

Examination of the *ACE* and *ACTN3* Genes in UTC Varsity Athletes and Sedentary Students

by

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ABSTRACT

Introduction: Growing evidence suggests that genetic variants of certain genes are linked to athletic performance. This study presents a comparative analysis of genetic variation of the *ACE* and *ACTN3* genes in varsity athletes (n=90) and sedentary students (n=48) at the University of Tennessee at Chattanooga. The *ACE* gene codes for the Angiotensin Converting Enzyme (ACE), which is an integral part of the Renin-Angiotensin system. It is responsible for regulating blood volume, arterial pressure, electrolyte balance, and cardiac and vascular function. Polymorphisms of the *ACE* gene affect serum and tissue levels of the enzyme, and are genetically distinguished by either an insertion (*I-allele*) or deletion (*D-allele*) of an alu repeat within intron 16. The Insertion allele is thought to be beneficial to endurance athletes. On the other hand, the *ACTN3* gene encodes the 901 amino acid alpha-actinin-3 protein found only in Type II (Fast-Twitch) muscle fibres. Its function is to provide structural support for the transmission of force during muscle contraction along the Z line and sustain the order of myofilaments and coordinate myofilament contraction (Yang et. al, 2003). The polymorphisms identified in exons 15 and 16 are thought to be important to *ACTN3* gene function. Although not clearly shown to be linked to athletic performance, a point mutation in exon 15 of the *ACTN3* gene introduces an Arginine(R)→Glutamine(Q) substitution at amino acid residue 523 (designated R523Q) with possible functional implications. A stronger correlation exists for genetic variants involving mutations in exon 16 of the *ACTN3* gene. A polymorphism in exon 16 manifests as a premature stop codon,

Arginine(R)→Stop(X) at position 577 of the protein (R577X). The R allele of Exon 16 is thought to be advantageous to athletes that require short and forceful bursts of power due to full-length and functional ACTN3 proteins. Each sample was scored for the *ACE* and *ACTN3* genotypes and data was analyzed in a population of 90 UTC athletes and 48 sedentary controls by PCR and RFLP analysis.

Results: The genotypic frequencies of the *ACE* gene deviated from the Hardy-Weinberg Equilibrium whereas exons 15 and 16 of the *ACTN3* gene did not. The *I* allele of the *ACE* gene was observed in a notably increased frequency among endurance athletes (24.1%) when compared to the non-endurance athletes (7.9%) and the sedentary group (12.5%). There were no significant differences between the athletes (endurance or non-endurance) and the control subjects with respect to exon 15 of the *ACTN3* gene. The frequency of the 577R allele of exon 16 was found to be significantly higher among UTC athletes (58.5%) than in the sedentary students (36.5%). In addition, there was a linear increase in the 577R allele from the sedentary group (36.5%), endurance athletes (55.6%), and non-endurance athletes (61.4%).

Conclusion: The *ACE I/D* and *ACTN3* polymorphisms have been related to athletic performance in elite athletes. This project examined an athlete population at the NCAA Division I level. Both of the favourable variants of these polymorphisms were observed in this project. It can be concluded that *I allele* of the *ACE* gene occurs at a greater frequency in endurance athletes when compared to non-endurance athletes and sedentary subjects. In addition, the 577R allele in exon 16 of the *ACTN3*

gene is found in an increased frequency in athletes and specifically non-endurance athletes when compared to the sedentary subjects. Furthermore, these results are consistent with previous studies of elite and Olympic caliber athletes and support the hypothesis that the *I allele* of the *ACE* gene is beneficial to endurance athletes whereas the *577R* allele of the *ACTN3* gene is advantageous to non-endurance athletes.

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INTRODUCTION

Heritability plays an important role in human performance. Researchers estimate that performance related traits important to elite athletes have heritability values of ~50% for maximal oxygen uptake (VO₂ Max), 42-46% for cardiac output, 40-50% for muscle fibre type proportions, and 67% for explosive muscle power (Macarthur et. al, 2004). Heritability reflects how much phenotype variation of a trait is due to inherited factors, where zero is independent of genetics and one (100%) is completely dependent. Thus, it is favourable to possess the right blend of genes that are conducive to an athlete's specific discipline (eg. sprinting or endurance running).

An increasing level of competition in sports has caused athletes to look for a competitive edge. Unfortunately, with recent developments in gene manipulation, there is a growing concern that scientists and athletes alike may be able to abuse this innovative technology to engineer individuals that permanently express desirable genes for athletic performance. Officials are worried that "gene doping" will become a reality. The identification of a collection of genes that control physical performance traits will optimistically aid scientists to determine whether an athlete's DNA has been altered to express advantageous genotypes such as increased endurance or muscular strength.

The purpose of this study is to determine whether a correlation exists between allelic variants of the endurance/athletic performance associated genes *ACE* or *ACTN3* in a group of UTC varsity athletes when compared to a group of sedentary university students. In recent years several studies have been published, examining

performance gene frequencies among elite athletes; however this represents only a small percentage of the greater population. Optimistically these studies and this report will initiate more research into this area of human performance genetics.

Specific allelic variants of the *ACE* and *ACTN3* genes are known to produce favourable traits with respect to athletic performance. The genes were chosen from The Human Gene Map for Performance and Health Related Fitness Phenotypes which was most recently compiled by Wolfarth et. al (2005) in *Medicine & Science in Sports & Exercise*. This is an annual publication that presents a collection of scientific reports relating to cardiorespiratory endurance, elite endurance athlete status, muscle strength, other performance traits, and exercise intolerance of variable degrees. The first year for this publication was in 2000 and reported 29 loci genetic links to athletic performance. By 2004 this value increased fivefold with a total of 140 autosomal gene entries, 4 sex-linked genes, and 16 mitochondrial genes (Wolfarth et. al, 2005). The *ACE* and *ACTN3* genes were chosen for examination in this study with respect to current literature because they have opposing effects in the human body; variants of the *ACE* gene are thought to confer a greater advantage in endurance activities or sports, whereas, variants of the *ACTN3* gene are thought to present an advantage to power athletes that require short bursts of intense strength and power.

Genomic Arrangement of the ACE Gene

The *ACE* gene is located on the long arm of Chromosome 17q 23.2 as shown in Figure 1. It covers approximately 20,546 bases of genomic DNA and is composed

of 25 exons. After post-transcriptional splicing and removal of non-coding introns the transcript is represented by a 4,195 base mRNA that directs synthesis of the final 1,306 amino acid protein.

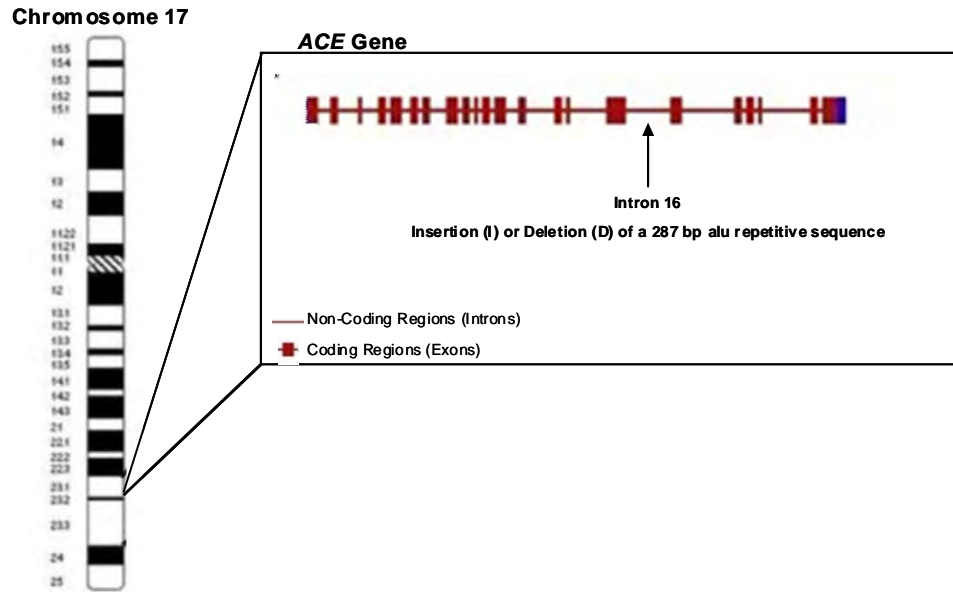


Figure 1 The *ACE* gene is located on the long arm (q) of Chromosome 17 on the band 23.2. The *ACE I/D* Polymorphism is characterized by an insertion (*I*) or deletion (*D*) of a 287 bp alu sequence at intron 16.

Functional Characteristics of the ACE Gene

The Angiotensin Converting Enzyme is an integral enzyme in the Renin-Angiotensin system (Figure 2), which is important in regulating blood volume, arterial pressure, and cardiac and vascular function (Tanriverdi, et. al 2005). The kidneys secrete the hormone renin into the bloodstream under sympathetic stimulation or hypotension (Kem et. al, 1990). Renin functions to cleave a ten amino-acid protein off of the inactive liver peptide Angiotensinogen. The resulting intermediate peptide, Angiotensin I is then converted to Angiotensin II by ACE (Sonna et. al, 2001). Angiotensin II is a multifunctional peptide that acts indirectly to

increase vasoconstriction, vascular resistance, and blood pressure by hindering the synthesis of Nitric Oxide, which is a vasodilator (Tanriverdi, et. al 2005). In addition vasoconstriction is indirectly mediated by ACE through degradation (proteolytic cleavage) of the vasodilator bradykinin (Kem, et. al 1990). Angiotensin II triggers the increase of blood volume through two main actions. It stimulates the cerebral cortex to release the hormone aldosterone that in turn signals the kidneys to enhance the retention of sodium and fluids. It also triggers the posterior pituitary gland to release vasopressin, which causes the kidneys to increase fluid absorption (Kem et. al, 1990).

The *ACE I/D* polymorphism corresponds to the presence (*I*) or absence (*D*) of a 287 bp alu repetitive sequence. The *D* (Deletion) allele is associated with a 190 bp PCR fragment targeting the polymorphism site in intron 16 of the *ACE* gene. Individuals homozygous for the *D*-allele have elevated ACE serum levels (Sonna et. al, 2001) as shown in Figure 2. Higher levels of ACE increase the conversion of Angiotensin I to II and accordingly vasoconstriction is the result. The *I* (Insertion) allele is associated with a 490 bp PCR fragment and is related to lower ACE serum levels.

Renin-Angiotensin System

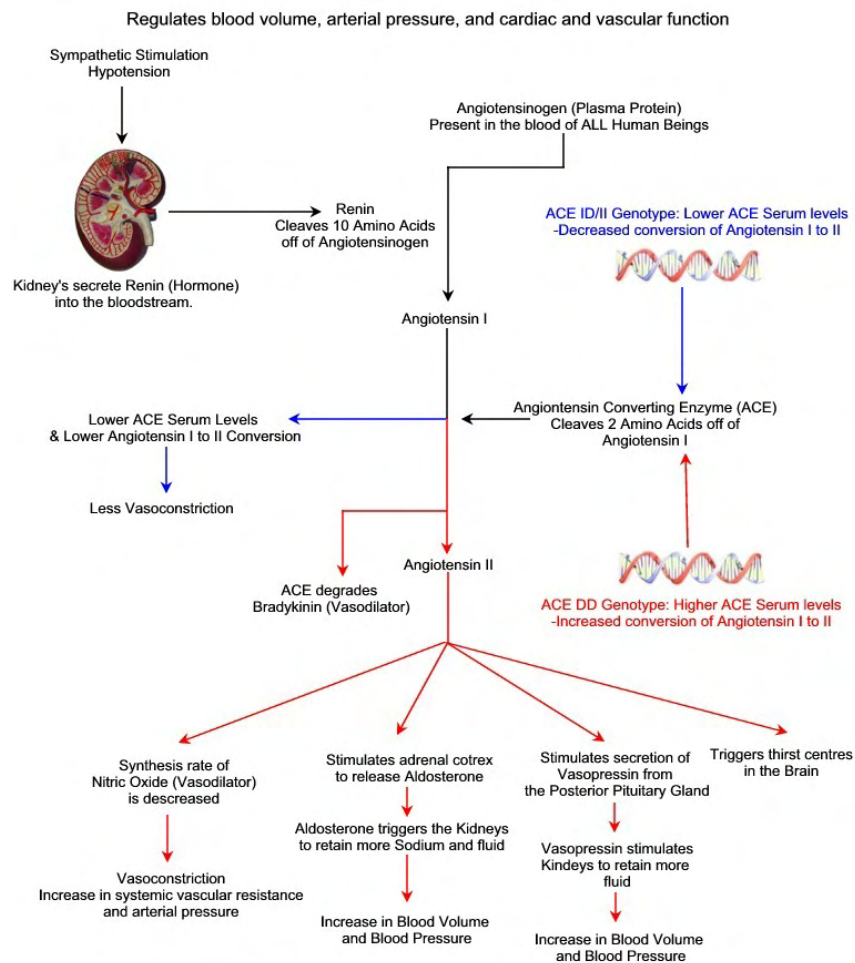


Figure 2 Effect of the *ACE* Gene on the Renin-Angiotensin System

The *ACE I/D* Polymorphism has been examined extensively in the field of medicine for a large number of conditions such as Diabetes, Alzheimer's, and many cardiovascular diseases. It has also been studied in populations all over the world because of its central role in the overall functioning of the human body. The importance of *ACE* has led researchers to examine its effects on elite athletes to determine if the variants of the *ACE* gene are related to human performance.

Relationship of Genetic Variants of ACE Gene with Athletic Performance

The *I* allele is functionally characterized as having reduced ACE serum levels and activity. This allele is considered a favourable mutation because lower ACE activity results in less vasoconstriction and thus an increased delivery of oxygenated blood to the working muscles. Therefore, individuals that possess an *I* allele or the *II* genotype are thought to present a greater advantage in endurance activities such as running, cycling, and swimming where the demand for oxygen during their duration is crucial.

ACE I/D Polymorphism in Endurance Athletes: In a series of reports examining the allelic and genotypic frequencies of the *ACE* gene the experimental groups have consisted primarily of elite athletes competing at the National, Olympic, and World Championship levels. A common occurrence among several of the studies is that there is not a significant difference in allelic frequencies when the athletes (eg. power and endurance) are combined in a group and compared to a control group. However, when the athlete group is further divided into athletes that are ‘elite’ or ‘outstanding’ performers based on the length of their athletic discipline (eg. sprint/power versus endurance events) a difference of the *I* allele and *II* genotype arises.

In a group of British Olympic caliber athletes the frequency of the *I* allele increased from 35% in sprint athletes ($\leq 200\text{m}$) to 65% in distance athletes ($\geq 5,000\text{m}$) (Myerson et. al, 1999). In a similar study by Nazarov et. al (2001) of Russian athletes (swimmer, skiers, triathletes, and track & field) there was an excess of the *D* allele (72%) in the short distance group and an excess of the *I* allele (63%) in the middle

distance group. Scanavini et. al (2002) examined a selection of Italian athletes (aerobic and anaerobic sports) designated as 'candidate Olympic athletes.' When the athletes were further classified into groups based on Olympic participation, performance, and VO₂ Max, the *II* genotype was found in 30.3% of Olympic aerobic athletes compared to 5.3% in anaerobic athletes and 12.5% in the controls. These findings suggest that the *I* allele is related to endurance athletes and it is difficult to detect an association of the *ACE I/D* polymorphism amongst a heterogeneous group of mixed athletic disciplines.

The researchers Rankinen et. al (2000) examined the genotypes of endurance runners, skiers, and cyclists. The *II* genotype was not statistically prevalent in any of the groups based on sport when compared to a group of controls. In addition, the athletes were divided into groups based on Maximal Oxygen Uptake (VO₂ Max) values. VO₂ Max is the maximal capacity for oxygen consumption by the body during maximal exertion (Wilmore, p.707). This value is a reliable measurement of an endurance athlete's ability to process oxygen per kilogram of body weight per minute. The quartile and decile values of athletes yielded similar *II*, *ID*, and *DD* genotype frequencies to the athletes with lower VO₂ Max values.

Athletes have been examined for the *ACE I/D* polymorphism based on placing within an athletic competition. The top finishers at the 2000 and 2001 South African Ironman Triathlons were tested for the *ACE I/D* polymorphism. The researchers found a significant linear trend for the *I* allele distribution among the fastest

finishers (51.5%), slowest 100 finishers (47.5%) and control subjects (42.4%) (Collins et. al, 2004).

Kenya has been the dominant nation in endurance running for the past 15 years. In 2005, 29 out of the top 50 roadrunners and 23 out of the top 50 distance runners (3000m-10000m) were from Kenya (IAAF rankings, 2005). Exercise physiologists and scientists have marveled at the dominance of Kenyans in endurance running competition. The explanation of their success is a collection of scientific hypotheses and a group of researchers (Scott et. al, 2005) examined the *ACE* gene in a cohort of elite Kenyan runners and Kenyan control subjects. It was determined that *I* allele frequency was similar in international (38%) and national (42%) level athletes and control subjects (38%). In addition, when both levels of athletes were combined and compared to controls there were no significant differences. A similar study by Moran et. al (2004) examined Ethiopian distance runners. Ethiopians have recently become distance-running power on the world-class level. The *II* genotype was found in 22.7% of male marathoners and only 4.3% in the control group. In addition, the *I* allele was found in a greater proportion (43%) when compared to the control group (26%) that was representative of the Ethiopian population. The studies on African endurance runners provide a mixed explanation to the success of their runners on the international level.

ACE I/D Polymorphism in the Heterogeneous Population: The *ACE* gene has been examined in the heterogeneous population to some extent in relation to athletic performance. A study performed by Sonna et. al (2001) examined a group of US

army recruits with relation to the *ACE I/D* polymorphism and physical performance during basic army training. It was concluded that the *ACE* genotype did not have an effect on aerobic power or muscular endurance in the army recruits. However, Myerson et. al (1999) found that the *I* allele was related to increased duration of repetitive biceps flexion after a program of general physical training in British Army recruits. In addition, a group of non-elite Turkish subjects were studied for their performance in several running tests. With respect to performance in the 60m sprint and middle-distance running tests, 56.7% of the ‘superior’ subjects had the *DD* genotype as compared to 37.9% in the ‘poor’ group (Cam et. al, 2005). These studies may suggest that in the non-elite population the *ACE I/D* polymorphism may have an effect on performance.

Genomic Arrangement of the ACTN3 Gene

The *ACTN3* gene is located on the long arm of chromosome 11q 13.2 as shown in Figure 3. It spans approximately 16,407 bases of genomic DNA and is composed of 21 exons. After post-transcriptional splicing and removal of non-coding introns the transcript is represented by a 2,858 base mRNA that directs synthesis of the final 901 amino acid α -actinin-3 protein. There are two sections of the gene that are being examined for genetic variations in this project. Polymorphisms of exons 15 and 16 are created by point mutations within the coding regions of the DNA molecule and are hypothesized to influence *ACTN3* gene function and potentially athletic performance.

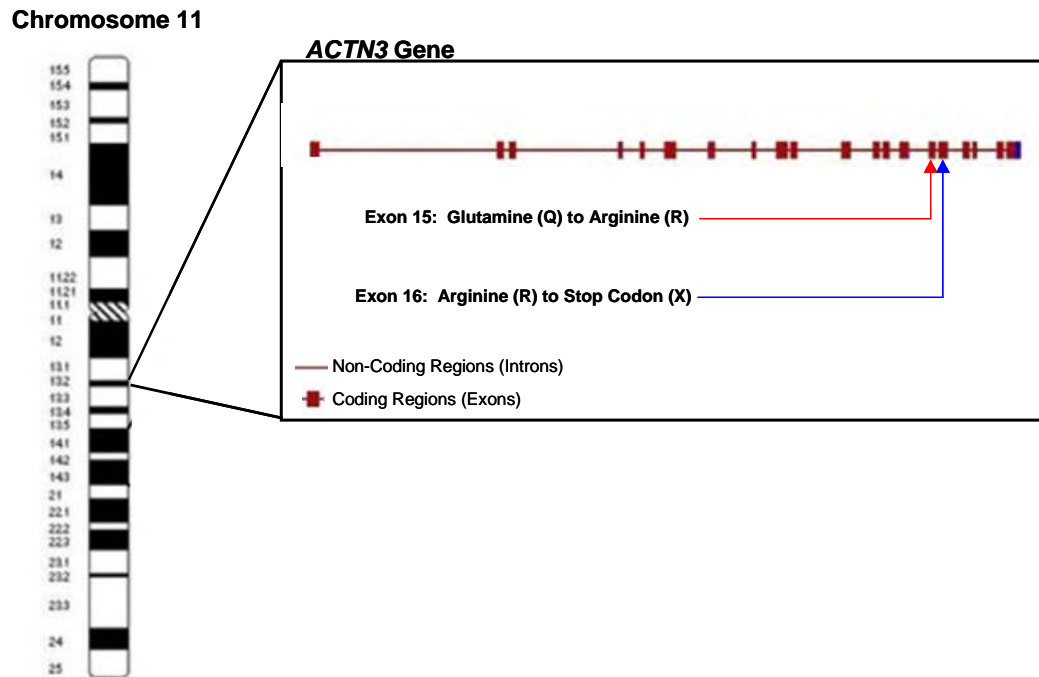


Figure 3 The *ACTN3* gene is located on the long arm (q) of Chromosome 11 on band 13.2. Exons 15 and 16 are the coding regions of the DNA that are transcribed into an mRNA molecule. A transition mutation in exon 15 has created the Q523R polymorphism whereas a nonsense point mutation in exon 16 has created the R577X polymorphism.

Functional Characteristics of the ACTN3 Gene

The expression of α -actinin-3 is limited to Type II (fast-twitch) muscle fibres (Mills et. al, 2001). Alpha-actinin-3 is a part of the sarcomeric α -actinins, which are major components of the Z line, where its function is twofold, to connect with actin filaments and sustain the order of myofilaments and coordinate myofilament contraction (Yang et. al, 2003). The Z line (Figure 4) is an important structure within the sarcomere and its function is to provide structural support for the transmission of force when the muscle fibres are activated (Wilmore, p. 100). Researchers believe that α -actinin-3 may be optimized to decrease the damage induced by eccentric muscular contraction (Yang et. al, 2003). This support is particularly important

during forceful contractions, which are prevalent in type II (fast twitch) muscle fibres and is shown in Figure 4.

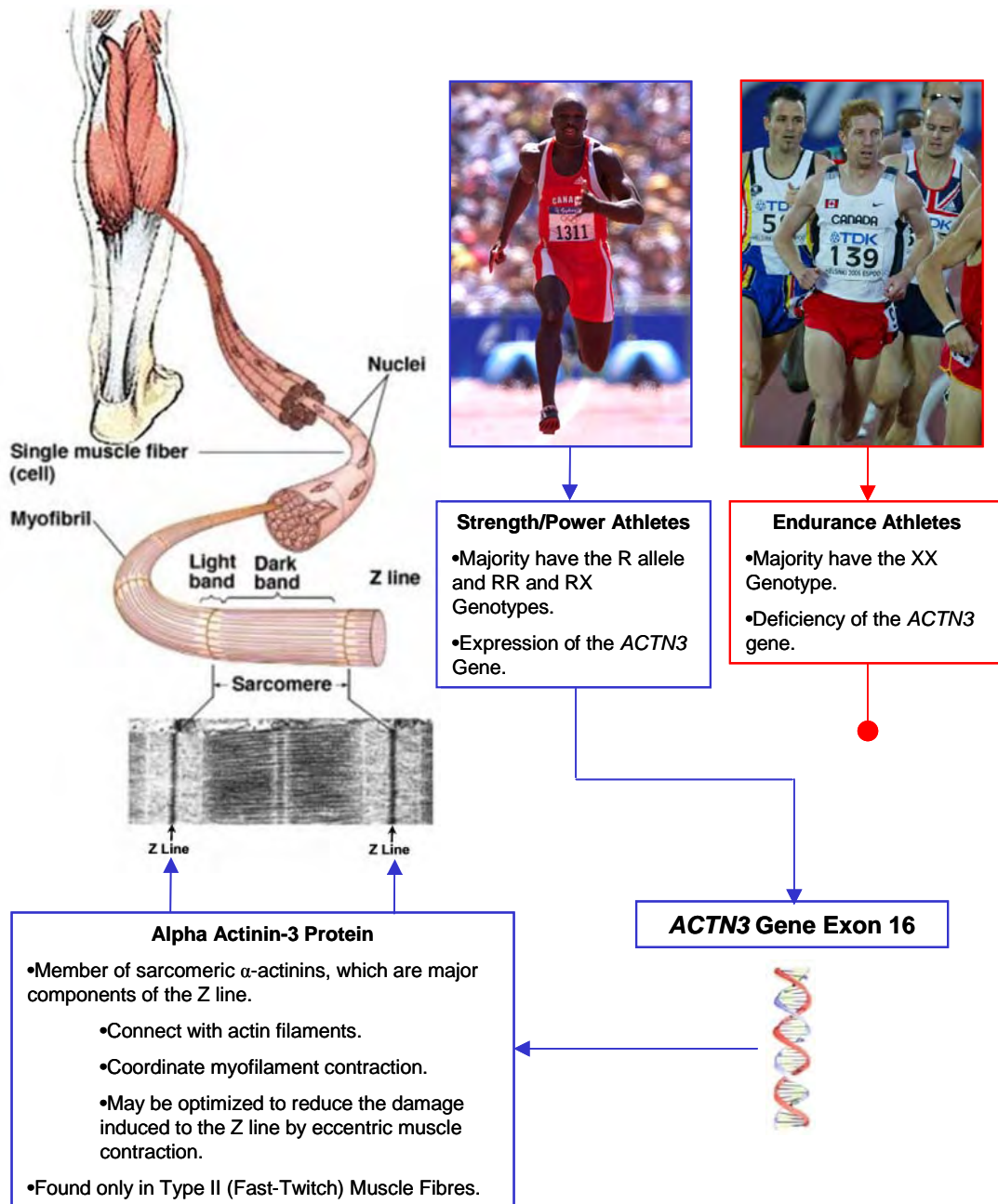


Figure 4 Effect of the *ACTN3* Gene on the Muscular System

Two independent polymorphisms in exons 15 and 16 of the *ACTN3* gene are proposed functional variants with possible influence on athletic performance. In exon 15 an A→G transition mutation occurs, which changes the nucleotide codon sequence from CAG to CGG. The CAG codon that once coded for Glutamine (Q) now codes for Arginine (R). Fortuitously, this nucleotide alteration introduces a cleavage recognition site for the restriction enzyme *MspI* in the 523R allele, and thus allelic variants can easily be distinguished by Restriction Fragment Length Polymorphism (RFLP) analysis (Figure 5). This method of analysis allows for the rapid detection of point mutations by the creation or destruction of restriction sites. For example, in exon 15, gel electrophoresis easily identifies the mutations, with the 523Q allele yielding two DNA fragments whereas the 523R allele (with the extra restriction site) has three fragments.

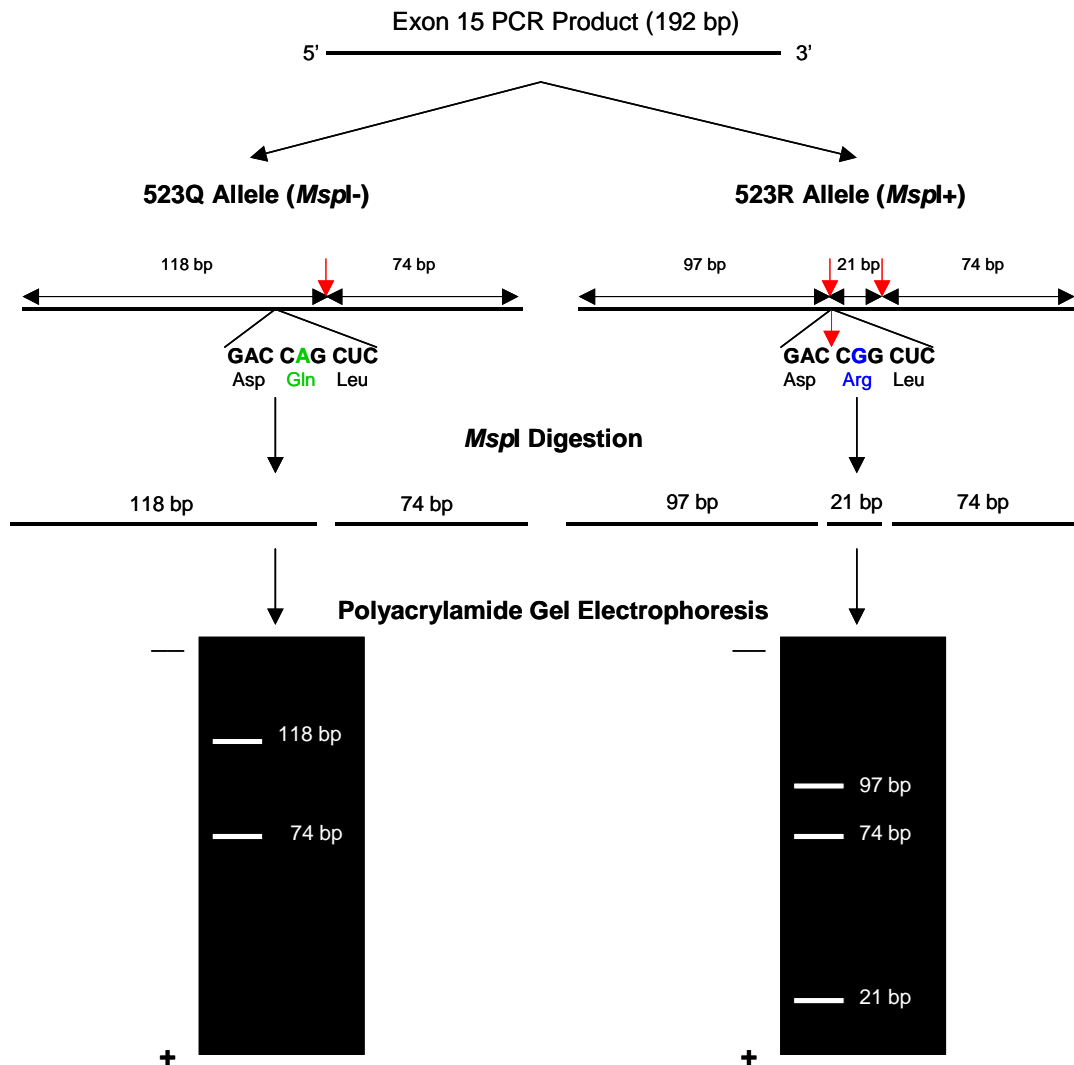
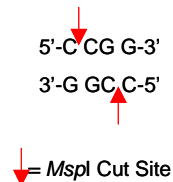


Figure 5 RFLP Genetic Analysis of Exon 15 Polymorphism.

The 523Q and 523R alleles of *ACTN3* Exon 15 are distinguished by a single nucleotide polymorphism (SNP) that is marked by the creation of a restriction cut site for the enzyme *MspI* in the 523R allele. Digestion of the 192 bp PCR product cleaves the 523Q (*MspI*-) allele into two fragments of 118 and 74 bp respectively. Likewise, *MspI* digestion of the 523R (*MspI*+) allele generates fragments of 97, 74, and 21 bp.

***MspI* Restriction Cut Site**



In exon 16, a nonsense point mutation (C→T) alters a codon in the exon sequence from **CGA** to **TGA**. This changes the amino acid from an Arginine (**R**) to a premature stop codon (**X**). The result is a deficiency of the α -actinin-3 protein, which is predicted to be present in 16% of the world population (Yang et. al, 2003). The α -actinin-2 protein, encoded by the *ACTN2* gene, is structurally and functionally similar to the α -actinin-3 protein and is present in all muscle fibre types. It is thought that the α -actinin-2 protein is able to compensate for the deficiency of α -actinin-3 in fast twitch muscle fibres (North et. al, 1999). The variant (577R) that codes for the functional α -actinin-3 protein is thought to be beneficial to athletes that utilize Type II muscle fibres during high velocity activities such as sprinting. Similar to the exon 15 polymorphism the nucleotide alteration within exon 16 introduces a cleavage recognition site for the restriction enzyme *DdeI* in the 577X allele, and thus allelic variants can easily be distinguished by RFLP analysis (Figure 6).

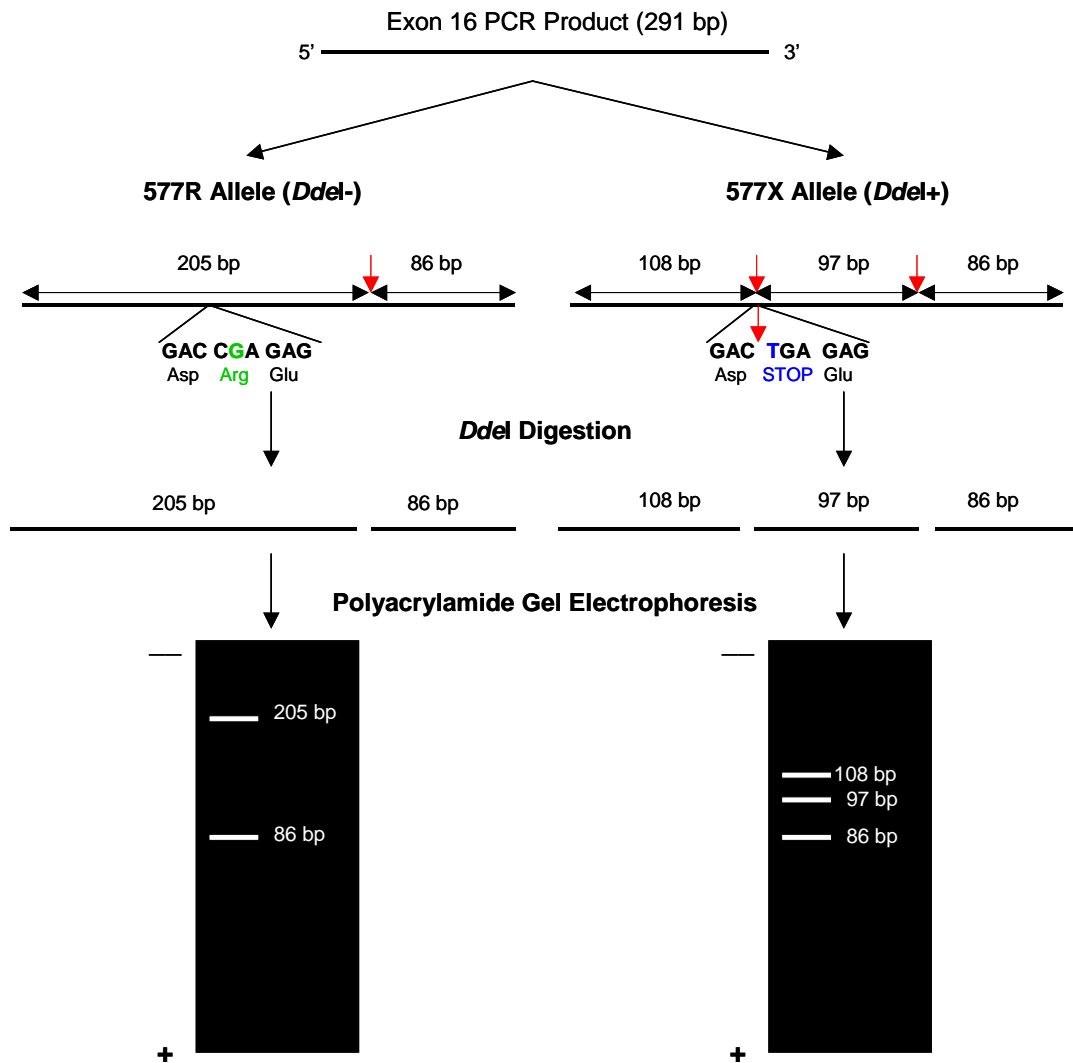
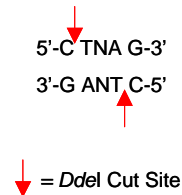


Figure 6 RFLP Genetic Analysis of Exon 16 Polymorphism.
 The 577R and 577X alleles of *ACTN3* Exon 16 are distinguished by a single nucleotide polymorphism (SNP) that is marked by the creation of a restriction cut site for the enzyme *Ddel* in the 577X allele. Digestion of the 291 bp PCR product cleaves the 577R (*Ddel*-) allele into two fragments of 205 and 86 bp respectively. Likewise, *Ddel* digestion of the 577X (*Ddel*+) allele generates fragments of 108, 97, and 86 bp.

***Ddel* Restriction Cut Site**



Relationship of Genetic Variants of the ACTN3 Gene with Athletic Performance

The *ACTN3* gene was first examined by North et. al (1999) when they discovered a transition mutation in Exon 16 that resulted in a deficiency of the α -actinin-3 protein. They examined muscle biopsies of patients with neuromuscular disorders and discovered that the α -actinin-3 deficiency occurred in only 19% of the samples, which led the researchers to believe that the deficiency was not related to a disease. In addition, exon 15 was examined and it was determined that the transitions in both exons 15 and 16 were independent of each other and certain variants of each exon were not related. Thus, North et. al concluded that there was no disease phenotype associated with the α -actinin-3 protein deficiency.

The lack of a disease phenotype related to the α -actinin-3 deficiency triggered Mills et. al (2001) to examine the evolution of the *ACTN3* gene. As mentioned, α -actinin-2 is found in all types of skeletal muscle in humans and is 80% identical and 90% similar to α -actinin-3 (Mills et. al, 2001). The divergence of the two proteins occurred over 300 million years ago and since then they have changed very little (Mills et. al, 2001). Due to their similarity it led the scientists to believe that α -actinin-3 is functionally redundant, which occurs when two genes carry out similar functions and when one is inactivated there is minute or no effect on the genotype. While they perform similar functions in muscle, it is thought that α -actinin-3 performs more effectively within Type II muscle fibres. While this does not have an effect on the general phenotype it may have an effect in certain populations and under extreme conditions (eg. elite sprinters). The results from this study intrigued this

research group and other researchers to examine this gene in populations such as elite sprint athletes that require short and forceful bursts of muscular power during a race.

A study was conducted by Yang et. al (2003) with a group of Australian athletes and control subjects. When the athlete group as a whole was compared to the controls, there was no significant allele or genotype dissimilarities. Furthermore, the athletes were divided into two groups: power/sprint athletes and endurance athletes. There was a lower frequency of the XX genotype in the male (8%) and female (0%) sprint athletes when compared to the control group (16% for males and 20% for females). The sprint athlete group had a higher frequency of the RR genotype (53%) whereas the elite endurance athletes had a slightly higher frequency of the XX genotype (24%) when compared to the control subjects (RR 30%, XX 18%). The main finding from this study was that the allele and genotype frequencies did not differ significantly when the athletes and the controls were compared. However, when the athletes were further stratified into groups based on sprint versus endurance the results digressed in opposite directions and differed appreciably in both males and females. This finding suggests that the 577R allele may be beneficial to sprint athletes.

The researchers mentioned above conducted a similar study with elite endurance athletes from Ethiopia and Kenya to determine whether the XX genotype appeared at an increased frequency as it did in the previous study. The XX genotype in the elite Ethiopian (9%) and Kenyan (1%) runners did not differ significantly from

the respective Ethiopian (11%) and Kenyan (1%) control groups that were representative of the populations (Yang et. al, 2005).

ACE I/D and ACTN3 R577X Polymorphisms and Athletic Performance

Two studies (Chiu et.al, 2005 and Hsieh et. al, 2005) have been completed in a similar method to this project. A group of Taiwanese athletes from the 2002 Asian games team and elite Taiwanese swimmers were tested for the *ACE I/D* and *ACTN3 R577X* polymorphisms. In both studies the *ACE* and *ACTN3* genotypes were not significantly different between the athletes and the control group. Additionally, there were not any athletes that had both the *ACTN3 XX* and the *ACE DD* genotypes, which means that multiple genetic traits may influence speed and endurance.

Purpose of Project

In previous studies, the allelic and genotypic frequencies have been examined in extreme and specialized populations such as elite endurance runners or sprinters and compared to control groups of sedentary individuals. From the examination of many different scientific journals, it appeared that there was very little research of human performance gene frequencies for both male and female collegiate athletes. The athletes were considered to be ‘regional’ to ‘national’ competitors at the Division I level of the National Collegiate Athletic Association (NCAA). This project examined whether there are any significant frequencies of the *ACE* and *ACTN3* allelic variants in UTC varsity athletes versus a group of UTC sedentary students. Furthermore, the athletes were divided into endurance athletes (eg. Men’s and Women’s Track & Field and Cross-Country athletes) and non-endurance athletes (eg.

Men's and Women's Basketball, Men's and Women's Tennis, Men's Golf, Men's Wrestling, and Women's Softball) and compared to the sedentary subjects with respect to the aforementioned genes.

Hypothesis

It may be hypothesized with respect to the *ACE* gene, that the *I* allele will be observed with a greater frequency in the varsity athletes when compared to the sedentary group. Specifically, the *I* allele is expected to be found in an increased frequency in endurance athletes when compared to non-endurance athletes and sedentary subjects. For Exon 16 of the *ACTN3* gene, the 577R allele is predicted to be more prevalent in the varsity athletes when compared to the sedentary group. In addition, the R allele is expected to exist at a higher frequency in non-endurance athletes when compared to endurance athletes and sedentary subjects. To date, there have not been any reports evaluating polymorphisms in exon 15 of the *ACTN3*, thus this study will also establish if there is a correlation to its variants and athletic performance in the UTC sample population.

MATERIALS AND METHODS

The participants in this project were divided into an athlete and sedentary student group. Both groups were selected from the UTC student population and ranged in age from 18 to 26 years-old. All participants voluntarily signed an informed consent form describing the study and potential risks, if any, prior to participation in the study (Appendix B). For the athlete group, the individual had to be on an official 2005-06 team roster of at least one of the UTC varsity sports. A call for participants was announced prior to team physicals during the beginning of the fall 2005 semester. A station was setup during athlete physicals to collect buccal samples providing the source of DNA for downstream molecular analysis. The sedentary control group was selected from UTC students on a voluntary basis during the fall 2005 semester.

Each subject was assigned a number associated with their sex, age, race, height, weight and varsity sport (not applicable to controls). The subject did not have access to the assignment code. DNA extraction was completed by using a small brush to gently scrape buccal cells from the inside of the subject's mouth. The extraction was performed under sterile conditions, ensuring that new materials and equipment were used for each subject to prevent contamination and harm to the subject. All experimental protocols involving human subjects were approved by the University of Tennessee Institutional Review Board (IRB# 05-055).

Project Overview

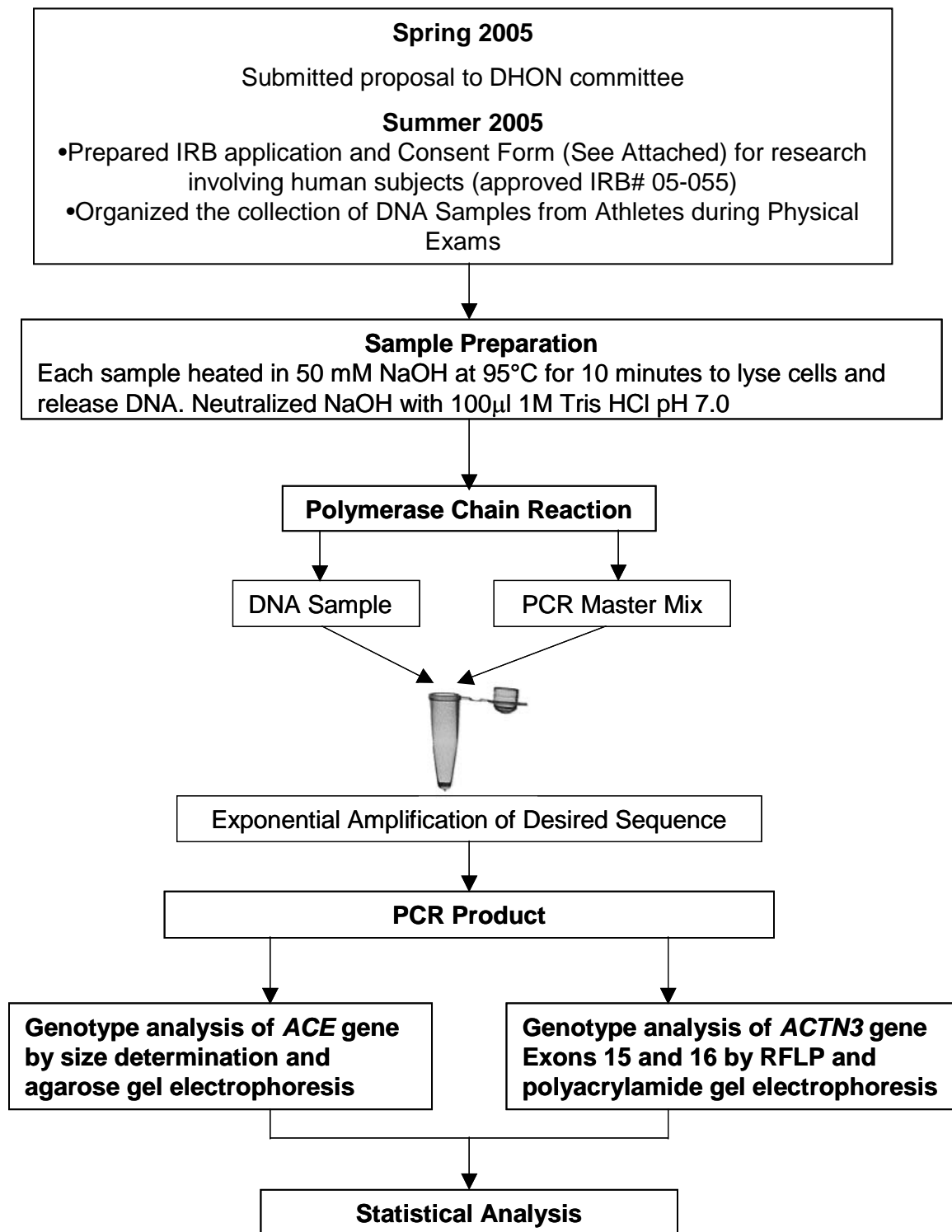


Figure 7 Flow-Chart of the Project Overview

Subjects

A total of 117 UTC varsity athletes consisting of 47 females and 70 males voluntarily donated their DNA samples. Of the total number of samples taken, 90 samples tested reliably in molecular analyses and were chosen for all subsequent tests (Table 1). Samples were taken from members of the Men's Basketball ($n=6$), Women's Basketball ($n=8$), Men's Cross-Country and Track & Field ($n=12$), Women's Cross-Country and Track & Field ($n=15$), Men's Golf ($n=6$), Men's Tennis ($n=6$), Women's Tennis ($n=6$), Women's Softball ($n=12$), and Men's Wrestling ($n=19$) teams.

Table 1 Representation of UTC Varsity Athletes Based on Team and Sex.

Varsity Sport	Number of Athletes
Men's Basketball	6
Women's Basketball	8
Men's Cross-Country/Track & Field	12
Women's Cross-Country/Track & Field	15
Men's Golf	6
Men's Tennis	6
Women's Tennis	6
Women's Softball	12
Men's Wrestling	19
Total	90
Total Females: 42 Total Males: 48	

Altogether, 48 samples were obtained from volunteer UTC sedentary students from Biology laboratory classes. There were 21 females and 27 males included within the sedentary group. The protocol of acquiring the DNA samples was identical to that of the athletes and each student was given an assignment code to protect their identity.

Subject Sample Preparation

DNA was isolated from the buccal cells of each subject by following a standard protocol. Briefly, each sample was denatured in 600 µl of 50mM NaOH at 95°C for 10 minutes. Additional cells were removed from the brush by vortexing for 10 seconds. Sample pH was neutralized by the addition of 100 µl 1M Tris•HCl, pH 7.0. Samples were stored at -20°C between tests.

Molecular Analysis of ACE and ACTN3 Genes

Each sample was analyzed for the *ACE* and *ACTN3* (Exon 15 and 16) gene polymorphisms. PCR was utilized to amplify the specific polymorphic region of each gene in preparation for molecular analysis. A schematic of the PCR amplification reaction is outlined in Figure 8 (Davis et. al, 2005). In this process, multiple rounds of primer extension reactions occur in which complementary strands of a defined region of a DNA molecule are simultaneously synthesized by a thermostable DNA polymerase isolated from *Thermus aquaticus* (*Taq*). During repeated rounds of these reactions, the number of newly synthesized DNA strands increases exponentially so that after 20 to 30 reaction cycles, the initial template DNA will have been replicated several million-fold.

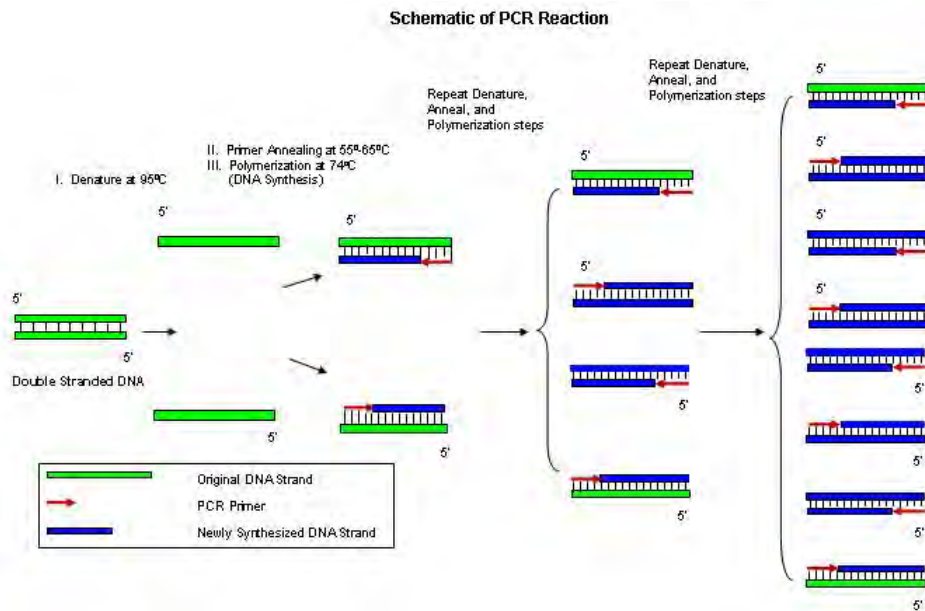


Figure 8 Outline of a PCR Reaction

Description of PCR Amplification Primers

Three sets of PCR primers were employed to target the *ACE I/D*, *ACTN3* Exon 15 and *ACTN3* Exon16 polymorphisms (Table 2). The PCR reactions and primers were adapted from the sources cited in this table. All primers were purchased from Sigma Genosys (The Woodlands, Tex.)

Table 2 DNA Primers used for *ACE* and *ACTN3* genes in PCR analysis.

Primer†	Sequence (5'→3')	PCR Product	T _m ‡ (°C)	Source
<i>ACE</i> FP	CTGGAGACCACTCCATCCTTTCT	190/490 bp	69.4	Rigat et. al, 1992
<i>ACE</i> RP	GATGTGGCCATCACATTCGTCAGAT	190/490 bp	71.8	Ueda et. al, 1996
<i>ACTN3</i> Exon 15 FP	GGTGGGTAGGTGGGTGAGGC	192 bp	69.9	Mills et. al, 2001
<i>ACTN3</i> Exon 15 RP	GAGTGTACCAGCCACACGTCC	192 bp	66.9	
<i>ACTN3</i> Exon 16 FP	CTGTTGCCTGTGGTAAGTGGG	291 bp	67.3	
<i>ACTN3</i> Exon 16 RP	TGGTCACAGTATGCAGGAGGG	291 bp	66.5	Mills et. al, 2001

‡T_M=annealing temperature; †FP=forward primer, RP=reverse primer

ACE Gene PCR Reaction: Standard PCR reaction conditions for the *ACE* gene consisted of 1X PCR buffer (51mM KCl, 1.5mM MgCl₂, 10mM Tris•HCl, pH 9.2), 10% Dimethylsulfoxide (DMSO), 800µM dNTPs (deoxyribonucleotide triphosphates: 200mM each of dATP, dCTP, dGTP, dTTP), 0.8 µM of appropriate primer pair (Table 2), and 1-2 units (*Taq*) DNA polymerase. The master mix was added to 5 µl of the original DNA sample in a thin-walled PCR tube. A drop of mineral oil was added to each PCR mixture to prevent condensation of the sample during the PCR reaction. Dimethylsulfoxide was not an original component of the *ACE* PCR reaction, however it was subsequently included upon review of the literature wherein Ueda et. al (1996) determined that common errors in mistyping the products of the *ACE* gene could be avoided by optimization of the reaction to include 10% DMSO.

Table 3 *ACE* Gene PCR Reaction Conditions

Reactants	Stock Solution Concentration	Final Concentration in Reactions	Volume (µl) (1X)	Master Mix Volume (25X)
Deionized Water	-	-	10.9	272.5
Buffer (ACE #10)	10X	1X	2.5	62.5
dNTPs	10 mM	800 µM*	2.0	50.0
ACE FP	20 µM	0.8 µM	1.0	25.0
ACE RP	20 µM	0.8 µM	1.0	25.0
<i>Taq</i> Polymerase	5 units/µl	1 unit/µl	0.2	5.0
DMSO	100%	10%	2.5	62.5
DNA Sample	-	-	5.0	-
Total Volume			25.0	502.5

*200µM each of dATP, dCTP, dGTP, dTTP

ACE Gene PCR Cycle: The PCR cycle for the *ACE* gene consisted of 7 minutes at 95°C followed by 50 cycles of 30 seconds at 95°C, 30 seconds at 58°C and 1 minute at 72°C, with a final step of 7 minutes at 72°C. After the final extension, the block

temperature was held at 4°C until the samples could be removed and refrigerated until further examination.

ACE Gene Agarose Gel Electrophoresis and Analysis: Allelic analysis of the *ACE* gene was examined by size fractionation of PCR product through a 1.5% agarose/1X TAE (0.04M Tris•Acetate, 0.001M EDTA) gel containing 0.5 µg/ml ethidium bromide. The ethidium bromide is a fluorescing dye, that intercalates between base pairs of the DNA molecule and allows the sample to be viewed under a long wave UV light. A 100 bp ladder purchased from (Fisher-Scientific) was added in the first well to provide a standard to measure the molecular weight of bands in experimental samples. For each sample, a 20 µl volume of the PCR reaction was taken and mixed with 1.5 µl of 6X loading dye (30% glycerol, 0.25% Bromophenol Blue, 0.25% xylene cyanol). Electrophoresis proceeded for 4 hours at 150 volts or until adequate separation of the *ACE* alleles. The gel was then viewed under a long wave UV light with the Gel Doc-It Imaging System (Bioimaging Systems, Upland, Ca.) using the Labworks Program (Bioimaging Systems, Upland, Ca.). The size of the amplification product for the *I* allele is 490 bp and 190 bp for the *D* allele.

ACTN3 Gene PCR Reaction: The Exon 15 and 16 master mix (Table 4) consisted of 1X PCR Buffer (16mM (NH₄)₂SO₄, 1.5mM MgCl₂, 0.01% Tween 20, 68mM Tris•HCl, pH 8.8), 1.5mM Magnesium Chloride (MgCl₂), 0.8µM of the appropriate primer pairs (Table 2), 800µM dNTPs, and 1 unit *Taq* polymerase. The master mix was added to 3 µl of the original sample in a thin-walled PCR tube. A drop of mineral oil was added to each PCR mixture.

Table 4 *ACTN3* Exon 15 and 16 PCR Reaction

Reactants	Stock Solution Concentration	Final Concentration in Reactions	Volume (µl) (1X)	Master Mix Volume (25X)
Deionized Water	-	-	13.8	345.0
10X PCR Buffer	10X	1X	2.5	62.5
MgCl ₂	25 mM	1.5 mM	1.5	37.5
dNTPs	10 mM	800 µM	2.0	50.0
Exon 15/16 FP	20 µM	0.8 µM	1.0	25.0
Exon 15/16 RP	20 µM	0.8 µM	1.0	25.0
<i>Taq</i> Polymerase	5 units/ul	1 unit/ul	0.2	5.0
DNA Sample	-	-	3.0	-
Total Volume			25.0	550.0

***ACTN3* PCR Cycle:** The PCR cycle for both Exons 15 and 16 of the *ACTN3* gene consisted of 94°C for 5 minutes, 50 cycles of 94°C for 30 seconds and 60°C for 60 seconds, with a final extension of 72°C for 7 minutes. After the final extension, the block temperature was lowered to 4°C until the samples could be removed and refrigerated until further examination.

***ACTN3* Gene Agarose Gel Electrophoresis:** Prior to RFLP analysis PCR samples were prescreened by agarose gel electrophoresis to confirm the presence of appropriate sized PCR products, as described for the *ACE* gene. Amplification of Exons 15 and 16 produced 192 bp and 291 bp DNA fragments, respectively.

Restriction Enzyme Digestion: The samples that expressed the expected PCR products were digested with the restriction enzymes *MspI* and *DdeI* for exon 15 and 16, respectively, according to manufacturers instruction (Promega, Madison, Wisc.). A 10 µl quantity of the PCR reaction for each sample was combined with a Digestion Master Mix in a thin walled PCR tube and incubated at 37°C for 24 hours to ensure complete digestion (Table 5).

Table 5 *ACTN3* Gene Exon 15 and 16 Restriction Enzyme Digestion Master Mix.

Exon 15		Exon 16	
	Volume (μ l)/Reaction		Volume (μ l)/Reaction
Deionized Water	5.5	Deionized Water	5.5
10X NEB #2	2.0	10X NEB #3	2.0
10X BSA	2.0	10X BSA	2.0
<i>MspI</i> Restriction Enzyme (5 units)	0.5	<i>DdeI</i> Restriction Enzyme (5 units)	0.5
PCR Reaction Sample	10.0	PCR Reaction Sample	10.0
Total Volume	20.0	Total Volume	20.0

***ACTN3* Polyacrylamide Gel Electrophoresis:** Allelic analysis of the *ACTN3* gene was examined by size fractionation through a 10% polyacrylamide/1X TBE (0.045M Tris•Borate, 0.001M EDTA) gel. It contained 26 ml deionized water, 4 ml 10X TBE buffer solution, 10 ml 40% Acrylamide Stock, 140 μ l 25% APS, and 20 μ l TEMED (Tetramethylethylenediamine). Samples were prepared for loading on the gel by adding a volume 4 μ l of 6X Buffer/Dye to 20 μ l of the digestion sample. Electrophoresis proceeded for 4 hours at 150 volts, at which time the gel was stained for 30 seconds to 1 minute in 0.5 μ g/ml ethidium bromide solution. The gel was then destained in water until the desired resolution of the bands was reached. The gel and resultant RFLP patterns were visualized under long wave UV light using the Gel Doc-It Imaging System and the Labworks Program. The restriction enzyme products for Exon 15 were 118 and 74 bp for the 523Q allele and 21, 74, and 97 bp for the 523R allele. For Exon 16 the restriction enzyme products yielded were 205 and 86 bp for the 577R allele and 86, 97, and 108 bp for the 577X allele.

Statistical Analysis

A contingency Chi-Square analysis was used to determine if there were any significant frequencies of the different genotypes and alleles of the *ACE* gene and exons 15 and 16 of the *ACTN3* gene between the athletes and sedentary group. An α value of 0.01 was used to establish the critical value. The textbook “Statistics: A Biomedical Introduction” (Brown & Highlander, 1977) was used to determine the critical values for the corresponding degrees of freedom value for each test. In addition the athletes and sedentary group were analyzed to establish if there were any deviations from the Hardy-Weinberg (H-W) Equilibrium for the *ACE* and *ACTN3* gene polymorphisms. Accordance with the H-W equilibrium test implies that the gene and genotype frequencies are invariable from generation to generation.

RESULTS

Hardy-Weinberg Equilibrium Test

There was no substantial deviation from the Hardy-Weinberg Equilibrium among the athletes and the sedentary group for exons 15 and 16 of the *ACTN3* gene. The athletes were in H-W equilibrium for the *ACE* gene. However, there was a significant digression from the H-W Equilibrium ($\chi^2_{[df=1]}=18.394$; $P<0.01$) for the sedentary group with respect to *ACE* gene.

Allelic and Genotypic Analysis of the ACE Gene

PCR analysis of the *ACE* gene yielded a 190 bp fragment for the *D* allele and a 490 bp fragment for *I* allele (Figure 9). Genotypic data was compiled for both athletes and the sedentary group by scoring each sample as *II*, *DD*, or *ID* (Appendix A).

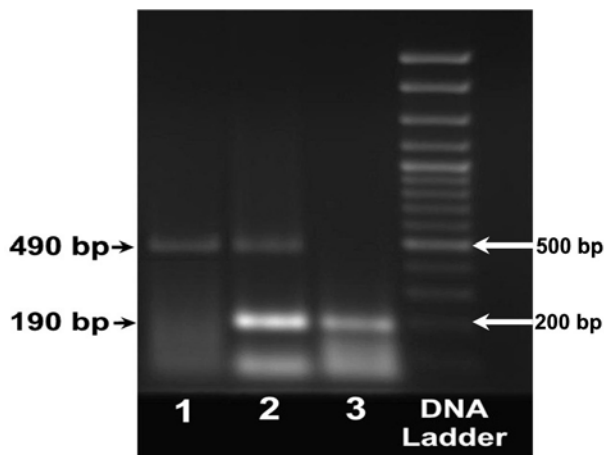


Figure 9 Agarose gel electrophoresis of the three possible genotypes of the *ACE* gene. Lane 1 represents a homozygous *II* genotype, Lane 2 heterozygous *ID* genotype, and Lane 3 the homozygous *DD* genotype.

The *ACE* genotyping data from the athletes and control groups is summarized in Table 6 and Figure 10. There were no significant allele ($\chi^2_{[df=1]}=0.00480$; $P>0.01$) or genotype ($\chi^2_{[df=2]}=3.546$; $P>0.01$) frequency differences between the athlete group as a whole and the sedentary group. However, when the athletes were divided into

endurance and non-endurance groups and compared to the sedentary group there was evidence of allele frequency variation ($\chi^2_{[df=2]}=10.037$; $P<0.01$). The endurance athletes had a highest percentage of the *I* allele (24.1%) when compared to the non-endurance athletes (7.1%) and sedentary subjects (12.5%).

Table 6 Number and Frequency (%) of *ACE* Genotypes and Alleles in Athletes and Sedentary Subjects.

Variables	Endurance Athletes (n=27)	Non-Endurance Athletes (n=63)	Athletes (n=90)	Sedentary Subjects (n=48)
Genotype				
II	3 (11.1%)	0 (0.0%)	3 (3.3%)	4 (8.3%)
ID	7 (25.9%)	9 (14.3%)	16 (17.8%)	4 (8.3%)
DD	17 (63.0%)	54 (85.7%)	71 (78.9%)	40 (83.4%)
Allele				
I	13 (24.1%)	9 (7.1%)	22 (12.2%)	12 (12.5%)
D	41 (75.9%)	117 (92.9%)	158 (87.8%)	84 (87.5%)

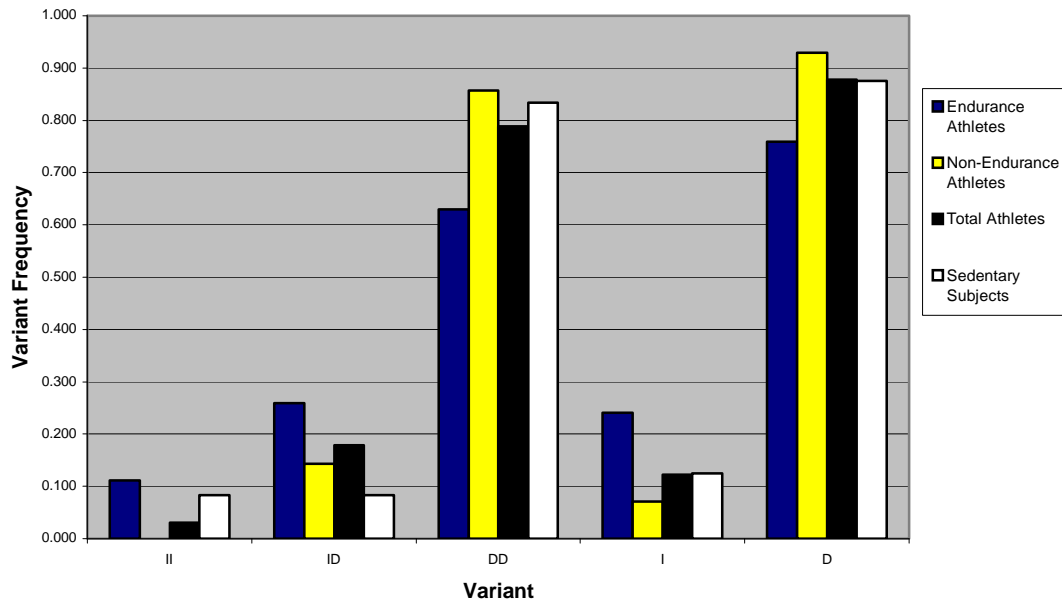


Figure 10 ACE genotype and allele frequency in endurance athletes, non-endurance athletes, total athletes and sedentary subjects. There are similar frequencies of each genotype between all of the groups. There is a significant allele frequency difference between the endurance athletes, non-endurance athletes, and sedentary subjects group.

Allelic and Genotypic Analysis of the ACTN3 Gene

Two polymorphic sites within the *ACTN3* gene were examined for genetic variation among athletes and sedentary controls. *MspI* RFLP analysis of Exon 15 yielded 118 and 74 bp fragments for the 523Q allele and 21, 74, and 97 bp fragments for 523R allele (Figure 9). Genotypes were scored for each sample and compiled for both athletes and the sedentary group (Appendix A).

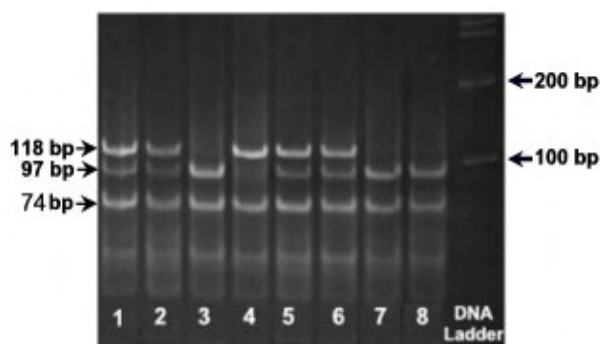


Figure 11 RFLP analysis of *ACTN3* Exon 15 demonstrating the three possible genotypes using polyacrylamide gel electrophoresis. Lanes 3, 7, and 8 represent the homozygous RR genotype. Lanes 1, 2, 5, and 6 represent the heterozygous RQ genotype. Lane 4 represents the homozygous QQ genotype.

Exon 15 of the *ACTN3* gene genotyping data from the athletes and sedentary group is summarized in Table 7 and Figure 12. There were no considerable genotype ($\chi^2_{[df=2]}=2.917$; $P>0.01$) or allele ($\chi^2_{[df=1]}=0.00865$; $P>0.01$) frequency differences between the athlete group as a whole and the sedentary group. Furthermore, when the athletes were divided into two groups and compared to the sedentary group, the genotype ($\chi^2_{[df=4]}=3.115$; $P>0.01$) and allele ($\chi^2_{[df=2]}=0.0875$; $P>0.01$) frequencies were not significant. The 523R allele values between endurance athletes (46.3%), non-endurance athletes (46.9%), and sedentary subjects (44.8%) were observed. The 523Q allele yielded very similar results as well with values of 53.7%, 53.1% and 55.2% for the endurance athletes, non-endurance, and sedentary subjects respectively.

Table 7 Number and Frequency (%) of *ACTN3* Exon 15 Genotypes and Alleles in Athletes and Sedentary Subjects.

Variables	Endurance Athletes (n=27)	Non-Endurance Athletes (n=49)	Athletes (n=76)	Sedentary Subjects (n=48)
Genotype				
RR	6 (22.2%)	13 (26.5%)	19 (25.0%)	8 (16.7%)
RQ	13 (48.1%)	19 (38.8%)	31 (40.8%)	27 (56.2%)
QQ	8 (29.7%)	17 (34.7%)	26 (34.2%)	13 (27.1%)
Allele				
R	25 (46.3%)	45 (46.9%)	69 (45.4%)	43 (44.8%)
Q	29 (53.7%)	51 (53.1%)	83 (54.6%)	53 (55.2%)

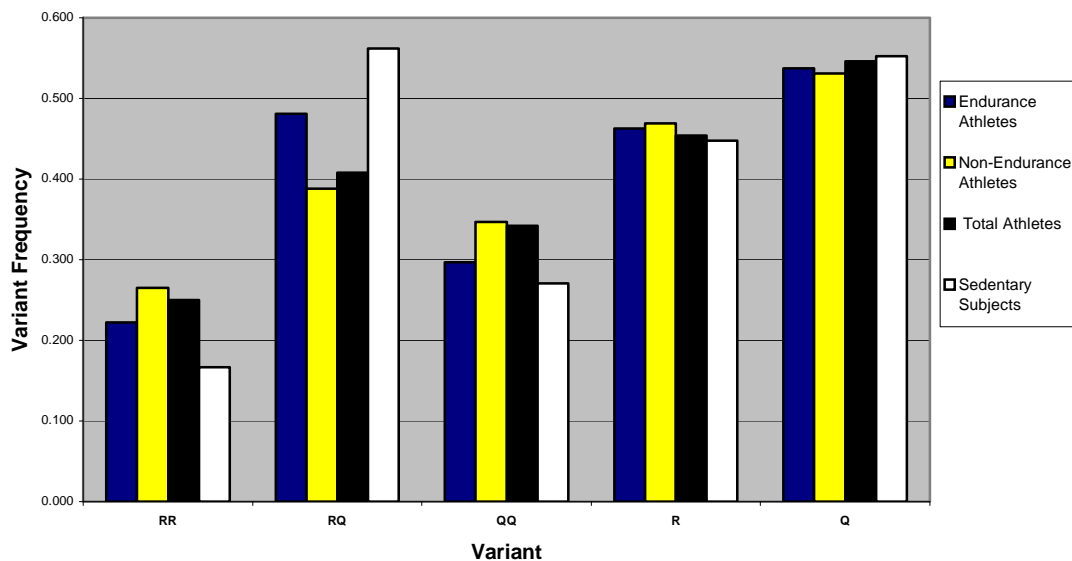


Figure 12 *ACTN3* Exon 15 genotype and allele frequency in endurance athletes, non-endurance athletes, total athletes and sedentary subjects. There are similar genotypic and allelic frequencies between all groups.

DdeI RFLP analysis of Exon 16 yielded 205 and 86 bp fragments for the 577R allele and 86, 97, and 108 bp fragments for the 577X allele (Figure 13). Genotypes were scored for each sample and compiled for both athletes and the sedentary group (Appendix A).

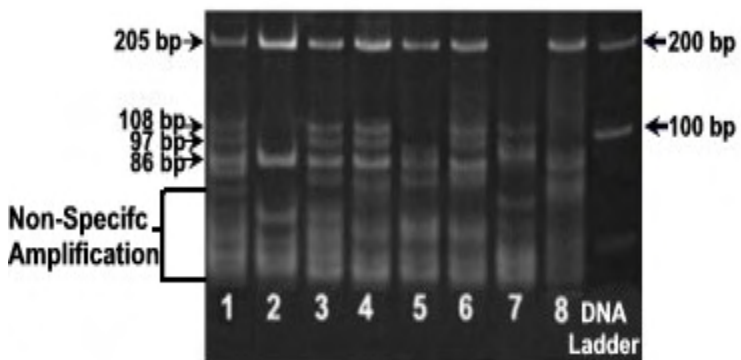


Figure 13 RFLP analysis of *ACTN3* Exon 16 demonstrating the three possible genotypes using polyacrylamide gel electrophoresis. Lanes 1, 3, 4, and 6 represent the RX genotype. Lanes 2, 5, and 8 represent the RR genotype. Lane 7 represents the XX genotype.

Exon 16 of the *ACTN3* gene genotyping data from the athletes and control groups is summarized in Table 8 and Figure 14. There were no significant genotype

($\chi^2_{[df=2]}=3.133$; $P>0.01$) frequency differences between the athlete group as a whole and the sedentary group. However, there was strong evidence of allele frequency variation ($\chi^2_{[df=1]}=11.083$; $P<0.01$) between the athletes and sedentary group. The athletes had a higher frequency of the 577R allele (58.5%) when compared to the sedentary subjects (36.5%).

A significant difference in genotype frequency ($\chi^2_{[df=4]}=5.755$; $P>0.01$) was not observed between the endurance and non-endurance athletes and the sedentary group. The increased frequency of the 577R allele was prominent in endurance athletes (55.6%) and non-endurance athletes (61.4%) and significant ($\chi^2_{[df=2]}=12.187$; $P<0.01$) when compared to the sedentary group. There was linear increase in the 577R allele from the sedentary group (36.5%), endurance athletes (55.6%), and non-endurance athletes (61.4%). In a similar trend, there was a linear decrease of the X allele in the sedentary group (63.5%), endurance athletes (44.4%), and non-endurance athletes (38.6%).

Table 8 Number and Frequency (%) of *ACTN3* Exon 16 Genotypes and Alleles in Athletes and Sedentary Subjects.

Variables	Endurance Athletes (n=27)	Non-Endurance Athletes (n=44)	Athletes (n=71)	Sedentary Subjects (n=48)
Genotype				
RR	8 (29.6%)	15 (34.1%)	22 (31.0%)	12 (25.0%)
RX	14 (51.9%)	24 (54.5%)	39 (54.9%)	23 (47.9%)
XX	5 (18.5%)	5 (11.4%)	10 (14.1%)	13 (27.1%)
Allele				
R	30 (55.6%)	54 (61.4%)	83 (58.5%)	35 (36.5%)
X	24 (44.4%)	34 (38.6%)	59 (41.5%)	61 (63.5%)

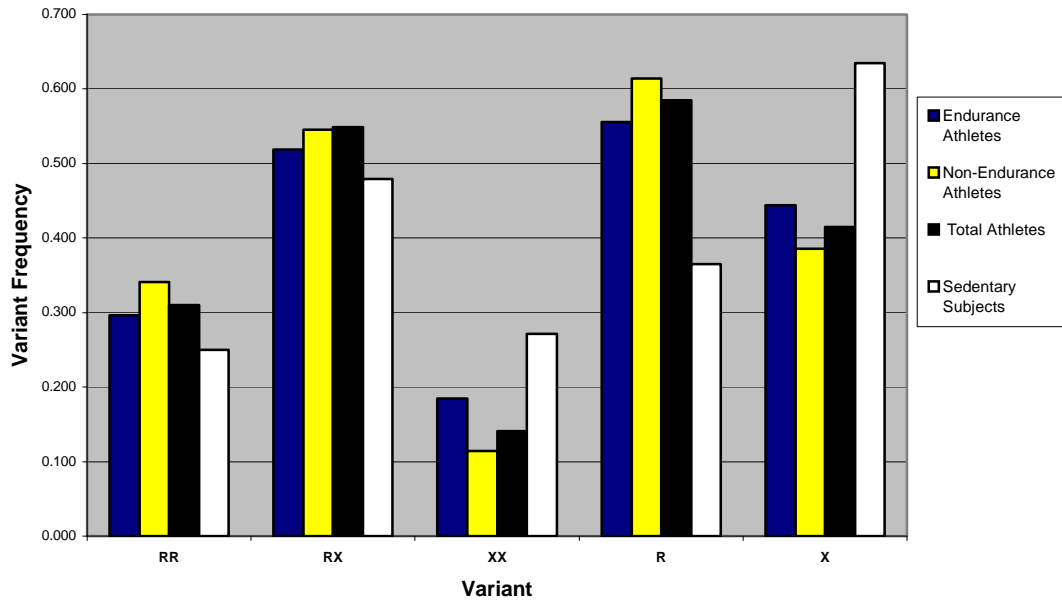


Figure 14 *ACTN3* Exon 16 genotype and allele frequency in endurance athletes, non-endurance athletes, total athletes and sedentary subjects. There is a marked increase of the 577R allele in the athlete group when compared to the sedentary group. The 577R allele occurred in the highest frequency among non-endurance athletes, second highest in endurance athletes and lowest in sedentary subjects.

DISCUSSION

In the past 5-10 years extensive research has been completed with respect to Human Performance Genetics. Scientists and doping officials are worried that the next form of performance enhancement will stem from this area of research to create athletes that are genetically modified through 'gene doping' to sprint faster, run longer, or jump higher. This project aimed to examine the athletic performance related *ACE* and *ACTN3* genes in a group of NCAA athletes from the University of Tennessee at Chattanooga and a group of sedentary students from the same institution. The athletes were compared to the sedentary group as a whole and then subdivided into endurance athlete and non-endurance athlete groups. The endurance athlete group consisted of the men's and women's track & field and cross-country teams whereas the non-endurance athlete group consisted of the rest of the athletics teams (Men's and Women's Basketball, Men's and Women's Tennis, Men's Wrestling, Men's Golf, and Women's Softball) examined in the study.

The *ACE* gene codes for the Angiotensin Converting Enzyme, which is responsible for regulating blood volume, arterial pressure, electrolyte balance, and cardiac and vascular function. The *I* allele of the gene is thought to be beneficial to endurance athletes. On the other hand, the *ACTN3* gene encodes the 901 amino acid alpha-actinin-3 protein found only in Type II (Fast-Twitch) muscle fibres. Its function is to provide structural support for the transmission of force during muscle contraction along the Z line and sustain the order of myofilaments and coordinate myofilament contraction (Yang et. al, 2003). Exons 15 and 16 were examined in this

project because of their importance to *ACTN3* gene function. A point mutation in exon 15 of the *ACTN3* gene introduces an Arginine(R)→Glutamine(Q) substitution at amino acid residue 523. A polymorphism in exon 16 exhibits as a premature stop codon, Arginine(R)→Stop(X) at position 577. The R allele of Exon 16 is thought to be advantageous to athletes that require short and forceful bursts of power due to full-length and functional *ACTN3* proteins.

ACE Gene

The *I* allele of the *ACE* gene is thought to present beneficial effects to athletes that compete in endurance events such as distance running, cycling, and swimming. In this study of 90 UTC athletes and 48 sedentary students, the frequency of the *I* allele was not found to be notably different between the two groups, however when the athletes were separated into endurance and non-endurance groups statistical analysis revealed a significant difference among the endurance athlete and non-endurance athlete groups and the sedentary students. The endurance athletes, non-endurance athletes, and sedentary students had *I* allele frequencies of 24.1%, 7.1%, and 12.5% (Table 6). The results suggest that combination of the endurance and non-endurance athletes that have increased frequencies of the *I* and *D* alleles respectively, essentially cancelled each other out thereby masking any significant differences present in specific groups of athletes.

These results were consistent with previously reported findings by Myerson et. al (1999) who did not find significant differences of *I* allele when sprint and distance runners were combined in a single group and compared to a control group.

However, they further stratified the athletes into different groups based on race distance and found *I* allele frequencies of 32% in sprint/power, 62% in middle (400m-3,000m) and distance ($\geq 5,000$ m) Olympic standard runners. The values from this project were 7.1% for non-endurance athletes and 24.1% for endurance athletes for the *I* allele. Thus, the lower frequency of the *I* allele may have occurred because it may not play as important of a role in determination of performance at a collegiate level. To confirm this statement a larger sample size from a collection of NCAA varsity athletic programs would have to be examined. In addition, the championship winning teams for each athletic discipline (eg. Cross-Country, Track & Field, Wrestling) could be examined to determine whether there was a difference between championship programs and less successful programs.

Conversely, the endurance and non-endurance athlete and control group genotype frequencies were different than previously published report by Scanavini et. al. (2002). There was a lower incidence of the *II* genotype in all endurance athletes (3.3%), non-endurance athletes (0%) and the sedentary group (8.3%) in this project when compared to the aerobic (endurance) athletes (21.1%), anaerobic (non-endurance) athletes (5.5%) and control group (12.5%) in the report by Scanavini et. al (2002). It is difficult to compare the athlete values with other literature values because there are not other studies on the NCAA collegiate level athletes. Furthermore, the athletes in most of the studies were of elite status and it is probable that their success has a greater dependence on their genetic makeup.

The control data was difficult to analyze because it deviated from the H-W Equilibrium for the *ACE* gene. Scanavini et. al (2002) had frequencies of 12.5%, 43.4%, and 44.1% for *II*, *ID*, and *DD* genotypes respectively for their control group. Collins et. al (2004) found frequencies of 42.2% and 57.8% for the *I* and *D* alleles in their control group. The results from this project indicate much lower values in both genotypic (8.3%, 8.3%, and 83.4% for *II*, *ID*, *DD*) and allelic (12.5% and 87.5% for *I* and *D*) frequencies for the control groups (Table 6). The deviation from the literature values could have been caused by a smaller sample size of 48 in this project compared to 152 (Scanavini et. al, 2002) and 199 in the report by Collins et. al (2004). Thus, a smaller control group decreases the probability of variation of genotypes.

It was hypothesized that the *I* allele would be found more frequently in athletes when compared to the sedentary group. This prediction was not observed in the study. It was also theorized that the *I* allele would be found in a greater frequency in the endurance athlete group. This hypothesis was observed in the results. The men's and women's track & field and cross-country teams consist primarily of runners that compete in the races that range in distances from 800m to 10,000m. Both teams are successful within the Southern Conference, NCAA Regional level, and even have several members that compete at the national level. Thus, from the effect the *I* allele has on the Renin-Angiotensin system and ultimately the increased Oxygen delivery to working muscles, these athletes would benefit from this allele.

ACTN3 Gene

Exons 15 and 16 of the *ACTN3* gene were examined in this project. Exon 15 had never been reported in relation to athletic performance. The results from the exon 15 data were insignificant between the athletes, subdivided athletic groups and the sedentary students (Table 7 and Figure 12). The equality of the variants of exon 15 has several possible explanations. The exon 15 polymorphism results in allelic variants distinguished by encoding either an Arginine or Glutamine at amino acid residue 523 in the full-length protein. Whereas Arginine is positively charged and Glutamine is uncharged, both of these amino acids carry polar side chains. It is possible that this substitution does not significantly alter the structure or function of the protein despite their charged nature, and thus the variants act similarly within the cell. Alternatively, the exon 15 polymorphism simply may not represent a heritable factor that influences phenotypic variation of athletic performance, or may only present an effect in conjunction with other genetic variations.

On the contrary, the 577R allele of exon 16 has been connected with athletes that utilize primarily Type II (Fast Twitch) muscle fibres during their events that require short and forceful bursts of power. There was a significant difference in the allele frequency between the athlete and sedentary groups (Table 8, Figure 14). The athletes had a 577R allele frequency of 58.5% compared to 36.5% in the sedentary students (Table 8). In addition, the RR genotype was found in 31.0% of the athletes and only 25.0% in the sedentary students.

Furthermore, stratification of the athlete group into endurance athletes and non-endurance athletes and when these groups were compared to the sedentary students revealed strong evidence of allele variation among these groups. The 577R allele occurred in frequencies of 61.4%, 55.6%, and 36.5% in the non-endurance athletes, athletes, and sedentary students (Table 8, Figure 14).

It was hypothesized that the 577R allele would be found at an increased frequency among the athletes as a whole when compared to the sedentary group because it represents a functional allele of the *ACTN3* gene. While not all athletes utilize Type II muscle fibres all of the time, the ability to have a fully functional α -actinin-3 protein may benefit all types of athletes during training and competition. The results obtained for exon 16 were comparable to those observed by Yang et. al (2003) in which the RR and RX genotypes of the sprint (non-endurance) athletes was 50% and 45% compared to the controls 30% and 50%. In addition their study showed that the 577R allele was found in greater frequency in the sprint group when compared to the endurance group. Interestingly, they observed deviations of the alleles in different directions between the sprint and endurance groups such that the differences between the groups “cancelled each other out” when they were combined as one group. This trend was not observed between the endurance athletes and non-endurance athletes in this report. Whereas the non-endurance athletes had the highest frequency of the R allele (61.4%) the endurance athletes had a relatively similar value of 55.6% and both values were higher than the sedentary group (36.5%) (Table 8, Figure 14). Thus, the results of this report are not as extreme as the data in the study

by Yang et. al. This may be due to the fact that the Australian athletes in the study by Yang. et. al were Olympic and International caliber. As a result, the data obtained from UTC athletes followed a similar trend with the 577R allele more prevalent in non-endurance athletes but not to the same degree as compared to the elite level athletes. Thus, it can be concluded that from the data obtained in this project, it appeared that the 577R allele is related to athletic performance and is more common in non-endurance athletes.

Conclusion

The field of human performance genetics is an area of science that is continually growing with the advent of increased competition of professional sports. Athletes and scientists alike are looking toward genetics to explain athletic abilities such as endurance, speed, and strength. Through publications such as The Human Gene Map for Performance and Health Related Fitness Phenotypes (Wolfarth et. al, 2004) many different genetic variants have been associated to the aforementioned athletic traits. The *ACE* and *ACTN3* genes are two of the most widely studied genes because of their opposite effects on the human body. The results of this project concur with previous studies in that the *I* allele of the *ACE* gene was found more frequently in endurance athletes and more non-endurance athletes have the 577R allele of Exon 16 in the *ACTN3* gene. The athletes examined in this project represent a very small percentage of the 156,420 NCAA Division I athletes (ncaa.org). A more broad and extensive study of this level of athletic competition is needed before definite conclusions can be made on whether the genetic variants of each gene are

found in a significant frequency between different types of athletes and the sedentary student population. The results obtained in this study provide insight into the possible effects that the variants of the *ACE I/D* and *ACTN3* polymorphisms have on athletic performance.

FUTURE CONSIDERATIONS

The results obtained in this project provide useful information for the area of the Human Performance Genetics. Optimistically, this report is to be published in a peer reviewed scientific journal. In order for this goal to be achieved, further statistical analysis is needed to determine if a correlation exists between the genetic variants of the *ACE* and *ACTN3* genes. Specifically the analysis will examine if there is a potential gene interaction among the 3 polymorphic sites that have 27 possible genotype combinations. For example, the *ACE DD*, *ACTN3 523QQ*, *577XX* genotype combination may occur in a higher frequency in the sedentary group when compared to the athletes.

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APPENDIX A: Athlete and Sedentary Group Data

UTC Athletes

Subject Number	Sex	Sport	ACE Gene	ACTN3 Gene	
				Exon 15	Exon 16
15	M	Wrestling	DD	x	x
16	M	Wrestling	DD	RQ	x
34	M	Wrestling	DD	QQ	x
37	F	W Basketball	DD	QQ	x
109	F	W Softball	DD	QQ	x
32	M	Wrestling	DD	RR	x
17	M	Wrestling	DD	RQ	RR
41	F	W Basketball	DD	QQ	RR
46	F	W Basketball	DD	QQ	RR
50	M	M Basketball	DD	QQ	RR
55	M	M Basketball	DD	QQ	RR
57	M	M Basketball	DD	QQ	RR
80	F	W Track/CC	DD	QQ	RR
103	F	W Track/CC	DD	QQ	RR
77	F	W Track/CC	DD	RQ	RR
79	F	W Track/CC	DD	RQ	RR
100	M	M Track/CC	DD	RQ	RR
104	F	W Softball	DD	RQ	RR
110	F	W Track/CC	DD	RQ	RR
68	M	M Tennis	DD	RR	RR
82	F	W Track/CC	DD	RR	RR
105	F	W Softball	DD	RR	RR
3	M	Wrestling	DD	x	RR
42	F	W Basketball	DD	QQ	RX
81	F	W Track/CC	DD	QQ	RX
91	F	W Softball	DD	QQ	RX
92	F	M Track/CC	DD	QQ	RX
96	M	M Track/CC	DD	QQ	RX
106	F	W Softball	DD	QQ	RX
7	M	Wrestling	DD	RQ	RX
19	M	Wrestling	DD	RQ	RX
48	F	W Basketball	DD	RQ	RX
49	M	Wrestling	DD	RQ	RX
53	M	M Basketball	DD	RQ	RX
56	M	M Basketball	DD	RQ	RX
64	M	M Golf	DD	RQ	RX
71	M	M Tennis	DD	RQ	RX
74	M	M Tennis	DD	RQ	RX
83	F	W Tennis	DD	RQ	RX
93	F	W Track/CC	DD	RQ	RX
98	F	W Track/CC	DD	RQ	RX
99	M	M Track/CC	DD	RQ	RX
102	M	M Track/CC	DD	RQ	RX
111	F	W Softball	DD	RQ	RX
112	F	W Softball	DD	RQ	RX

113	F	W Softball	DD	RQ	RX
116	F	W Softball	DD	RQ	RX
72	F	W Tennis	DD	RR	RX
73	F	W Tennis	DD	RR	RX
75	F	W Tennis	DD	RR	RX
84	F	W Tennis	DD	RR	RX
95	F	W Track/CC	DD	RR	RX
1	M	Wrestling	DD	x	RX
26	M	Wrestling	DD	QQ	x
2	M	Wrestling	DD	RQ	x
5	M	Wrestling	DD	x	x
13	M	Wrestling	DD	x	x
24	M	Wrestling	DD	x	x
60	M	M Golf	DD	x	x
114	F	W Softball	DD	x	x
115	F	W Softball	DD	x	x
76	F	W Tennis	DD	QQ	XX
58	M	M Basketball	DD	RR	XX
67	M	M Tennis	DD	RR	XX
78	F	W Track/CC	DD	RR	XX
101	M	M Track/CC	DD	RR	XX
6	M	Wrestling	DD	x	XX
8	M	Wrestling	DD	x	x
10	M	Wrestling	DD	x	x
12	M	Wrestling	DD	x	x
107	F	W Softball	DD	x	x
69	M	M Tennis	ID	QQ	x
44	F	W Basketball	ID	QQ	RR
70	M	M Tennis	ID	QQ	RR
65	M	M Golf	ID	RR	RR
66	M	M Golf	ID	RR	RR
47	F	W Basketball	ID	QQ	RX
62	M	M Golf	ID	QQ	RX
86	M	M Track/CC	ID	QQ	RX
87	M	M Track/CC	ID	RQ	RX
94	F	W Track/CC	ID	RQ	RX
97	F	W Track/CC	ID	RQ	RX
39	F	W Basketball	ID	RR	RX
108	M	M Track/CC	ID	QQ	XX
63	F	W Track/CC	ID	RQ	XX
61	M	M Golf	ID	RR	XX
85	M	M Track/CC	ID	RR	XX
89	M	M Track/CC	II	QQ	RR
117	M	M Track/CC	II	RQ	RX
88	F	W Track/CC	II	RR	RX

Sedentary Subjects

Subject Number	Sex	ACE Gene	ACTN3 Gene	
			Exon 15	Exon 16
118	F	DD	RR	RR
124	M	DD	QQ	RR
129	M	DD	RR	RR
130	M	DD	QQ	RR
134	F	DD	QQ	RR
136	M	DD	QQ	RR
147	F	DD	RQ	RR
150	M	DD	RQ	RR
154	F	DD	RQ	RR
155	F	DD	RQ	RR
120	F	DD	QQ	RX
121	F	DD	RR	RX
128	M	DD	QQ	RX
133	M	DD	QQ	RX
137	M	DD	QQ	RX
139	M	DD	RQ	RX
140	M	DD	RQ	RX
141	F	DD	RQ	RX
142	F	DD	RQ	RX
143	F	DD	RQ	RX
144	F	DD	RQ	RX
145	M	DD	RQ	RX
146	M	DD	RQ	RX
148	M	DD	RQ	RX
151	M	DD	RQ	RX
152	F	DD	RQ	RX
153	F	DD	RQ	RX
156	M	DD	RQ	RX
157	F	DD	RQ	RX
159	M	DD	RQ	RX
119	F	DD	RR	XX
122	M	DD	QQ	XX
123	F	DD	RR	XX
125	F	DD	RR	XX
126	M	DD	QQ	XX
127	F	DD	RR	XX
132	F	DD	QQ	XX
135	F	DD	QQ	XX
149	F	DD	RQ	XX
158	M	DD	RQ	XX
160	M	ID	RQ	RR
162	F	ID	RQ	RR
131	M	ID	RR	XX
161	F	ID	RQ	XX
138	F	II	QQ	RX

163	F	II	RQ	RX
164	F	II	RQ	RX
165	F	II	RQ	XX



**INFORMED CONSENT FOR PARTICIPATION IN RESEARCH
ACTIVITIES**

Title of Project: **Examination of the ACE and ACTN3 Genes in UTC
Varsity Athletes and Sedentary Students**

*The Institutional Review Board of the University of Tennessee at Chattanooga
(FWA00004149) has approved this research project (UTC IRB# 05-055)*

- 1.a) You are invited to participate in a research study conducted by Ian Mayne and Dr. Margaret Kovach. The overall purpose of this research is to examine whether there is a relationship between two specific genes and athletic performance when compared to a control group.
- b) Your participation will involve the completion of this consent form that will permit the researchers to extract your DNA using a small brush to remove a number of the cells from the inside of your mouth.
- c) The amount of time involved in your participation will be very brief, no longer than 10 minutes.
2. There are no known risks associated with this type of research; there will be no discomfort to the subject as a result of the DNA extraction.
3. There are no known benefits to your participation in this research other than the enhancement of scientific knowledge.
4. Your participation is entirely voluntary and you may choose not to participate in this research study or withdraw your consent at any time.
5. We will do everything we can to protect your privacy. As part of this effort, your identity will not be revealed in any publication that may result from this study. In rare instances, a researcher's study must undergo an audit or program evaluation by an oversight agency (such as the Office for Human Research Protection) that would lead to disclosure of your data as well as any other information collected by the researcher. If this were to occur, such information would only be used to determine whether the researcher conducted this study properly and adequately protected your rights as a human participant. Importantly, any and all auditing agencies would maintain the confidentiality of any information reviewed by their office(s).
6. If you have any questions or concerns regarding this study, please contact Ian Mayne at 423-266-5478 or Dr. Kovach at 423-425-4397.
7. Information about potential subject:

Sex: _____

Age: _____

Ethnicity: _____

Height: _____

Weight: _____

Varsity Sport: _____ (Not applicable to control subjects)

I have read this consent form and have been given a chance to ask questions. I agree to participate in the research study described above, titled “Examination of the ACE and ACTN3 Genes in the UTC Varsity Athletes and Sedentary Students.”

Signature of Research Participant

Date (MM/DD/2005)

Printed Name of Research Participant

Signature of person obtaining consent

Date (MM/DD/2005)

from Research Participant

Printed Name of person obtaining consent from Research Participant