29]. Immediately after finishing the football match, a significantly higher increase in CK, cortisol and testosterone levels was observed in players with the RR and RX genotypes compared to those with the XX genotype.

Like the previous study by Coelho, D.B., *et al.* 2019[26], the study by Massidda, M., *et al.* was also conducted on professional football players. This time participants were grouped by genotype and compared to a control group (healthy non-athletes). No differences were found in the frequencies of the different genotypes between the non-athletes and the football players. However, a greater tendency to suffer muscle injuries was observed among players with the XX genotype compared to RR[30].

Only one study[31], conducted on 18 healthy young men, found no significant differences between the different genotypes of the ACTN3 R577X polymorphism when studying different parameters related to injury risk. The participants performed two episodes of 50 jumps, separated by two weeks, each jump from a height of 40 cm to a 90-degree knee bend. Muscle pain was reported using an ordinal scale of 0-10, where 0 meant no pain and 10 very intense pain, and muscle damage percentages were obtained by collecting blood samples and analysing CK. CK increased immediately after exercise with no significant differences between genotype groups and decreased on finishing the two sets of 50 jumps with no significant differences between the different groups. The differences between the RR, RX and XX genotypes could be called modest and no association was found between the different genotypes and the risk of injury.

Conclusion

The SNP ACTN3 R577X expressed in fast-twitch muscle fibres and its protein α-actinin-3 play an important role in muscle metabolism, injury severity and incidence. Despite the heterogeneity and low number of studies, it is hypothesised that individuals with the XX genotype have an increased risk of injury because the X allele is associated with an α-actinin-3 deficiency in the muscle. α-actinin-3 is a protein necessary for the formation of fast-twitch muscle fibre, and its absence seems to negatively affect muscle capacity, leading to a greater propensity to injury. By contrast, the RR genotype of the ACTN3 R577X polymorphism is likely to play a protective role in skeletal muscle functions, which would be reflected in a lower risk of injury.

Conflict of interest

The authors declare that they are not subject to any type of conflict of interest.phy

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