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John P. Wagle  
*East Tennessee State University*

Kevin M. Carroll  
*East Tennessee State University*

Aaron J. Cunanan  
*East Tennessee State University*

Alexander Wetmore  
*East Tennessee State University*

Christopher Taber  
*Sacred Heart University, taberc@sacredheart.edu*

*See next page for additional authors*

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Preliminary Investigation into the Effect of ACTN3 and ACE Polymorphisms on Muscle and Performance Characteristics

John P. Wagle,1 Kevin M. Carroll,1 Aaron J. Cunan,1 Alexander Wetmore,1 Christopher B. Taber,2 Brad H. DeWeese,1 Kimitake Sato,1 Charles A. Stuart,3 and Michael H. Stone1

1Department of Sport, Exercise, Recreation, and Kinesiology, Center of Excellence for Sport Science and Coach Education, East Tennessee State University, Johnson City, Tennessee; 2Department of Physical Therapy and Human Movement Science, Sacred Heart University, Fairfield, Connecticut; and 3Department of Internal Medicine, Quillen College of Medicine, East Tennessee State University, Johnson City, Tennessee

Abstract

Wagle, JP, Carroll, KM, Cunan, AJ, Wetmore, A, Taber, CB, DeWeese, BH, Sato, K, Stuart, CA, and Stone, MH. Preliminary investigation into the effect of ACTN3 and ACE polymorphisms on muscle and performance characteristics. J Strength Cond Res XX(X): 000–000, 2018—The purpose of this investigation was to explore the phenotypic and performance outcomes associated with ACTN3 and ACE polymorphisms. Ten trained men (age = 25.8 ± 3.0 years, height = 183.3 ± 4.1 cm, body mass = 92.3 ± 9.3 kg, and back squat to body mass ratio = 1.8 ± 0.3) participated. Blood samples were analyzed to determine ACTN3 and ACE polymorphisms. Standing ultrasonography images of the vastus lateralis (VL) were collected to determine whole muscle cross-sectional area (CSA-M), and a percutaneous muscle biopsy of the VL was collected to determine type I–specific CSA (CSA-T1), type II–specific CSA (CSA-T2), and type II to type I CSA ratio (CSA-R). Isometric squats were performed on force platforms with data used to determine peak force (IPF), allometrically scaled peak force (IPFa), and rate of force development (RFD) at various timepoints. One repetition maximum back squats were performed, whereby allometrically scaled dynamic strength (DSa) was determined. Cohen’s d effect sizes revealed ACTN3 RR and ACE DD tended to result in greater CSA-M but differ in how they contribute to performance. ACTN3 RR’s influence seems to be in the type II fibers, altering maximal strength, and ACE DD may influence RFD capabilities through a favorable CSA-R. Although the findings of the current investigation are limited by the sample size, the findings demonstrate the potential influence of ACTN3 and ACE polymorphisms on isometric and dynamic strength testing. This study may serve as a framework to generate hypotheses regarding the effect of genetics on performance.

Key Words fiber type, genetics, genotype, muscle size, strength, rate of force development

Introduction

Athletic potential and performance outcomes are believed to be the result of a combination of several factors related to training and recovery strategies. Genotype, however, is likely the largest contributor to athletic potential and performance, with heritability estimated to be responsible for as much as 66% of performance (7). Human gene mapping has been especially insightful in the identification of candidate genes related to certain phenotypic characteristics. Two of the most extensively explored in athletics are the α-actinin-3 (ACTN3) and angiotensin-converting enzyme (ACE) genes. ACTN3 encodes for the skeletal muscle α-actinin-3, which is expressed predominantly in sarcomeres of fast-twitch, glycolytic muscle fibers (8,16). The expressed protein is believed to enhance structural integrity of the Z-line within these sarcomeres, consequently enhancing its force production capabilities. A polymorphism of the ACTN3 gene that may influence the performance outcomes occurs at amino acid 577 (16). The replacement of arginine (R) with a stop codon (X) at that location within chromosome 11 creates the most notable gene variants pertaining to strength-power performance outcomes (6). The R allele and the RR polymorphism have well-established relationships to strength-power performance outcomes in a variety of populations, including soccer players (24), rowers (6), and sprinters (21). Furthermore, in ACTN3 knockout mouse models, a decreased fiber-
specific cross-sectional area (CSA) was observed in type II fibers with a concomitant reduction in strength (17).

ace has several polymorphic sites, but of interest are the presence (insertion, I allele) or absence (deletion, D allele) of a 287-base pair (bp) Alu element fragment at intron 16. Fragment absence, the D allele, has been most associated with strength-power related phenotype (13), particularly in sprinters (20). This may be due to the increased localized ACE activity within the muscle observed in the presence of the D allele, ultimately leading to a greater conversion of angiotensin I to angiotensin II. A greater amount of angiotensin II has been associated with cell growth in endothelial, cardiac, and vascular smooth muscle cells. Because of the recently increasing evidence of localized renin-angiotensin systems within the muscle, it is possible that the D allele is associated with increased muscle growth, which would be advantageous for strength-power athletes (5).

The observed outcomes of certain ACTN3 and ACE polymorphisms within the context of sport performance primarily address prevalence within certain athletic populations and the implications for talent identification. Few studies address the specific effects of polymorphisms on mechanistic strength-related characteristics (9,10). Although valuable, these investigations often focused on the untrained (3,9,10) or elderly (19). Therefore, there is a gap in the current literature exploring the potential effect of various polymorphisms of these 2 candidate genes have on mechanistic physical outputs—especially considering trained, strong subjects. Furthermore, to the authors’ knowledge, no study has simultaneously examined the influence that ACTN3 and ACE polymorphisms have on muscle characteristics. Therefore, the purpose of this investigation was to explore the phenotypic physiological and performance outcomes associated with the respective ACTN3 and ACE polymorphisms in trained subjects. Specifically, the authors aimed to provide a rationale for further investigation of (a) the potential effect that ACTN3 and ACE polymorphisms have on whole muscle and fiber-specific characteristics and (b) the effect that ACTN3 and ACE polymorphisms have on isometric and dynamic performance capabilities.

**Methods**

**Experimental Approach to the Problem**

To explore the phenotypic physiological and performance outcomes associated with ACTN3 and ACE polymorphisms, subjects were asked to complete a testing series beginning with a whole blood draw, which would eventually be used for genotyping. Immediately thereafter, subjects completed standing ultrasonography measurements and a one-time subcutaneous muscle biopsy—both of the vastus lateralis (VL). After 48 hours of rest, subjects returned to complete isometric squat (ISQ) testing performed on dual force platforms to assess isometric strength and rate of force development (RFD) capabilities. Finally, subjects completed a 1 repetition maximum (1RM) back squat after another 48-hour rest period.

**Subjects**

Ten well-trained men (mean ± SD; age = 25.8 ± 3.0 years, height = 183.3 ± 4.1 cm, body mass = 92.3 ± 9.3 kg, and back squat to body mass ratio = 1.8 ± 0.3) volunteered for the current investigation. Subjects, most of whom were former athletes including Division I and professional status, were required to have spent at least the past year engaging in a strength training program that included back squats. Each subject’s hydration status (urinary specific gravity) was determined using a refractometer (Atago, Tokyo, Japan) before any data collection to ensure that hydration status would not influence the results. All subjects read and signed a written informed consent form, and the procedures were approved by East Tennessee State University’s Institutional Review Board.

**Procedures**

**Genotyping**

A 10-ml blood sample was drawn into 2 separate 4-ml EDTA tubes (BD Vacutainer K2 EDTA; Franklin Labs, NJ, USA) by venipuncture from certified personnel. The whole blood samples were stored at –80 °C until subsequent analysis. Automated DNA extraction was performed using the manual processing protocol of the QIAaamp DNA Blood Mini Kit (Qiagen, Crawley, United Kingdom). Real-time polymerase chain reaction (PCR) was performed to determine the genotype of the ACTN3 and ACE polymorphisms in each subject, with reactions performed on 96-well microtiter plates. Each 50 μl of reaction volume contained 25-μl Platinum SuperFi PCR Master Mix (ThermoFisher, Waltham, MA, USA), 10-μl 5X SuperFi GC Enhancer (ThermoFisher, Waltham, MA, USA), 2.5 μl of both the respective forward and reverse primers for ACTN3 and ACE, and 12.5 μl of subject DNA combined with nuclease-free water at a concentration of approximately 250 ng·μl⁻¹.

For ACTN3, the 290-bp fragment of exon 15 was amplified using the forward primer CTGTTGCCCTGTGTAAGTGGG and the reverse primer TGGTCACAGTATGCAGGAGGG. Polymerase chain reaction was performed for 35 cycles (30 seconds of denaturation at 94 °C, 30 seconds of annealing at 65 °C, and 60 seconds of extension at 72 °C), final extension at 72 °C for 5 minutes, and held at 4 °C. Amplified products were then electrophoresed on 0.5% agarose gel stained with ethidium bromide to confirm primer adherence. Samples were then purified using QIAquick PCR Purification Kit (Qiagen, Calgary, United Kingdom). After purification, ACTN3 polymorphisms were determined using an automated DNA sequencer (CEQ 8000 Genetic Analysis System; Beckman Coulter, Indianapolis, IN, USA).

The ACE PCR amplification followed identical procedures of those used for ACTN3 except for the substitution of specific primers for ACE—the forward primer CGTGAGCACCATCCCATCCTTTCT and reverse primer GATGTGGCCATCA-CATTGTCAGA. To determine polymorphism, amplified products were electrophoresed and visualized by using agarose

![Image](https://via.placeholder.com/150)
gels stained with ethidium bromide. The products were assessed for the presence of a 490 bp fragment (I allele), a 190 bp fragment (D allele), or both (I/D heterozygote) (Figure 1). Genotyping was performed in accordance with published genotyping and quality control recommendations including external control samples and internal controls of genotyping samples in duplicates (23,29).

**Muscle Biopsy Sampling and Processing.** Immediately after ultrasonography and blood draw procedures, all subjects received a one-time percutaneous biopsy. Biopsies of the superficial region of right VL at a depth of approximately 3 cm were obtained using the Bergström (2) technique and a 5-mm biopsy needle with suction with 1% lidocaine as a local anesthetic. A portion of the muscle tissue was immediately mounted on cork under a microscope to orient the specimen for transverse sectioning, frozen in a slurry of isopentane cooled by liquid nitrogen, and stored at −80°C until subsequent processing. The samples were sectioned on a cryostat (Leica, Wetzlar, Germany) at a thickness of 14 μm and affixed to a microscope slide in preparation for immunohistochemical analysis.

After sectioning and mounting, tissues were fixed with acetone at −20°C for 2 sets of 5 minutes each. All samples were then blocked for 2 hours in a 10% normal goat serum. Sections were incubated overnight in monoclonal antibodies specific to myosin heavy chain (MYH) isoforms: MYH2 for type IIA fibers (IgG1, 1:100 dilution) and MYH7 for type I fibers (IgG2b, 1:200 dilution) (Developmental Studies Hybridoma Bank; University of Iowa, Iowa City, IA, USA). Finally, samples were then incubated for 2 hours using goat antimouse AlexaFluor 550 (IgG1) and AlexaFluor 555 (IgG2b), each at 1:200 dilution (Invitrogen, Carlsbad, CA, USA).

A series of photographs were taken of the slides at ×10 magnification using an Olympus BX41 microscope (Olympus America, Inc., Melville, NY) and imaged using an Olympus Q-Color3 camera (Olympus America, Inc., Melville, NY). Fibers were classified, counted, and sized using the ImageJ software (National Institute of Health, USA). Using the color composite feature within the software, fiber types were identified and sized objectively based on the color-specific staining intensity within each. Type I–specific CSA (CSA-T1), type II–specific CSA (CSA-T2), and type II to type I CSA ratio (CSA-R) were calculated from the collected data.

**Standing Ultrasonography Measurement.** Standing ultrasonography measures began with the application of a water-soluble transmission gel to the measurement site and a 16-Hz probe oriented in the short-axis, perpendicular to the VL muscle, while not depressing the skin. Subjects were upright and bearing weight on the opposite leg, which was positioned on a 5-cm tall platform, unweighting the measured leg and creating an internal knee angle of 160 ± 10° (30). Cross-sectional area (CSA-M) was obtained using a panoramic image sweep in the transverse plane perpendicular to the muscle (31). A straight-edge was placed along the skin to ensure that the probe remained along the previously established midline. Three images were obtained and saved for subsequent analysis using the software provided within the ultrasonography device.

**Isometric Strength Assessment.** Subjects completed a standardized general warm-up sequence before beginning the isometric strength assessment. Isometric strength was assessed using the ISQ using an adapted protocol from McBride et al. (18). Subject bar heights were set such that an internal knee angle of 100° existed, which was assessed through goniometer (18). Data were collected using a dual force platform design (2 × 91 cm × 45.5 cm of force platforms; RoughDeck HP, Rice Lake, WI, USA) inside a custom-built apparatus with data sampled at 1,000 Hz.

Participants completed warm-up trials at 50 and 75% of their perceived maximal effort before performing a minimum of 2 maximal effort trials. If a countermovement of greater than 200 N was observed, or trials differed by more than 250 N, subjects were required to complete an additional trial (15). Participants were also instructed to push “as fast and hard as possible” and were strongly verbally encouraged during trials (18). A 3-minute seated rest interval was prescribed between each of the ISQ trials. LabVIEW (Version 7.1; National Instruments, Austin, TX, USA) was used for collecting and ForceDecks (Version 1.2.6464; NMP Technologies Ltd., London, United Kingdom) for processing kinetic data (4). Peak force (IPF), allometrically scaled peak force (IPFa), and RFD over 50 ms (RFD-50), 100 ms (RFD-100), and 200 ms (RFD-200) were calculated from the collected data.

**Dynamic Strength Assessment.** Dynamic strength was measured using a 1RM back squat. Dynamic strength testing was completed after ISQ and after 48 hours of rest to ensure that
subjects were adequately recovered. Before testing, each subject performed a general dynamic warm-up.

After the general warm-up, bar and safety bar heights in the squat rack were adjusted as needed to best accommodate each subject. Subjects warmed up with progressively heavier loads of 30, 50, 70, 80, and 90% of their self-reported 1RM for 5, 3, 2, 1, and 1 repetitions, respectively, before maximal attempts. Each subject attained their 1RM back squat by attempting progressively heavier loads until they could not complete a successful repetition. For a repetition to be considered successful, the subject’s hip crease must have been below the patella at the bottom of the descent during the back squat and was verified by multiple certified strength and conditioning coaches. One repetition maximum back squat and allometrically scaled dynamic strength (DSa) were calculated from the collected data.

**Statistical Analyses**

Subjects were grouped by polymorphism for both *ACTN3* and *ACE* for analysis. Descriptive statistics including mean and SD were calculated. Within-subject reliability for each variable was assessed using intraclass correlation coefficients (ICCs) (12). Between-group Cohen’s *d* effect sizes were calculated for each dependent variable to determine the magnitude and meaningfulness of performance differences across polymorphisms. Effect sizes were interpreted with magnitude thresholds of 0–0.2, 0.2–0.6, 0.6–1.2, 1.2–2.0, and 2.0 and above as trivial, small, moderate, large, and very large (11). Statistical analyses were performed using Microsoft Excel (Redmond, WA, USA).

**RESULTS**

All performance-dependent variables in the current investigation returned acceptable ICC values (11). The frequency of RR, RX, and XX *ACTN3* genotypes was 70% (*n* = 7), 30% (*n* = 3), and 0% (*n* = 0), respectively. The frequency of DD, ID, and II *ACE* genotypes was 30% (*n* = 3), 50% (*n* = 5), and 20% (*n* = 2), respectively (Figure 1).

A moderate between-group effect (*d* = 0.61) favored *ACTN3* RR compared with *ACTN3* RX for CSA-M. In addition, a small between-group effect favored *ACTN3* RR for CSA-T1 (*d* = 0.21), CSA-T2 (*d* = 0.42), and CSA-R (*d* = 0.58). Isometric and dynamic performance outcomes also favored *ACTN3* RR over *ACTN3* RX, yielding moderate between-group effect magnitudes for IPF (*d* = 0.73), IPFa (*d* = 0.94), RFD-200 (*d* = 0.64), and 1RM (*d* = 0.99), along with a large effect for DSa (*d* = 1.51) (Table 1).

A moderate between-group effect for CSA-M favored *ACE* DD compared with *ACE* ID (*d* = 0.67) and *ACE* ID over *ACE* II (*d* = 0.65), along with a large positive effect in *ACE* DD over *ACE* II (*d* = 1.37). A moderate unfavorable effect for CSA-T1 was observed in *ACE* ID compared with *ACE* DD (*d* = −0.83) and *ACE* II compared with *ACE* DD (*d* = −0.80)—meaning that CSA-T1 was smallest in *ACE* DD. Conversely, small effects were present favoring *ACE*
ID over ACE II ($d = 0.35$) in CSA-T1. ACE DD had a moderate effect difference over ACE II in CSA-R ($d = 0.88$). Furthermore, large favorable effects were present comparing ACE DD with ACE ID in CSA-R. ($d = 1.42$). Trivial effects were observed comparing all ACE polymorphisms for CSA-T2 (Table 1). Considering ACE DD with respect to ACE ID, a moderate favorable effect was observed for IPF ($d = 0.70$), 1RM ($d = 1.14$), and DSa ($d = 1.06$). ACE ID had a moderate favorable effect for RFD-100 ($d = 0.69$) relative to ACE II. Finally, moderate effects favored ACE DD over ACE II in RFD-100 ($d = 0.66$), 1RM ($d = 0.93$), and DSa ($d = 0.62$).

**DISCUSSION**

The purpose of this investigation was to explore the potential physiological and performance outcomes associated with ACTN3 and ACE polymorphisms. Specifically, the authors aimed to examine (a) the potential effect that ACTN3 and ACE polymorphisms have on muscle characteristics including whole muscle, fiber-specific morphology and fiber-specific CSA distribution and (b) the effect that ACE polymorphisms have on isometric and dynamic performance capabilities. The main results of this investigation have shown that subjects possessing the ACTN3 RR polymorphism had larger whole muscle and fiber-specific CSA as well as a greater CSA-R compared with ACTN3 RX. Furthermore, our results indicate that individuals with the ACTN3 RR variant were stronger under both isometric and dynamic conditions and may possess greater RFD capabilities. Although ACE DD had the largest whole muscle CSA, a moderate between-group effect favored ACE ID and ACE II variants for CSA-T1. However, no meaningful effects were observed for CSA-T2.

Whole muscle CSA, often used as an indicator of force production capabilities (26), is affected by both inherited (i.e., candidate gene polymorphism) and environmental factors (i.e., training and nutrition). Because of the α-actinin-3 protein’s role as an actin anchor within the Z-line of fast muscle and ACE’s role in the synthesis of angiotensin II and cell growth, both provide mechanistic rationale for a larger muscle phenotype. The ACTN3 R allele has been associated with larger whole muscle size in previous literature (32), which agrees with the findings of the current investigation. However, Zempo et al. (32) compared the presence of the R allele (i.e., ACTN3 RR and RX) with ACTN3 XX. In the current investigation, no X allele homozygotes were present, but the findings do reveal the potential that R allele homozygotes (ACTN3 RR) possess a greater whole muscle size in comparison with heterozygotes (ACTN3 RX).

The ACE DD polymorphism presents a less clear mechanistic rationale as it relates to muscle size (10), although previous literature has indicated that the D allele is associated with greater changes in muscle CSA after resistance training (23). It has been postulated that, because there is a high prevalence of ACE within the muscle, that the generation of angiotensin II (a potent growth regulator in cardiac and smooth muscle) provides a link to larger muscle sizes. The presence of the ACE II genotype has been associated with high-level endurance performance (20), which typically favors athletes with lesser muscle mass than strength-power athletes (1). In addition, previous longitudinal research has suggested a preference for ACE DD and ACE ID variants for the gaining of muscle mass over ACE II (5). Indeed, the results of the current cross-sectional investigation indicated that muscle mass was from greatest-to-least: ACE DD > ACE ID > ACE II. Although training, nutrition, and other factors may ultimately determine the muscle size as an adult, the presence of one or both polymorphisms may provide for a greater potential for muscle hypertrophy, and therefore a greater force production capability.

Strength potential is also closely related to the composition of the muscle. This includes fiber-type specific CSA and the CSA-R. The current investigation provided interesting considerations in this regard, demonstrating the potential that ACTN3 RR may have a small effect on both CSA-T1 and CSA-T2 compared with ACTN3 RX. The ACTN3 RR genotype has been linked to elite strength-power performance in track and field (22,25). Therefore, the findings of the current investigation lend support for previous findings, especially considering ACTN3’s function within fast, glycolytic fibers. Interestingly, the ACE DD genotype was associated with a moderate decrease in CSA-T1 compared with the other genotypes and no effect in CSA-T2. However, there was a small between-group effect favoring ACE DD over ACE ID and a moderate effect supporting ACE DD over ACE II, which creates a potentially favorable scenario for force production abilities. Considering the potential combination of ACTN3 RR (i.e., larger CSA-T2) and ACE DD (i.e., smaller CSA-T1), there may be a situation where the CSA-R may be maximized. Greater CSA-R may minimize the drag effect that T1 fibers have on T2 fibers during whole muscle contraction, potentially increasing the maximum contraction velocity (14,28). A higher contraction velocity would be beneficial to performances in strength-power events, particularly in sprinting and jumping, which involve high RFDs and dynamic strength.

The ACTN3 RR and ACE DD genotypes were simultaneously present in 2 subjects in the current investigation. Although there are technical limitations of only having 2 subjects with these genotypes simultaneously, it is interesting to note that these subjects yielded among the greatest scores on isometric and dynamic strength as well as RFD. More specifically, one of the subjects had the highest CSA-M, greatest CSA-R, and ranked first in each performance measure collected including absolute and relative measures of strength performance and RFD at all considered timepoints. The other subject possessing both genotypes had the second highest CSA-M, was second in 1RM, third in IPF and DSa, and fourth in IPFa. The RFD capabilities of the second subject increased in rank as the timepoint expanded, moving from seventh in RFD-50 up to fourth in RFD-200 among...
all subjects. The lower ranking in the early RFD timepoints may be partly due to this subject's seventh-ranked CSA-R, which has been previously connected to RFD capabilities (28). This variability in RFD may indicate the importance of training, as the subjects had different athletic backgrounds. It may also suggest that there are other genes and their respective polymorphisms that must be taken into consideration that more drastically influence RFD capabilities than ACTN3 and ACE. This list may contain more than 40 candidate genes (27) including, but not limited to MCT1 (monocarboxylate transport 1), MYLK (myosin light chain kinase), COL1-A1 (collagen α-1 chain type I), insulin-like growth factor-related genes, or myostatin-related genes (27). As demonstrated, the factors influencing strength performance are robust and comprise bioenergetic, structural, and regulatory aspects.

Although the findings of the current investigation are limited by the sample size, it is the first of the authors' knowledge to investigate the potential influence of ACTN3 and ACE polymorphisms on isometric and dynamic strength testing. This research has the potential to act as a framework for the generation of future hypotheses within strength and conditioning research as it relates to the influence of genetics. The current investigation suggests that the ACTN3 RR and ACE DD tend to result in greater whole muscle size but differ in how they contribute to performance capacities. While ACTN3 RR's influence seems to be in the T2 fibers and therefore alters gross isometric and strength performance, ACE DD seems to influence RFD capabilities through creating a favorable CSA-R. Future investigations should continue to explore the individual and combined effects of these 2 genotypes as well as the inclusion of other heritable characteristics and their relative contributions to performance potential and outcomes.

**Practical Applications**

The findings of the current investigation provide unique considerations for talent identification of strength-power athletes. Although previous investigations have explored the general physical qualities associated with these 2 candidate genes and their respective polymorphisms, the current investigation is the first to provide specific effect magnitudes on mechanistic strength qualities, albeit with a limited sample size. This may be valuable for organizations and governing bodies with long-term athlete development models that guide younger athletes toward certain sports in which they have a higher likelihood for success.

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