The Science Behind the ACTN3 Polymorphism

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Abstract

A common polymorphism in the alpha-actinin-3 (ACTN3) gene results in the lack of ACTN3 protein expression in fast twitch muscle fibers in ~16% of the human population (1). This genetic change has been linked with muscle performance in humans (2) but does not cause any known muscle disease (1). We have developed a series of laboratory modules that provide an authentic classroom research experience and which address the connection between science and society by examining the implications of ACTN3 genetic testing to improve sports training and performance. This article accompanies the lesson “The ACTN3 Polymorphism: Applications in Genetics and Physiology Teaching Laboratories,” and summarizes background information that an instructor would need to implement the project in class.

INTRODUCTION

In humans, athletic performance is a complex trait influenced both by environmental factors, such as diet and training, as well as heritable genetic factors. However, the relative contribution of environment and genetics, as well as the interaction between the two, is not entirely understood. Many genes have been associated with human athletic performance in various studies; this review focuses on the human alpha-actinin-3 (ACTN3) gene, which encodes the protein alpha-actinin-3 (ACTN3). The ACTN3 protein is expressed in fast-twitch muscle fibers, which are primarily responsible for rapid and forceful contractions during sprint and power activities (1,3). A common genetic variant in the gene, in which the ancestral C nucleotide is replaced by T, results in a change from arginine (R) to a stop codon (X) at amino acid position 577 (R577X). The R577X allele prevents the production of functional ACTN3 protein (1,4,5).

GENETICS OF ACTN3

Alleles and dominance

Both variants of ACTN3 are common in the general population, with approximately 60% of the global population carrying the C allele (which encodes functional ACTN3) and 40% carrying the T allele (which encodes non-functional ACTN3) (Table 1). Every human inherits two copies of the ACTN3 gene, one maternal and one paternal. Therefore, there are three possible combinations, or genotypes, at this locus: the C/C or RR genotype, in which both copies of the ACTN3 protein are functional; the C/T or RX genotype, in which only one copy of the ACTN3 protein is functional; and the T/T or XX genotype, in which both copies of the ACTN3 are non-functional. Throughout this review article, we follow the convention in the scientific literature of designating the functional ACTN3 genotype as RR or RX, and the non-functional ACTN3 genotype as XX. It should be noted that XX refers to two premature stop codons in the ACTN3 protein, not genetic female sex chromosomes.

The dominance relationship among the R allele and the X allele is not fully understood, as the gene affects many different phenotypes related to athletic function. However, several human association studies of muscle performance support an additive, or incompletely dominant, model in which heterozygous individuals exhibit intermediate phenotypes for the traits studied (2,6). Similar trends have also been observed in studies of mouse models: heterozygous RX mice showed intermediate muscle mass and endurance capacity relative to RR and XX mice (7).

| Population          | C    | T    | n   |
|---------------------|------|------|-----|
| African             | 0.885| 0.115| 1322|
| Admixed American    | 0.428| 0.572| 694 |
| East Asian          | 0.557| 0.444| 1008|
| European            | 0.566| 0.434| 1006|
| South Asian         | 0.413| 0.587| 978 |
| Global              | 0.599| 0.401| 5008|

Table 1. Allele Frequency by Population, 1000 Genomes Project

Population genetics

Among different regional populations, the frequency distribution of alleles and genotypes is variable at the ACTN3 locus. For example, over 85% of individuals in African populations harbor the ancestral C allele, whereas nearly 60% of South Asian individuals carry the non-functional T allele (Table 1). Remarkably, approximately ~16% of the global population carries two non-functional alleles of ACTN3 (XX...
genotype), which results in a complete deficiency of ACTN3 protein in skeletal muscle (8). However, this frequency is as high as 28% in individuals of South Asian descent (Table 2). The majority of individuals in African populations (~75%) have two copies of the functional R allele (RR genotype) whereas most other populations have 15-30% of individuals with this genotype (Table 2). More information on the ACTN3 polymorphism can be found in the National Center for Biotechnology Information Single Nucleotide Polymorphism Database (NCBI dbSNP, https://www.ncbi.nlm.nih.gov/snp/) under SNP identification number rs1815739.

### Table 2. Genotype Frequency by Population, HapMap Project

| Population          | C/C | C/T | T/T | n  |
|---------------------|-----|-----|-----|----|
| African             | 0.747 | 0.241 | 0.013 | 790 |
| Admixed American    | 0.120 | 0.600 | 0.280 | 100 |
| East Asian          | 0.341 | 0.455 | 0.204 | 510 |
| European            | 0.288 | 0.512 | 0.200 | 972 |
| South Asian         | 0.138 | 0.586 | 0.276 | 174 |
| Global              | 0.424 | 0.425 | 0.151 | 2546 |

The fact that nearly ~16% of the world’s population (over one billion humans) lacks a functional skeletal muscle ACTN3 protein but does not experience any obvious muscle disease suggests that other proteins can compensate for the loss of ACTN3. This functional redundancy is most likely accomplished by the closely related protein alpha-actinin-2 (ACTN2) (9).

**ACTN3: IMPLICATIONS FOR A ROLE IN ATHLETIC PERFORMANCE**

Because of its role in skeletal muscle function and its frequency variation among different populations, many studies have indicated that ACTN3 may contribute to variation in human muscle performance. In particular, some researchers have hypothesized that ACTN3 deficiency primarily affects the function of fast twitch muscle fibers, and thus would likely have a greater influence in athletes competing in sprint or power activities, rather than endurance athletes whose performance relies more heavily on slow twitch muscle fibers (2,4,5).

To investigate this hypothesis, the association between ACTN3 genotype and athletic performance has been examined in many different populations. In one study, the frequencies of R577X alleles in elite athletes were compared to those of a group of non-athlete (sedentary) controls. Yang et al. identified a significant association between ACTN3 genotype and athletic performance: elite sprint athletes had significantly higher frequencies of the R allele (functional ACTN3) than a control population (5). Most other studies of elite athletes versus controls support the correlation between RR genotype and elite sprint or power athlete status (2). Although there has been some suggestion that the XX genotype is associated with endurance performance, data on this aspect are less robust (10-16).

Several other studies have examined the relationship between ACTN3 genotype and a variety of muscle functions in non-athlete populations (8,17,18), which are described in more detail below. Interestingly, across many of these studies the strength of association between ACTN3 genotype and muscle performance is different between genetic females and genetic males, further emphasizing the complex nature of this trait.

**MUSCLE PHYSIOLOGY: WHAT YOU NEED TO KNOW TO UNDERSTAND THE ROLE OF ACTN3**

### Cellular organization

Muscle fibers consist of single muscle cells or myocytes. Myocytes are multinucleated, surrounded by the sarcolemma, and fuse with a tendon at each end of the fiber. Myofibrils make up the muscle fibers and are contractile elements surrounded by the sarcoplasm. Cellular organelles such as mitochondria and the sarcoplasmic reticulum lie between myofibrils (Figures 10.3 and 10.4 at https://cnx.org/contents/FPtK1zmh@15.1-bfiqsxdB@6/10-2-Skeletal-Muscle).

### Molecular organization

The myofibril contractile elements are composed of myofilaments, which are primarily made up of the proteins actin and myosin. The sarcomere is the functional unit of the myofibril and is composed of thick and thin filaments (myosin and actin, respectively) and the filamentous structural protein titin (connectin). The sarcomeres are what give skeletal muscle its striated appearance due to the banding pattern visible by light microscopy. The sarcomere is made up of a complete A-band (myosin thick filaments) and the two halves of the I-bands (actin thin filaments) adjacent to it. The Z line (Z disc) demarcates the borders of the sarcomere and is in the middle of an I-band (Figures 10.4 and 10.5 at https://cnx.org/contents/FPtK1zmh@15.1-bfiqsxdB@6/10-2-Skeletal-Muscle).

### Structure and function

Skeletal muscle contraction results from the sliding action of interdigitating actin and myosin filaments. During contraction every sarcomere shortens, as do the myofibrils and the entire muscle cell. The myofilaments do not shorten; rather they slide by each other, overlapping as the I-bands and the H-zone (within the A-band) shorten, causing the Z lines to come closer together. The ACTN3 protein is a structural component of the Z line, therefore connecting with actin filaments and playing a role in coordinating myofilament contraction (Figure 10.5 at https://cnx.org/contents/FPtK1zmh@15.1-bfiqsxdB@6/10-2-Skeletal-Muscle).

### Muscle fiber types and ACTN3 expression and function

Skeletal muscle consists of two major types of fibers: Type I and Type II. Type I, or slow twitch fibers, have a high resistance to fatigue, increased oxidative metabolism, and are better for endurance activities. Type II, or fast twitch fibers, have low resistance to fatigue, primarily anaerobic metabolism, and an increased ability to generate speed and force. The alpha-actinins (ACTN2 and ACTN3) are structural components of the Z-line in skeletal muscle. The expression of the ACTN2 gene is ubiquitous in skeletal muscle while the ACTN3 gene is expressed only in Type II (fast twitch) muscle fibers where it has been shown to play roles in structural, metabolic, signaling, and calcium handling properties. While no skeletal muscle phenotype has been documented in relation to ACTN2 mutation, ACTN3 deficiency disrupts the aforementioned...
aspects of fast twitch fiber function, causing these fibers to behave more like slow twitch fibers (19).

**MUSCLE FUNCTIONS POTENTIALLY AFFECTED BY ACTN3 GENOTYPE**

A number of muscle-related phenotypes have been studied in relation to ACTN3 genotype including maximal oxygen consumption (VO\textsubscript{2max}), jumping ability, grip strength, and muscle fatigue.

**VO\textsubscript{max}**

Slow twitch muscle fibers are red due to a high capillary density, have high myoglobin levels resulting in increased oxygen binding capacity, contain high numbers of mitochondria, and show increased levels of oxidative metabolic enzymes (20). Therefore, individuals with a higher number of slow twitch fibers have improved endurance and higher VO\textsubscript{2max} (21). VO\textsubscript{2max} is defined as the maximal volume of oxygen consumed by the body during incremental exercise. VO\textsubscript{2max} is a complex phenotype dependent on both oxygen delivery (lung & heart function, blood composition, circulation) and utilization (metabolism in target cells, primarily muscle during exercise).

Based on the evidence that fast twitch muscle fibers behave more like slow twitch muscle fibers in ACTN3-deficient individuals, some researchers have hypothesized that ACTN3-deficient (XX) individuals have higher VO\textsubscript{2max} than individuals who express functional ACTN3 (RX or RR). A number of published studies have tested this hypothesis with inconsistent results. A study done on male professional soccer players from Brazil indicated that VO\textsubscript{2max} was higher in XX individuals than in RR individuals (22). In addition, a study on non-professional athletes had similar results. In a population of male police recruits, XX individuals had higher VO\textsubscript{2max} levels than RR individuals both before and after endurance training (23). Additional studies on male endurance cyclers and runners, elderly women, and male and female university students did not indicate any significant differences in VO\textsubscript{2max} levels by ACTN3 genotype (24-26), but one study on male and female university students indicated a higher VO\textsubscript{2max} in RR individuals compared to XX individuals (27). Among the studies that did report significantly increased VO\textsubscript{2max} in RR individuals, a small effect was observed. For example, untrained male police recruits with the RR genotype had an average VO\textsubscript{2max} of 47.2 +/- 7.5 mL/kg/min as compared to 52.8 +/- 6.2 mL/kg/min for the XX genotype. After training, RR male police recruits had an average VO\textsubscript{2max} of 52.6 +/- 5.9 mL/kg/min, compared to 56.1 +/- 5.6 mL/kg/min. for XX individuals (23), similar to the values reported for the male professional soccer players (22). Therefore, it is likely that ACTN3 genotype contributes only a small amount to oxygen consumption, if at all.

**Jump height**

It has been demonstrated in previous studies that individuals are able to jump higher in a countermovement jump (CMJ) than in other types of jumps (28). During a CMJ, the individual starts from an upright standing position, makes a preliminary downward movement by flexing at the knees and hips, and then immediately extends the knees and hips again to jump vertically off the ground. The greater jump height associated with the CMJ has been attributed to the idea that the countermovement during flexion of the knees and hips allows individuals to achieve greater joint movements at the start of the push-off (28). Specifically, Bobbert et al. (28) demonstrated that the preparatory countermovement allows muscles to build up a high level of active-state myosin-actin cross-bridges, and therefore the muscles are able to produce more force.

Jumping performance has been studied previously in relation to the ACTN3 R577X polymorphism. Orysiak, et al. (14) studied Polish male athletes and used the CMJ to indirectly measure lower limb power. In this study it was demonstrated that subjects with the RR genotype had significantly higher jump heights than subjects with the XX genotype. Further studies of Polish athletes of various sports also demonstrated RR genotype subjects had significantly greater power output during a spike jump test compared to XX individuals (29). An additional study performed in non-athletic young men showed similar results, where men with the RR genotype jumped 5% higher during CMJ tests (30).

**Grip strength**

Grip strength is defined as the force applied by the hand to pull on or suspend from objects and can be used as an estimator for whole body strength. Grip strength requires the use of fast-twitch (type II) muscle fibers responsible for generating rapid and powerful contractions that are less resistant to fatigue. Broos et al. (30) demonstrated a significant difference in grip strength between individuals with the RR genotype and those with the XX genotype (RR genotype had 6% improved grip strength). These findings also confirm what has been demonstrated in mouse models. Previous studies investigating the forelimb grip strength of Actn3 knockout (Actn3 +/- (KO)) mice, Actn3 heterozygous (Actn3 +/- (HET)) mice, and Actn3 homozygous (Actn3 +/- (WT)) mice (note: mouse gene symbols only capitalize the first letter compared to human gene symbols, which use all upper-case letters. Therefore, in humans the gene symbol is ACTN3, whereas in mice it is Actn3), showed that the Actn3 genotype significantly affected grip strength (7). Specifically, Actn3 KO mice displayed significantly reduced grip strength compared with HET or WT mice (7).

**Muscle fatigue**

The ACTN3 R577X polymorphism may negatively impact the performance of type II muscle fibers that are attributable to muscle mass, force generation, and resistance to fatigue (5). Additionally, activities that require endurance and have been more closely associated with ACTN3 deficiency require more fatigue-resistant slow-twitch muscle fiber activity (31). While fewer studies have examined resistance to fatigue and its association with ACTN3 genotype, Broos et al. (30) studied the fatigue index of a group of healthy young men. The fatigue index is a measure of endurance and defined as the rate at which power declines. This study demonstrated that individuals with the XX genotype had a significantly lower fatigue index, meaning these individuals took a longer time to fatigue and lose power/strength (30). These observations suggest that the improved fatigue index of XX individuals may be beneficial for endurance activities, such as rowing, swimming or long distance (10,11).
CONCLUSION

The R577X variant of ACTN3 prevents the production of functional ACTN3 protein in fast twitch muscle fibers. In humans who do not express ACTN3 (XX), fast twitch muscle fibers behave more like slow twitch muscle fibers, and many studies have tested the effect of ACTN3 deficiency on various muscle-related functions including maximal oxygen consumption (VO\textsubscript{max}), jumping ability, grip strength and muscle fatigue. Other studies have also examined the correlation of ACTN3 genotype and athletic performance in sprint or endurance activities. The findings in these studies are frequently heterogeneous, emphasizing the complex nature of this trait.

In this article, we have provided instructors with information and references related to ACTN3 genetics, muscle physiology, and muscle functions potentially affected by ACTN3 genotype. These topics encompass background information to accompany the lesson “The ACTN3 Polymorphism: Applications in Genetics and Physiology Teaching Laboratories.” The link to this lesson is https://www.coursesource.org/courses/the-actn3-polymorphism-applications-in-genetics-and-physiology-teaching-laboratories.

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