# THE ASSOCIATION OF THE ACTN3 R577X GENOTYPES AND ATHLETE STATUS – A META-ANALYSIS

A Thesis by

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Bachelor of Science, University of Kansas, 2016

Submitted to the Department of Human Performance Studies
and the faculty of the Graduate School of
Wichita State University
in partial fulfillment of
the requirements for the degree of
Master of Education

May 2019

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The following faculty members have examined the final copy of this thesis for form and content, and recommend that it be accepted in partial fulfillment of requirements for the degree of Master of Education with a major in Exercise Science.

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# ABSTRACT

This study is a meta-analysis interested in research that investigated ACTN3 genotype frequencies present in different athlete types – endurance, middle distance, or power related. One of the many factors that determine athletic ability is genetics. One heavily studied gene in that regard is the ACTN3 R577X polymorphism. ACTN3 is a protein-encoding gene that is only expressed in type II muscle fibers. Three different hypotheses were formulated: 1) homozygous ACTN3 RR genotype will show a positive association with power related sports 2) no significant association of the homozygous minor allele XX will be shown with endurance athletes 3) no significant association will be shown between middle distance athletes and the heterozygous RX genotype. The analysis regarding hypothesis 1 revealed a statistically significant association between the RR genotype and power oriented athletes (OR = 1.4680, 95%CI = 1.0113; 2.1308, Z = 2.02, p = .04). Results for the analysis regarding hypothesis 2 showed no significant association between endurance athletes and the XX genotype (OR = .96, 95%CI = .72; 1.29, Z = .25, p = .80). Analysis for hypothesis 3 also demonstrated a non-significant association between RX genotypes and middle distance athletes (OR = 0.74, 95%CI = 0.38; 1.45, p = .38).

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# CHAPTER 1

# INTRODUCTION

## 1.1 The α-actin-3 Protein

One of the many factors that determine athletic ability is genetics. One heavily studied gene in that regard is the ACTN3 R577X polymorphism. Human skeletal muscles are composed of several categorized fiber types based on their chemical properties. These categories include: Type I (slow-twitch oxidative), Type IIa (fast-twitch oxidative), and Type IIb (fast-twitch glycolytic). The different cellular compositions allow for the different aerobic and anaerobic capabilities seen in human performance. The Type I fibers rely primarily on the aerobic metabolism while the Type IIb fiber type relies primarily on anaerobic metabolism. The Type IIa has a combination of chemical properties so is indistinguishable on which metabolism it will rely on (Scott, Stevens, and Binder–Macleod, 2001). ACTN3 is a protein-encoding gene that is only expressed in type II muscle fibers.

ACTN3 encodes for an actin binding protein called α-actin-3, which is a structural component of the z-disk in the sarcomere of the human skeletal muscle (Vincent et al., 2007). The sarcomeres are the functional units inside striated muscle cells. Sarcomeres are repeated segments within each cell, and each sarcomere runs from Z-disk to Z-disk. The Z-disk acts as an anchoring point for the actin filament. The sarcomeres are made from a thin filament called actin and a thick filament called myosin. These filaments work together to slide past each other to allow muscles to contract (Powers & Howley, 2007).

The R577X of the ACTN3 R577X gene title refers to the non-sense mutation at nucleotide 577 of the α-actin-3 DNA sequence (rs1815739, rs = reference single nucleotide polymorphism). During the cellular process of protein synthesis a DNA sequence that encodes

for a protein is transcribed into a messenger RNA (mRNA) molecule and transferred to ribosome where it is translated to a protein by linking together amino acids in a specific order specified by the code of the mRNA. During translation transfer RNA molecules (tRNA) pair with a corresponding sequences of mRNA three-nucleotides at a time. Each sequence of mRNA called a codon pairs with its anticodon of the tRNA. Each codon sequence of mRNA codes for a specific anticodon tRNA molecule that is carrying a specific amino acid according to the codon sequence. The ribosome machinery then links together the amino acids in the specific order to form a protein. (Griffiths, Wessler, Carroll, & Doebley, 2012). A non-sense mutation like the one in the ACTN3 R577X polymorphism produces a premature stop codon sequence resulting in a truncated protein. The polymorphism results in two alleles commonly referred to as R (wild type) and X (truncated version) and three genotypes RR, RX, or XX.

### 1.2 The α-actin-3 Protein in Regards to Exercise Science

Current research in respect to genetics and exercise are mostly candidate gene studies, but for many traits the relationship between the phenotype and the genotype is immensely complex and does not rely solely on one gene. An example of this was in a study done by Yang et al. (2010) considering the genetic factors effecting human height, they showed that there are approximately 295,000 different small nucleotide polymorphisms (SNPs) contributing to only 45% of the variance seen in human height. The nature versus nurture argument is underlying question regarding athletic ability. In regards to the heritability of muscle mass and power, reported values have ranged anywhere from 15-90%, and the heritability of aerobic capacity has been documented around 50% (Stewart & Rittweger, 2006). Athletic ability is a complex trait heavily dependent on both environmental and genetic factors. An additional factor furthering the complication of genotype and phenotype relationships is the individual variance in epigenetic

factors including alterations in chromosome structure and gene expression alterations caused by sources other then DNA sequence changes (Tucker & Collins, 2012).

Even with the complexity between athletic ability and genetics there is potential benefit to further understanding the relationship. One motivation for this type of research is the development of a consumer genetic test that quantifies athletic performance characteristics and potential risks using a set of specific genes that indicate individuals potential in specific sports. Genetic markers of potential use might provide insight on traits like recovery rates, speed, endurance, heart arrhythmias, or hypertrophic cardiomyopathy (Collier, 2012).

In regards to the research of the ACTN3 gene there have been studies that have shown that the ACTN3 genotype RR is over represented in elite power athletes while the XX genotype has been shown to be beneficial in the endurance athletes (Yang et al., 2007). A previously done meta-analysis concentrated on only elite athletes defined by national or international competition and only power related sports (Weyerstraß, Stewart, Wesselius, and Zeegers, 2017). In contrast this study will look at other studies that associated the genotypes with athletic categories including endurance and middle distance in addition to power related sports. Genetic profiles that determine individual's abilities in sport performance is a future potential benefit to clinicians and coaches in order to guide individuals with any specific genetic potential.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Underlying Biology

One of the many factors that determine athletic ability is genetics. One heavily studied gene in this regard is the ACTN3 R577X polymorphism. The RR genotype has been associated with power-oriented athletes like sprinters and weightlifters (Yang et al., 2003). While the homozygous ACTN3 XX genotype has been hypothesized to be advantageous for endurance athletes, the results from a large study done by Papadimitriou (2018) showed no association. The associations with athletic performance, however, whether it is with power related sports or endurance sports are not seen across the board (Scott et al., 2010). These mixed results suggest the necessity of a weighted analysis to further quantify the effect size potential overall effects.

ACTN3 is a protein-encoding gene that is only expressed in type II muscle fibers. It encodes for an actin binding protein called α-actin-3, which is a structural component of the z-disk in the sarcomere of the human skeletal muscle (Vincent et al., 2007). The ACTN3 R577X (rs1815739, rs = reference single nucleotide polymorphism) is a non-sense mutation at nucleotide 577 within the protein. Non-sense mutations produce premature stop codons resulting in a truncated protein. The polymorphism results in two alleles of the ACTN3 gene commonly referred to as R (wild type) and X (truncated version) and three genotypes RR, RX, or XX. The truncated allele XX genotype does not however result in a disease phenotype or a functional impairment. According to Yang et al., (2003) about 18% of the population has this genotype. However, as stated by Fridén & Lieber (2001) the Z-disk is the most vulnerable structure in the sarcomere to exercise induced injury including damage to the α-actin proteins; suggesting that

the R allele offers an advantage during rapid contraction because of a greater absorptive and transmission capacity at the Z-disk.

## 2.2 Research on the ACTN3 Gene

There has been research that fails to demonstrate any associations with the ACTN3 gene and athletic performance as stated above. In research done by Eroğlu, O., Zileli, R., Nalbant, M. A., & Ulucan, K. (2018) the ACTN3 R577X polymorphism was shown to have no correlations with national level athletes (n = 84). They had a mixture of athletes genomic data tested including short distance, medium distance, and long distance runners and none of the athletes harbored the RR genotype. These results suggest a further complicating factor of human genetics; the different allele frequencies within sub populations around the world.

In the future genetic profiles that determine individual's abilities in sport performance might offer a potential benefit to enhance clinicians and coach's ability to assist athletes. It will potentially enhance the ability to guide individuals with any specific genetic potential on a training prescription in order to optimize their training process and success in their respective competitions. There isn't, however, a best practice guideline to use for athletes genetic information as of yet so further research will be required to develop these guidelines (Pickering, 2017). Therefore, the objective of this meta-analysis is to further quantify any advantage between the ACTN3 polymorphism and different types of athletic events. Additionally, ACTN3 is a gene of interest in other areas of exercise science research; there has been studies done that suggest that the polymorphism may impact a number of traits, including injury risk (Massidda, 2017), flexibility (Kikuchi, 2017), testosterone levels (Ahmetov, 2014) and training adaptation (Pimenta, 2012). The majority of research studies published examining ACTN3 do not include a meta-analysis.

## 2.3 Power Related Sports and Genotype RR

There have been studies that have shown that the ACTN3 genotype RR is over represented in explosive power athletes (Vincent et al., 2007). Yang et al. (2003) genotyped 107 Olympic level power athletes, 436 controls, and 194 Olympic level endurance athletes, and showed that sprint athletes had a lower frequency of XX genotype. 6% of all the sprint athletes had the XX genotype. The sprint group also had an increased frequency of RR compared with the control group (50% of sprinters versus 30% in controls), and none of the female sprinters had the XX genotype. Additionally all of the male sprinters had at least one functional R allele.

There have been several studies that have shown a relationship between power sports and the RR genotype (Yang et al., 2003; MacArthur & North, 2004). Another example of this was a study done by Druzhevskaya, Ahmetov, Astratenkova, & Rogozkin (2008). In this study they had recruited subjects (n = 486) that were regional or national competitors in power related sports including: alpine skiing, gymnastics, bodybuilding, figure skating, ice hockey, jumping events, sprinters, power lifters, weightlifters, wrestlers, and volleyball players. They also had a sample of 1197 control subjects who were unrelated citizens not participating in any sports. Results showed that the XX genotype was only present in 6.4% of the athletes versus 14.2% of the controls, which was statistically significant, suggesting that the R allele offers an advantage in the power related sports.

## 2.4 Middle Distance Sports and Genotype RX

The majority of the literature focuses on the association of the RR genotype with strength exercises or the XX genotype with endurance athletes; meanwhile, there is not a lot of research on the heterozygous RX genotype. Li et al., (2017) examined the presence of the ACTN3 polymorphism genotypes amongst athletes of middle distance swimmers, which is a sport

requiring both the aerobic and anaerobic metabolism. The genotype frequencies of 81 women and 79 men who compete at the national level in China were taken. Li et al., (2017) demonstrated that the homozygous RR genotype was the most prevalent amongst the elite middle distance swimmers, 41.8% for males and 46.9% for females. The RX frequencies were 38% and 34.6% for males and females, respectively. These results suggest that the RR genotype as well as having the R allele is the most advantages for middle distance sports.

Ben-Zaken, Eliakim, Nemet, Rabinovich, Kassem, & Meckel, (2015) conducted another study that used swimmers as their subjects as well as track athletes. In this study there was 137 runners and 91 swimmers, and they were tested for the ACTN3 genotype frequencies. The track athletes and swimmers were further split into groups based on the type of event the competed in i.e. long distance, middle distance, or short distance. The results showed that like Li et al. (2017) there was no correlation between the RX genotype and middle distance swimmers. There has been few studies researching the RX genotype and middle distance track athletes, and athletes categorized in middle distance sports in general. This could be due to the observation that most elite middle distance athletes could realistically qualify as relatively elite short distance or long distance events if trained.

### 2.5 Endurance Sports and Genotype XX

In a study by Niemi & Majamaa (2005) genotypes of elite Finnish track and field athletes (n = 52 for endurance athletes, n = 89 for sprint athletes) were analyzed at the ACTN3 gene. Athletes were included in this study if they competed at the national track and field championships or at the national level for cross-country. The results showed that the XX genotype had a higher frequency than the RR among endurance runners and that none of the top

sprinters harbored the XX genotype, suggesting that there might be benefits of the XX genotype for endurance athletes.

Grealy et al. (2013) theorized that the X allele decreases the capacity to contract muscles fast while increasing the ability to endure long duration exercise, and that race times therefore do not benefit because an individual might endure a longer event and still have a slow performance because the decreased speed. They conducted a study on the Ironman World Championships endurance athletes looking for any performance associations with the X allele. There were a total of 196 endurance athletes who participated in the study. Results showed that 23.5% of the athletes carried the XX genotype, however there was no association with a better race time.

An additional study was done by Yang et al. (2007) on the effect of the X allele on endurance athletes. This study included 76 elite Ethiopian endurance athletes, and 284 elite Kenyan endurance athletes. The results of the study did not support the hypothesis that the X allele is beneficial to endurance athletes, especially amongst the Kenyans where the XX genotype frequency was approximately 1%.

# CHAPTER 3

# METHODOLOGY

## 3.1 Information sources

Studies for potential inclusion were searched in the following databases: PubMed, SPORTDiscus, and MEDLINE (EBSCO). Additional studies were identified from Google Scholar or via cross-referencing.

## 3.2 Electronic search terms/strategy

A combination of key words: ACTN3, ACTN3 R577X, alpha-actin-3, genotypes, allele frequencies, polymorphism, athlete status, endurance, power, sprint or sport were used to search for potential articles.

## 3.3 Eligibility criteria

*Inclusion criteria.* Eligible papers included case-control or gene candidate studies that were not included in a previously published meta-analyses. Articles included healthy individuals, no animals, no diseased people, and no children as included participants. Articles must have reported genotype frequencies for the ACTN3 gene among athletes. A minimum of 14 articles was ruled necessary for this study.

*Exclusion criteria.* Data collection process excluded papers that did not include necessary gene frequency data, review articles, unoriginal articles, articles previously used in a different meta-analysis, and articles only available in another language other then English.

## 3.4 Data extraction

For all used studies the following data was extracted: authors, date published, type of experiment, statistical procedure used, frequencies of each polymorphism in subjects,

characteristics of the study sample population (gender, number, ethnicity, endurance or power athlete).

### 3.5 Statistical analysis

For each study selected, odds ratios were calculated to estimate the effect size of the association based on alleles, genotypes, and athlete types. For each hypothesis there will be a different meta-analysis conducted to quantify the pooled effect size. Hypothesis 1 is an analysis between power-oriented athletes and the genotype RR. This odds ratio will be as follows:

OR = (RR genotype | athlete) / (RR genotype | control) = [(RR | athlete) / (not RR | athlete)] / [(RR | control) / (not RR | control)]

There was also an allele-based analysis done for the association of power-oriented athletes and the R allele. This odds ratio will be calculated as follows:

OR = (odds of having R | an athlete) / (odds of having R | a control) = [(R | athlete) / (X | athlete)] / [(R | control) / (X | control)]

Hypothesis 2 is an analysis between endurance-oriented athletes and the association to the XX genotype. Each odds ratio in this analysis will as follows:

OR = (XX genotype | athlete) / (XX genotype | control) = [(XX | athlete)/(not XX | athlete)] / [(XX | control)/(not XX | control)]

Hypothesis 3 is an analysis of middle distance athletes associated with the RX genotype. The odds ratio for this was as follows:

OR = (RX | athlete) / (RX | a control) = [(RX | athlete)/(not RX | athlete)] / [(RX | control) / (not RX | control)]

A meta-analysis of the odds ratios was then carried out using a random effects model to add weight to each study, and to quantify the different associations being tested. A random

effects model assumes that the data being drawn comes from varying populations where unknown variables may be present, which allows different study outcomes to vary in a normal distribution. According to DerSimonian and Laird (1985) and Neyeloff, Fuchs, and Moreira (2012) random effects models are considered a more natural choice compared to fixed effect models which assume that all the studies were done on similar subjects and similar conditions, and are preferred even when heterogeneity is low. Therefore, based on the heterogeneous nature of this study it was necessary to use the random effects model.

Heterogeneity was quantified with the Cochran's Q statistic, and the $I^2$ statistic. The null hypothesis in a Q test says that all studies will be equal. The Q statistic is calculated by the weighted sum of squared differences between each study effects and pooled effect across studies. The weights are the ones used in the pooling method (Neyeloff et al., 2012). The Q is distributed as a chi-square statistic with the number of studies minus 1 degree of freedom. If the calculated critical value falls below the critical value than the null hypothesis is not rejected, and the studies are ruled homozygous enough for a fixed effect model to be executed. The $I^2$ statistic is a way to quantify the heterogeneity. It is expressed as a percentage of the total variability in a set of effect sizes due to between study variability. All statistical tests used a significance value of 5%. Statistics were carried out on Excel, RevMan, and CMA (comprehensive meta analysis) statistical software.

# CHAPTER 4

# RESULTS

## 4.1 Literature Search

Figure 1 summarizes the search and identification of included studies. A total of 519 articles were identified through searching MEDLINE (EBSCO), Pubmed, and SPORTDiscus. 72 articles remained after review based on the title and abstract. After removing duplicates, and reviewing full text 12 articles remained. An additional 5 studies were identified through other sources and cross-referencing. Articles that were reviewed and excluded based on the full text were excluded for one of the following reasons: not including a control group, not reporting the necessary genotype and allele frequency data, or athlete participants were not clearly defined.

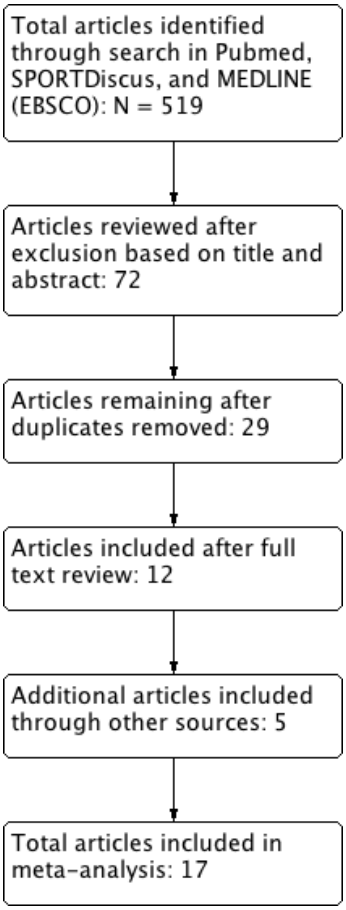


Figure 1. Identification of included studies.

Table 1 shows the descriptive data of the 17 included studies. The table includes the author, year, country where the research was conducted, ethnicity of the participants, total athletes included, and the total controls included.

TABLE 1

DESCRIPTIVE CHARACTERISTICS OF INCLUDED STUDIES

| Study | Country | Ethnicity | Total Athletes | Total Controls |
|---|---|---|---|---|
| Ben-Zaken 2015 | IL | Israeli | 228 | 217 |
| Eroglu 2018 | TR | Turkish | 50 | 34 |
| Galeandro 2017 | IT | Caucasian, African | 43 | 128 |
| Gineviciene 2017 | LT, RU | Caucasian | 161 | 1202 |
| Ginszt 2018 | PL, RU, AT | N/A | 100 | 100 |
| Guilherme 2018 | BR | Brazilian | 656 | 964 |
| Heffernan 2016 | UK, IE, ZA | Caucasian | 507 | 710 |
| Honarpour 2017 | IR | Iranian | 90 | 200 |
| Koku 2019 | TR | Caucasian | 100 | 100 |
| Li 2017 | CN | Asian | 160 | 206 |
| Mägi 2016 | EE | Caucasian | 58 | 322 |
| Peplonska 2017 | PL | Caucasian | 413 | 451 |
| Pimjan 2017 | TH | Asian | 117 | 99 |
| Salgueirosa 2017 | BR | Brazilian | 40 | 2016 |
| Wessner 2016 | AT | Caucasian | 142 | 216 |
| Yang 2017 | CN | Asian | 103 | 50 |
| Yusof 2016 | MY | Chinese, Indian, Malays | 180 | 180 |
| Abbreviations: IL = Israel, RU = Russia, UK = United Kingdom, PL = Poland, CN = China, TR = Turkey, IT = Italy, IR = Iran, BR = Brazil, EE = Estonia, LT = Lithuania, MY = Malaysia, TH = Thailand, IT = Italy, AT = Austria, IE = Ireland, ZA = South Africa | | | | |

## 4.2 Hypothesis 1

Hypothesis number 1 stated the power-oriented athletes would have higher odds of harboring the wild-type RR genotype in comparison to the XX genotype. The analysis of hypothesis 1 included only subjects harboring the wild type RR genotype or the XX genotype. Therefore this hypothesis was tested by taking the ratio of the odds of being an RR genotype given that one was an athlete versus the odds of being an RR given one was a control. Table 2

shows the results of the meta-analysis for these odds ratios (OR = 1.4680, 95%CI = 1.0113; 2.1308), and Figure 2 is a forest plot summarizing these results. The results indicate that the athletes were significantly more likely to have the RR genotype than the control group (Z = 2.02, p = .04).

TABLE 2

ODDS RATIOS BASED ON WILDTYPE RR AND HOMOZYGOUS GENOTYPE OF MINOR ALLLE XX

| | **RR** | | **XX** | | | |
|---|---|---|---|---|---|---|
| **Study** | **Athletes** | **Total** | **Athletes** | **Total** | **Weight** | **Odds Ratio M-H, Random, 95%CI** |
| Ben-Zaken 2015 | 45 | 93 | 17 | 57 | 7.30% | 2.2059 [1.0974, 4.4339] |
| Eroglu 2018 | 3 | 12 | 3 | 11 | 2.80% | .8889 [.1381, 5.7229] |
| Galeandro 2017 | 21 | 61 | 5 | 21 | 5.10% | 1.68 [.5402, 5.2247] |
| Gineviciene 2017 | 67 | 513 | 18 | 172 | 8.10% | 1.2853 [.7403, 2.2313] |
| Ginszt 2018 | 31 | 64 | 4 | 21 | 4.90% | 3.9924 [1.2092, 13.182] |
| Guilherme 2018 | 136 | 455 | 52 | 255 | 9.00% | 1.6643 [1.1556, 2.3970] |
| Heffernan, 2016 | 169 | 412 | 104 | 234 | 9.20% | .8693 [.6287, 1.2021] |
| Honarpour, 2017 | 37 | 87 | 20 | 55 | 7.30% | 1.295 [.6466, 2.5938] |
| Koku, 2019 | 29 | 62 | 15 | 33 | 6.50% | 1.0545 [.4518, 2.4613] |
| Mägi 2016 | 21 | 74 | 4 | 32 | 5.00% | 2.7736 [.8667, 8.8757] |
| Peplonska 2017 | 74 | 236 | 32 | 99 | 8.40% | .9564 [.5784, 1.5816] |
| Pimjan 2017 | 19 | 53 | 44 | 53 | 6.20% | .1143 [.0460, .2842] |
| Salgueirosa 2017 | 18 | 94 | 2 | 35 | 3.70% | 3.9079 [.8573, 17.8131] |
| Wessner 2016 | 22 | 87 | 12 | 61 | 6.80% | 1.3821 [.6240, 3.0609] |
| Yang 2017 | 29 | 42 | 3 | 20 | 4.20% | 12.6410 [3.1459, 50.79] |
| Yusof 2016 | 19 | 59 | 5 | 42 | 5.40% | 3.5150 [1.1915, 10.369] |
| **Total (95% CI)** | **2404** | | **1201** | | **100%** | **1.4680 [1.0113, 2.1308]** |
| Heterogeneity. $Tau^2$ = .37; $Chi^2$ = 59.71, df = 15 ($p$ < .00001); $I^2$ = 75% | | | | | | |
| Test for overall effect: Z = 2.02 ($p$ = .04) | | | | | | |

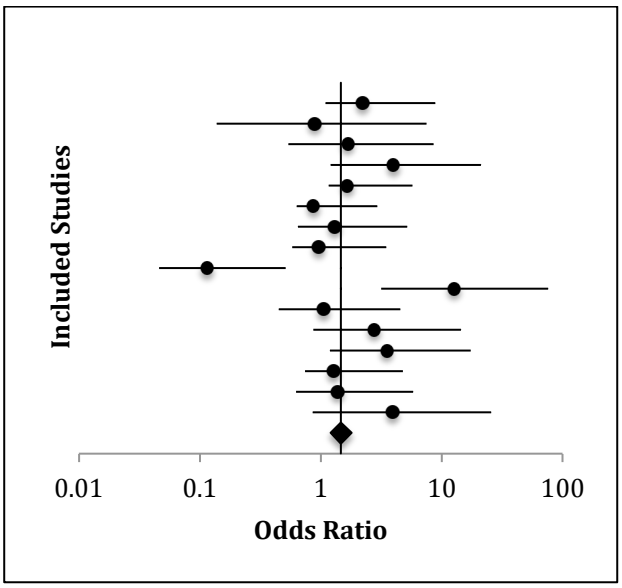


Figure 2. Forest plot of odds ratios based on RR genotype and XX genotype in power athletes.

Table 3 and figure 3 shows the results of another analysis regarding hypothesis 1. This analysis used the ratio of the odds of being RR given an athlete versus the odds of being RR given a control, but this analysis includes the heterozygous RX subjects. Therefore this analysis is an odds ratio of the RR genotype and not the RR genotype in power athletes and controls. The meta-analysis results of these odds ratios show that the athletes were 1.47 (95%CI = 1.15; 1.88) more likely to be a power athlete (Z = 3.05, p = .002).

TABLE 3

ODDS RATIO BASED ON RR GENOTYPE AND NOT RR GENOTYPE IN POWER ATHLETES

| | **RR** | | **RX/XX** | | | |
|---|---|---|---|---|---|---|
| **Study** | **Athletes** | **Total** | **Athletes** | **Total** | **Weight** | **Odds Ratio M-H, Random, 95% CI** |
| Ben-Zaken 2015 | 45 | 93 | 70 | 239 | 6.90% | 2.26 [1.38, 3.71] |
| Eroglu 2018 | 3 | 12 | 19 | 44 | 2.30% | 0.44 [0.10, 1.84] |
| Galeandro 2017 | 21 | 61 | 22 | 110 | 5.40% | 2.10 [1.04, 4.25] |
| Gineviciene 2017 | 67 | 513 | 94 | 850 | 8.10% | 1.21 [0.86, 1.69] |
| Ginszt 2018 | 31 | 64 | 19 | 86 | 5.40% | 3.31 [1.63, 6.72] |

TABLE 3 (continued)

| Study | RR | | RX/XX | | Weight | Odds Ratio M-H, Random, 95% CI |
|---|---|---|---|---|---|---|
| | Athletes | Total | Athletes | Total | | |
| Guilherme 2018 | 136 | 455 | 191 | 836 | 8.70% | 1.44 [1.11, 1.86] |
| Heffernan 2016 | 169 | 412 | 338 | 805 | 8.80% | 0.96 [0.76, 1.22] |
| Honarpour 2017 | 37 | 87 | 53 | 203 | 6.70% | 2.09 [1.24, 3.55] |
| Koku 2019 | 29 | 62 | 71 | 138 | 6.10% | 0.83 [0.45, 1.51] |
| Magi 2016 | 21 | 74 | 37 | 306 | 6.10% | 2.88 [1.56, 5.31] |
| Peplonska 2017 | 74 | 236 | 124 | 413 | 8.10% | 1.06 [0.75, 1.51] |
| Pimjan 2017 | 19 | 53 | 98 | 163 | 5.80% | 0.37 [0.19, 0.71] |
| Salgueirosa 2017 | 18 | 94 | 22 | 152 | 5.50% | 1.40 [0.71, 2.77] |
| Wessner 2016 | 22 | 87 | 34 | 185 | 6.10% | 1.50 [0.82, 2.77] |
| Yang 2017 | 29 | 42 | 30 | 67 | 4.70% | 2.75 [1.22, 6.20] |
| Yusof 2016 | 19 | 59 | 22 | 162 | 5.40% | 3.02 [1.49, 6.13] |
| **Total (95% CI)** | **2404** | | **4759** | | **100.00%** | **1.47 [1.15, 1.88]** |
| Heterogeneity. $Tau^2 = .17$; $Chi^2 = 59.01$, df = 15 ($p < .00001$); $I^2 = 75\%$ | | | | | | |
| Test for overall effect: Z = 3.05 ($p = .002$) | | | | | | |

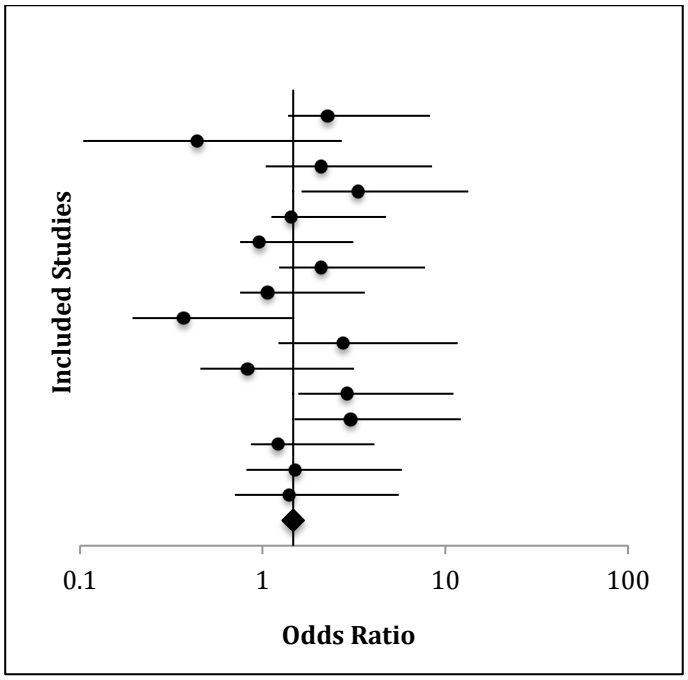


Figure 3. Odds ratios of RR genotype in power athletes and controls versus not RR genotype power athletes.

In addition to the genotype meta-analysis regarding hypothesis number 1 an allele based meta-analysis was conducted. This analysis was to test whether the power oriented athletes were more likely to harbor the R allele than the X allele (Table 4; Figure 4). Results show that the power athletes were 1.26 times more likely to have at least 1 R allele in their genotype (OR = 1.26, 95%CI = 1.05; 1.52, Z = 2.43, p = .02).

TABLE 4

ODDS RATIOS BASED ON MINOR ALLELE X AND MAJOR ALLEL R IN POWER ATHLETES

| | **R** | | **X** | | | |
|---|---|---|---|---|---|---|
| **Study** | **Athlete** | **Total** | **Athlete** | **Total** | **Weight** | **Odds Ratio M-H, Random, 95% CI** |
| Ben-Zaken 2015 | 143 | 368 | 87 | 296 | 7.00% | 1.53 [1.10, 2.12] |
| Eroglu 2018 | 22 | 57 | 22 | 55 | 3.60% | 0.94 [0.44, 2.01] |
| Galeandro 2017 | 59 | 211 | 27 | 131 | 5.20% | 1.50 [0.89, 2.51] |
| Gineviciene 2017 | 210 | 1704 | 112 | 1022 | 7.70% | 1.14 [0.90, 1.46] |
| Ginszt 2018 | 77 | 193 | 23 | 107 | 5.00% | 2.42 [1.41, 4.18] |
| Guilherme 2018 | 411 | 1491 | 243 | 1091 | 8.20% | 1.33 [1.11, 1.59] |
| Heffernan 2016 | 572 | 1395 | 442 | 1039 | 8.30% | 0.94 [0.80, 1.10] |
| Honarpour 2017 | 107 | 322 | 73 | 258 | 6.70% | 1.26 [0.88, 1.80] |
| Koku 2019 | 114 | 230 | 86 | 172 | 6.30% | 0.98 [0.66, 1.46] |
| Magi 2016 | 75 | 422 | 41 | 338 | 6.20% | 1.57 [1.04, 2.36] |
| Peplonska 2017 | 230 | 776 | 146 | 502 | 7.70% | 1.03 [0.80, 1.31] |
| Pimjan 2017 | 92 | 216 | 142 | 216 | 6.40% | 0.39 [0.26, 0.57] |
| Salgueirosa 2017 | 56 | 305 | 24 | 187 | 5.30% | 1.53 [0.91, 2.56] |
| Wessner 2016 | 66 | 298 | 46 | 246 | 6.10% | 1.24 [0.81, 1.88] |
| Yang 2017 | 85 | 131 | 33 | 87 | 4.90% | 3.02 [1.72, 5.31] |
| Yusof 2016 | 55 | 238 | 27 | 204 | 5.40% | 1.97 [1.19, 3.26] |
| **Total (95% CI)** | **8357** | | **5951** | | **100.00%** | **1.26 [1.05, 1.52]** |
| Heterogeneity: $Tau^2 = 0.10$; $Chi^2 = 70.60$, df = 15 ($p < 0.00001$); $I^2 = 79\%$ | | | | | | |
| Test for overall effect: Z = 2.43 ($p = 0.02$) | | | | | | |

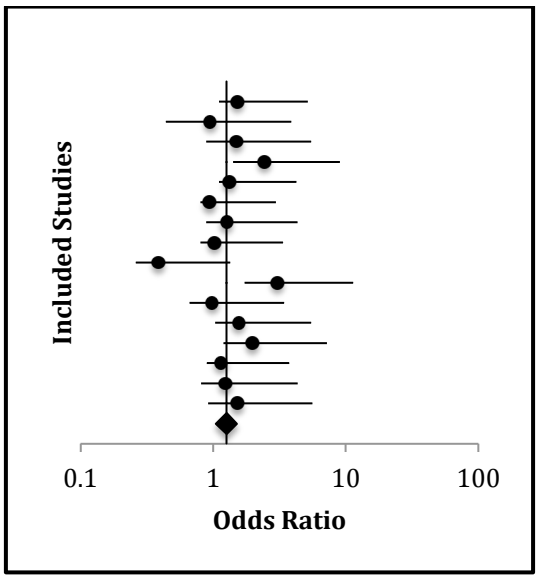


Figure 4. Odds ratio based on R allele and X allele in power athletes.

### 4.3 Hypothesis 2

Hypothesis 2 stated a statistical analysis would show no significant association of the homozygous ACTN3 XX genotype with endurance related sports. Table 5 and figure 5 illustrate the analysis of this hypothesis. An odds ratio was taken for each study included – the odds of being an endurance athlete given you have the XX genotype versus the odds of being a control given the XX genotype (OR = .96, 95%CI = .72; 1.29). The result shows no statistically significant advantage of the XX genotype among endurance athletes (Z = .25, p = .80).

TABLE 5

ODDS RATIO BASED ON THE XX GENOTYPE IN ENDURANCE ATHLETES

| | XX | | RR | | | |
|---|---|---|---|---|---|---|
| **Study** | **Athlete** | **Total** | **Athlete** | **Total** | **Weight** | **Odds Ratio M-H, Random, 95% CI** |
| Ben-Zaken 2015 | 35 | 75 | 31 | 79 | 15.40% | 1.35 [0.71, 2.57] |
| Eroglu 2018 | 4 | 12 | 0 | 9 | 0.90% | 10.06 [0.47, 215.56] |
| Ginszt 2018 | 12 | 29 | 13 | 46 | 7.80% | 1.79 [0.67, 4.77] |
| Guilherme 2018 | 66 | 269 | 95 | 414 | 30.40% | 1.09 [0.76, 1.56] |
| Peplonska 2017 | 25 | 92 | 83 | 245 | 19.90% | 0.73 [0.43, 1.24] |

TABLE 5 (continued)

| Study | XX | | RR | | Weight | Odds Ratio M-H, Random, 95% CI |
|---|---|---|---|---|---|---|
| | Athlete | Total | Athlete | Total | | |
| Wessner 2016 | 13 | 62 | 25 | 90 | 11.70% | 0.69 [0.32, 1.48] |
| Yang 2017 | 14 | 31 | 14 | 27 | 7.10% | 0.76 [0.27, 2.15] |
| Yusof 2016 | 6 | 43 | 14 | 54 | 6.80% | 0.46 [0.16, 1.33] |
| **Total (95% CI)** | **613** | | **964** | | **100.00%** | **0.96 [0.72, 1.29]** |
| Heterogeneity: Tau² = 0.04; Chi² = 9.18, df = 7 (P = 0.24); I² = 24% | | | | | | |
| Test for overall effect: Z = 0.25 (P = 0.80) | | | | | | |

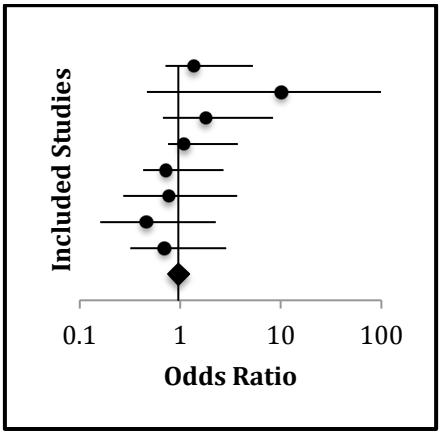


Figure 5. Odds ratio comparing the XX genotype relative to the RR genotype in endurance athletes.

### 4.4 Hypothesis 3

Hypothesis number 3 is in regards to the RX heterozygous genotype in middle distance athletes. The hypothesis was that the statistical analysis would show no significant association of the heterozygous ACTN3 RX genotype with middle distance related sports. Table 6 summarizes the odds ratios for included studies, and figure 6 summarizes the analysis in a forest plot. For this analysis there were only 3 available studies that met the inclusion criteria. After a meta-analysis was carried out the results were that there was no statistically significant association between being a middle distance athlete and harboring the RX genotype (OR = 0.74, 95%CI = 0.38; 1.45, p = .38).

TABLE 6

ODDS RATIOS BASED ON RX GENOTYPE FREQUENCIES IN MIDDLE DISTANCE ATHLETES

| Study | RX | | RR/XX | | Weight | Odds Ratio M-H, Random, 95% CI |
|---|---|---|---|---|---|---|
| | Athlete | Total | Athlete | Total | | |
| Eroglu 2018 | 10 | 27 | 2 | 19 | 12.70% | 5.00 [0.95, 26.31] |
| Li 2017 | 58 | 162 | 102 | 204 | 44.90% | 0.56 [0.37, 0.85] |
| Yusof 2016 | 45 | 148 | 60 | 137 | 42.40% | 0.56 [0.34, 0.91] |
| **Total (95% CI)** | **337** | | **360** | | **100.00%** | **0.74 [0.38, 1.45]** |
| Heterogeneity: $Tau^2 = 0.22$; $Chi^2 = 6.49$, df = 2 (P = 0.04); $I^2 = 69\%$ | | | | | | |
| Test for overall effect: Z = 0.88 (P = 0.38) | | | | | | |

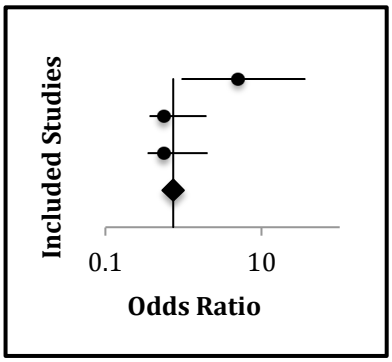


Figure 6. Odds ratio of RX genotype in middle distance athletes.

# CHAPTER 5

# DISCUSSION

The objective of this meta-analysis was to investigate the ACTN3R577 gene polymorphism associations with different types of athlete; specifically, power oriented athletes, endurance athletes, and middle distance athletes. The first hypothesis tested in this study was done on the homozygous RR genotype and its association with power athletes. A total of 16 studies were identified and used for the meta-analysis (Figure 2). The results show that the athletes were 1.46 times more likely to harbor the RR genotype over the XX homozygous genotype (OR = 1.4680, 95%CI = 1.0113; 2.1308, Z = 2.02, p = .04). This result is consistent with previous literature (Weyerstrab, 2016; Yang et al., 2003), which showed a positive association between power oriented athletes and genotype RR. To further analyze this relationship between the RR genotype and power oriented athletes the odds of being a power athlete and having the RR genotype versus the odds of not having the RR genotype was also calculated (Figure 3). The results of this meta-analysis are consistent with the first outcome (OR = 1.47, 95%CI = 1.15; 1.88, Z = 3.05, p = .002). These results suggest that individuals that are power-oriented athletes are statistically more likely to have the RR genotype in comparison to individuals with the XX homozygous genotype and also the heterozygous RX genotyped individuals.

To further analyze the relationship with power oriented athletes and the ACTN3 polymorphism an allele-based meta-analysis was conducted (Figure 4). These results of this analysis were consistent with the first 2 analysis regarding hypothesis 1 and suggests not only having the RR genotype but having just one copy of the R allele might offer a benefit to power oriented athletes.

Hypothesis 2 was in regards to endurance athletes with the XX genotype. The results of this meta-analysis included 8 studies and found no correlation with XX genotype in the endurance athletes (OR = .96, 95%CI = .72; 1.29, Z = .25, p = .8). There are, however, limitations surrounding this study. First, there were only 9 included studies, and each individual study had a relatively low sample size and a large heterogeneity between the study participants. The above limitations suggest further studies investigating the true effect of the XX genotype on athletes are warranted. It is possible that while the X allele and XX genotypes display no functional detriments in the non-athlete, they might not be of neutral function to the athlete. The results from hypothesis 1 and 2 suggest this possibility. In previous studies investigating the X allele and risk of injury the results have shown an increased probability of injury for athletes with the XX genotype (Belli, Crisp and Verlengia, 2017; Pimenta et al., 2012; Del Coso et al., 2017). This is further evidence that the RR genotype might offer an advantage in comparison to the XX genotype.

This information may be useful for genetic testing of athletes in the future. Knowledge of the XX genotype in an athlete might help guide training style or assist in setting required recovery times. Massidda et al., (2017) investigated the X allele and its association with injuries in football players concluding that the X allele did show risk of general injury. If further research continues to replicate similar results then genotyping ACTN3 polymorphism, along with other well-established genes, could be useful for athletes and other health professionals.

Hypothesis 3 of this study investigated the relationship between the heterozygous RX genotype and middle distance athletes. This analysis was limited by the lack of available studies as well as small sample sizes. There were, however, 3 studies investigating this hypothesis that included the necessary data for a meta-analysis. These studies included both track and field

athletes as well as swimmers. Figure 6 includes the results for this analysis, and results show no correlation between the RX genotype and the athletes (OR = .74, 95%CI = .38; 1.45, Z = .88, p = .38). The results of the study by Li et al., (2017), however, showed that the elite middle distance swimmers in China were more likely to have at least one R allele and that the RX genotype was associated with better performance. They also concluded that the RX could be used as a possible biomarker for selecting elite swimmers. These conclusions suggest further research needs to be conducted on this genotype.

# CHAPTER 6

# CONCLUSION

In conclusion, the RR genotype polymorphism was associated favorably with power athletes while the XX and RX genotypes failed to show association with endurance and middle distance athletes respectively. Based on previous literature, included studies of this meta-analysis, and the results of this meta-analysis; the RR genotype and R allele might offer advantages in any type of athletic event whether it is anaerobic or aerobic in nature. Further research needs to be conducted in order to make any comprehensive conclusions on the XX polymorphism and RX polymorphism. There is an undisputed effect genetics have on athletic performance. Emerging research on different genetic polymorphisms and biomarkers indicate a direct and quantifiable relationship with sport success. In the future these genes present the potential opportunity to individualize training guidelines, which will help maximize genetic potential and prevent injury. Further research, however, is needed to develop these guidelines and quantify the effects of genetic information on athletic performance.

# REFERENCES

REFERENCES


Ahmetov, I. I., Donnikov, A. E., & Trofimov, D. Y. (2014). Actn3 genotype is associated with testosterone levels of athletes. *Biology of Sport*, 31(2), 105–108.

Belli, T., Crisp, A. H., and Verlengia, R. (2017). Greater muscle damage in athletes with ACTN3 R577X (RS1815739) gene polymorphism after an ultra-endurance race: a pilot study. *Biology of Sport* 34, 105–110.

Ben-Zaken, S., Eliakim, A., Nemet, D., Rabinovich, M., Kassem, E., & Meckel, Y. (2015). ACTN3 polymorphism: comparison between elite swimmers and runners. *Sports Medicine Open*, 1(1), 13.

Collier, R. (2012). Genetic tests for athletic ability: Science or snake oil?.

DerSimonian R, Laird N. (1986). Meta-analysis in Clinical Trials. *Controlled Clinical Trials*. 7(3), 177-188.

de Macedo Salgueirosa, F., Rodrigues, P., Seniski, G. G., Wharton, L., & Osiecki, R. (2017). ACTN3 R577X and ACE I/D genotype frequencies of professional soccer players in brazil. *Journal of Exercise Physiology Online*, *20*(6), 129-139.

Del Coso, J., Salinero, J. J., Lara, B., Gallo-Salazar, C., Areces, F., Puente, C., et al. (2017a). ACTN3 X-allele carriers had greater levels of muscle damage during a half-ironman. *European Journal of Applied Physiology*. 117, 151–158.

Druzhevskaya, A. M., Ahmetov, I. I., Astratenkova, I. V., & Rogozkin, V. A. (2008). Association of the ACTN3 R577X polymorphism with power athlete status in Russians. *European Journal of Applied Physiology*, 103(6), 631-634.

Eroğlu, O., Zileli, R., Nalbant, M. A., & Ulucan, K. (2018). Prevalence of alpha actinin-3 gene (ACTN3) R577X and angiotensin converting enzyme (ACE) insertion/deletion gene polymorphisms in national and amateur Turkish athletes. *Cellular and Molecular Biology*, 64(5) 24-28.

Fridén, J., & Lieber, R. L. (2001). Eccentric exercise-induced injuries to contractile and cytoskeletal muscle fibre components. *Acta Physiologica Scandinavica*, 171(3), 321–326.

Galeandro, V., Notarnicola, A., Bianco, A., Tafuri, S., Russo, L., Pesce, V., ... & Petruzzella, V. (2017). ACTN3/ACE genotypes and mitochondrial genome in professional soccer players performance. *Journal of Biological Regulators and Homeostatic Agents*, *31*(1), 207-213.

Gineviciene, V., Jakaitiene, A., Aksenov, M. O., Aksenova, A. V., Astratenkova, A. D. I., Egorova, E. S., ... & Utkus, A. (2016). Association analysis of ACE, ACTN3 and PPARGC1A gene polymorphisms in two cohorts of European strength and power athletes. *Biology of Sport*, *33*(3), 199.

Ginszt, M., Michalak-Wojnowska, M., Gawda, P., Wojcierowska-Litwin, M., Korszeń-Pilecka, I., Kusztelak, M., ... & Majcher, P. (2018). ACTN3 genotype in professional sport climbers. *Journal of Strength and Conditioning Research*, *32*(5), 1311.

Grealy, R., Smith, C. L. E., Chen, T., Hiller, D., Haseler, L. J., & Griffiths, L. R. (2013). Original research: The genetics of endurance: Frequency of the ACTN3 R577X variant in Ironman World Championship athletes. *Journal of Science and Medicine in Sport*, 16, 365–371.

Griffiths, A. J. F., Wessler, S. R., Carroll, S. B., Doebley, J. (2012). *Introduction to genetic analysis*. New York, NY. W.H. Freeman and Company.

Guilherme, J. P. L. F., Bertuzzi, R., Lima-Silva, A. E., Pereira, A. D. C., & Lancha Junior, A. H. (2018). Analysis of sports-relevant polymorphisms in a large Brazilian cohort of top-level athletes. *Annals of Human Genetics*, *82*(5), 254-264.

Heffernan, S. M., Kilduff, L. P., Erskine, R. M., Day, S. H., McPhee, J. S., McMahon, G. E., ... & Cook, C. J. (2016). Association of ACTN3 R577X but not ACE I/D gene variants with elite rugby union player status and playing position. *Physiological Genomics*, 48(3), 196-201.

Honarpour, A., Mohseni, M., Ghavidel Hajiagha, S., Irani, S., & Najmabadi, H. (2017). Investigation of the Relationship Between a Genetic Polymorphism in ACTN3 and Elite Sport Performance Among Iranian Soccer Players. *Iranian Rehabilitation Journal*, 15(2), 149-154.

Kikuchi, N., Zempo, H., Fuku, N., Murakami, H., Sakamaki-Sunaga, M., Okamoto, T., ... & Miyachi, M. (2017). Association between ACTN3 R577X Polymorphism and trunk flexibility in 2 different cohorts. *International Journal of Sports Medicine*, 38(05), 402-406.

Koku, F. E., Karamızrak, S. O., Çiftçi, A. S., Taşlıdere, H., Durmaz, B., & Çoğulu, Ö. (2019). The relationship between ACTN3 R577X gene polymorphism and physical performance in amateur soccer players and sedentary individuals. *Biology of Sport*, 36(1), 9.

Li, Y. C., Wang, L. Q., Yi, L. Y., Liu, J. H., Hu, Y., Lu, Y. F., & Wang, M. (2017). ACTN3 R577X genotype and performance of elite middle-long distance swimmers in China. *Biology of Sport*, 34(1), 39.

MacArthur, D. G., & North, K. N. (2004). A gene for speed? The evolution and function of α-actinin-3. *Bioessays*, 26(7), 786-795.

Mägi, A., Unt, E., Prans, E., Raus, L., Eha, J., Veraksitš, A., ... & Kõks, S. (2016). The association analysis between ACE and ACTN3 genes polymorphisms and endurance capacity in young cross-country skiers: longitudinal study. *Journal of Sports Science & Medicine*, 15(2), 287.

Massidda, M., Voisin, S., Culigioni, C., Piras, F., Cugia, P., Yan, X., ... & Calò, C. M. (2017). ACTN3 R577X Polymorphism Is Associated With the Incidence and Severity of Injuries in Professional Football Players. *Clinical Journal of Sport Medicine: Official Journal of the Canadian Academy of Sport Medicine*, 29(1), 57-61.

Neyeloff, J. L., Fuchs, S. C., & Moreira, L. B. (2012). Meta-analyses and Forest plots using a microsoft excel spreadsheet: step-by-step guide focusing on descriptive data analysis. *BioMedCentral Research Notes*, 5(1), 52.

Niemi, A. K., & Majamaa, K. (2005). Mitochondrial DNA and ACTN3 genotypes in Finnish elite endurance and sprint athletes. *European Journal of Human Genetics*, 13(8), 965.

Papadimitriou, I. D., Lockey, S. J., Voisin, S., Herbert, A. J., Garton, F., Houweling, P. J., … Eynon, N. (2018). No association between ACTN3 R577X and ACE I/D polymorphisms and endurance running times in 698 Caucasian athletes. *BioMedCentral Genomics*, 19(1), 13.

Peplonska, B., Adamczyk, J. G., Siewierski, M., Safranow, K., Maruszak, A., Sozanski, H., ... & Zekanowski, C. (2017). Genetic variants associated with physical and mental characteristics of the elite athletes in the Polish population. *Scandinavian Journal of Medicine & Science in Sports*, 27(8), 788-800.

Pickering, C., & Kiely, J. (2017). ACTN3: More than just a gene for speed. *Frontiers in Physiology*, 8, 1080.

Pimenta, E. M., Coelho, D. B., Cruz, I. R., Morandi, R. F., Veneroso, C. E., de Azambuja Pussieldi, G., ... & Fernández, J. A. D. P. (2012). The ACTN3 genotype in soccer players in response to acute eccentric training. *European Journal of Applied Physiology*, 112(4), 1495-1503.

Pimjan, L., Ongvarrasopone, C., Chantratita, W., Polpramool, C., Cherdrungsi, P., Bangrak, P., & Yimlamai, T. (2017). A study on ACE, ACTN3, and VDR genes polymorphism in Thai weightlifters. *Walailak Journal of Science and Technology*, 15(9), 609-626.

Powers, S. K., & Howley, E. T. (2007). *Exercise physiology: Theory and application to fitness and performance* (pp. 303-308). New York, NY. McGraw-Hill.

Scott, R. A., Irving, R., Irwin, L., Morrison, E., Charlton, V., Austin, K., ... & Yang, N. (2010). ACTN3 and ACE genotypes in elite Jamaican and US sprinters. *Medicine and Science in Sports and Exercise*, 42(1), 107-112.

Scott, W., Stevens, J., & Binder–Macleod, S. A. (2001). Human skeletal muscle fiber type classifications. *Physical Therapy*, 81(11), 1810-1816.

Stewart, C. E. H., & Rittweger, J. (2006). Adaptive processes in skeletal muscle: molecular regulators and genetic influences. *Journal of Musculoskeletal and Neuronal Interactions*, 6(1), 73.

Tucker, R., & Collins, M. (2012). What makes champions? A review of the relative contribution of genes and training to sporting success. *British Journal of Sports Medicine*, 46(8), 555-561.

Vincent, B., De Bock, K., Ramaekers, M., Van den Eede, E., Van Leemputte, M., Hespel, P., & Thomis, M. A. (2007). ACTN3 (R577X) genotype is associated with fiber type distribution. *Physiological Genomics*, 32(1), 58-63.

Wessner, B., Stuparits, P., Fail, C., Pavic, F., Tschan, H., & Bachl, N. (2016). Genetic polymorphisms in alpha-actinin 3 and adrenoceptor beta genes in Austrian elite athletes and healthy controls. *Schweizerische Zeitschrift für Sportmedizin & Sporttraumatologie*, 64(4).

Weyerstraß, J., Stewart, K., Wesselius, A., & Zeegers, M. (2017). Nine genetic polymorphisms associated with power athlete status–a meta-analysis. *Journal of science and medicine in sport*, 21(2), 213-220.

Yang, J., Benyamin, B., McEvoy, B. P., Gordon, S., Henders, A. K., Nyholt, D. R., ... & Goddard, M. E. (2010). Common SNPs explain a large proportion of the heritability for human height. *Nature Genetics*, 42(7), 565.

Yang, N., MacArthur, D. G., Gulbin, J. P., Hahn, A. G., Beggs, A. H., Easteal, S., & North, K. (2003). ACTN3 genotype is associated with human elite athletic performance. *The American Journal of Human Genetics*, 73(3), 627-631.

Yang, N., Macarthur, D. G., Wolde, B., Onywera, V. O., Boit, M., Lau, S. Y. M. A., ... & North, K. N. (2007). The ACTN3 R577X polymorphism in East and West African athletes. *Medicine and Science in Sports and Exercise*, 39(11), 1985-1988.

Yang, R., Shen, X., Wang, Y., Voisin, S., Cai, G., Fu, Y., ... & Yan, X. (2017). ACTN3 R577X gene variant is associated with muscle-related phenotypes in elite Chinese Sprint/power athletes. *Journal of Strength and Conditioning Research*, 31(4), 1107-1115.

Yusof, H. A., Singh, R., Zainuddin, Z., Rooney, K., & Muhamed, A. M. C. (2016). Alpha-Actinin-3 (ACTN3) R/X Gene Polymorphism and Physical Performance of Multi-Ethnic Malaysian Population. *International Journal of Applied Exercise Physiology*, *5*(3), 18-30.