

# The polymorphic ACE gene and resulting genotypes and allele frequencies within specific groups

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## ABSTRACT

The polymorphic angiotensin I-converting enzyme (ACE) gene was one of the first genetic elements to demonstrate a significant impact on human performance. Since then, it has been shown to play a role in coronary artery disease. The functional genetic alleles have been determined. The ACE phenotypes have been associated with improvements in athletic performance, endurance, as well as association with blood pressure variations. The I allele has been connected with endurance events, while the D allele is associated with strength and power athletic events, as well as coronary disease. The objectives for this study were twofold, first to provide a guided inquiry research experience for undergraduate students based on a topic in class, and second, to examine the ACE gene alleles present in five distinct populations: Men's and Women's hockey teams, elite (Olympic level) bobsledders, Ironman Triathletes, members of the college's track team, and African American college students. The results showed significant differences ( $p < 0.05$ ) observed in the distribution of ACE genotype polymorphisms between the control group and both the men's and women's hockey teams, but not in the African American population and international bobsledders or track team members.

Key Words: *Ace gene, angiotensin I-converting enzyme, genotypes, alleles, insertions/deletions*

## INTRODUCTION

The gene of interest for this research investigation was the angiotensin-converting enzyme gene, better known as the ACE gene. The ACE gene influence has been widely explored and published in reference to athletic performance (MacArthur & North, 2005; Tsianos et al. 2004; Brutsaert & Parra, 2006; Costa et al., 2009; Puthuchery et al., 2011). It also appears that the ACE gene has a genetic linkage to blood pressure variations (Badaruddoza et al., 2009; Akgavaid et al., 2000; Cox et al., 2002; Morshed et al., 2002;). The ACE gene is found on chromosome 17. It is noted that the 16<sup>th</sup> intron has polymorphisms based on insertions or deletions. The insertion and/or deletion involves a 287 base pair Alu transposable element (Sayed-Tabatabaei et al., 2006) leading to three possible genotypes DD, D/I, or II (Figure 1). The DD genotype has been associated with coronary artery disease (Samani, et al., 1996; Mattu et al., 1995; Prasad et al., 2000). Phenotypically, elite, short-distance swimmers (Woods et al, 2001) and strength and power athletes (Myerson, et al., 1999; Folland et al., 2000; Puthuchery et al., 2011) have a higher frequency of the DD genotype. Conversely, the II genotype has been associated with endurance events, such as ironman triathlons (Collins et al., 2004), long distance running (Myerson et al. 1999) and endurance swimming (Tsianos et al., 2004).

This paper compiles the results from four undergraduate research students' examinations of the ACE genotype frequencies, using five very distinct populations: Men's and Women's hockey teams, African American college students, elite (Olympic level) bobsledders, Ironman triathletes and members of the college's track team. Students from the general genetics and general biology classes (100 students) served as the control. Each student investigator hypothesized that there would be a higher frequency of the DD

genotype found within their specific population of study, except for the Ironman triathletes. It was thought that triathletes would have a higher insertion (I) variant than the control, due to this variant being associated with performance in endurance athletes.

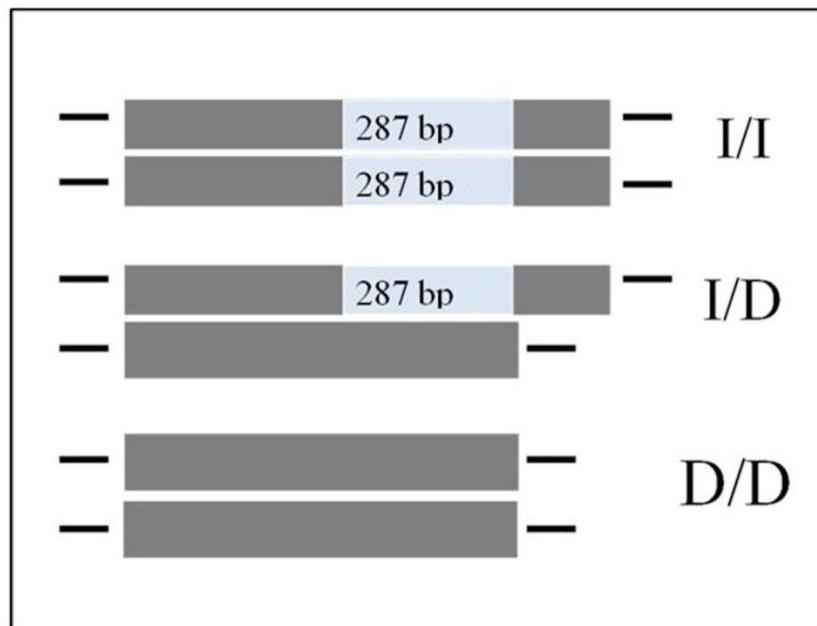


Figure 1. Schematic diagram of the ACE gene illustrating the insertion/insertion (I/I), insertion/deletion (I/D) and the deletion/deletion (D/D) genotypes. The size of the insertion is 287 base pairs (bp). Diagram created by Jeremy Schmidt.

## METHODS

Sterile saline solution (10 mL) was used to obtain buccal cells. One hundred samples were collected from college biology and genetics classes and served as the control; fifty samples were collected from African American college students, and thirty-five samples were collected from elite bobsledders when they were competing at the International Bobsled Championship in Lake Placid, NY; fifty-one samples were collected from the Plattsburgh State hockey teams, men (thirty samples) and women (twenty one samples); fifteen samples were collected from Ironmen triathletes competing in the Lake Placid Ironman Triathlon, and twenty samples collected from the Plattsburgh State track team.

DNA was isolated following the protocol “DNA Isolation by Saline Mouthwash” (Genetic Origins). The ACE genotypes were determined through DNA samples amplified through polymerase chain reaction (PCR) and using the following primers: 5’-CTGGAGAGCCACTCCCATCCTTTCT-3’ (forward) and 5’-GACGTCGCCATCACATTCGTCAGAT-3’ (reverse). The PCR reaction was set up in a final volume of 25  $\mu$ l, using 100 ng DNA (as measured using a Nanodrop spectrophotometer), 0.2  $\mu$ M of each primer, 200  $\mu$ M dNTPs, 1.5 mM MgCl<sub>2</sub> and 0.025 units of *Taq* polymerase.

After the initial denaturation at 95°C for 3 minutes, DNA samples were amplified for 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and primer extension at 72°C for 45 seconds. A final extension step at 72°C for 10 minutes was performed. The amplified PCR samples were separated via electrophoresis in a 1.5% agarose gel containing ethidium bromide (7 µl of a [10 mg/mL]/40mL of agarose gel), and visualized under UV light. The I allele was determined by the presence of a ~490 bp fragment, while the D allele was verified by a ~190 bp band (Figure 2).

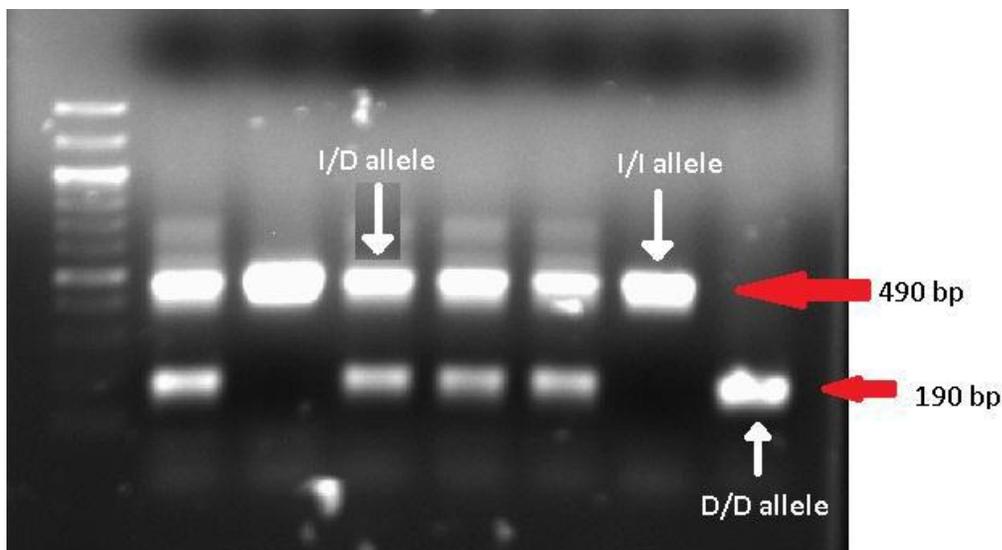


Figure 2. DNA gel electrophoresis genotype results for five samples. Samples with an insertion (I) are ~490 base pairs in length; samples with a deletion (D) are ~190 base pairs in length. M = the 100 base pair standard. Genotypes and allele frequencies for each test population were compared against the control using Chi-Square analysis (Alpha value of 0.05).

All protocols were approved through the Committee on the Protection of Human Subjects (COPHS). Each undergraduate research student also went through the Collaborative Institutional Training Initiative (CITI) course. In addition, each sample submitted had a signed consent form from the contributor.

## RESULTS

The ACE genotypes and allele frequencies are presented in Figures 3 and 4 respectively. The frequencies of the DD, ID, and II genotypes among the control (n = 100) were DD 32% (32), ID 44% (44), and II 24% (24). Whereas the African American group (n = 50) frequencies were DD 32% (16), ID 52% (26), and II 16% (8) for the same genotypes. The elite bobsledders (n= 37) were DD 42.86% (15), ID 45.71% (16), and II 11.43% (6); the men's hockey team's (n=30) frequencies were DD 30% (9), ID 66.67% (20) and II 3.33% (1), while the women's hockey team's (n = 21) frequencies were DD 14.29% (3), ID 76.19% (16) and II 9.52% (2). The Ironman athletes (n = 15) had 20% (3) DD, 46.7% (7) ID, and 33.3% (5) II. Finally, the track team members (n = 20) had 20% (4) DD, 55% (11) ID, and 25% (5) II.

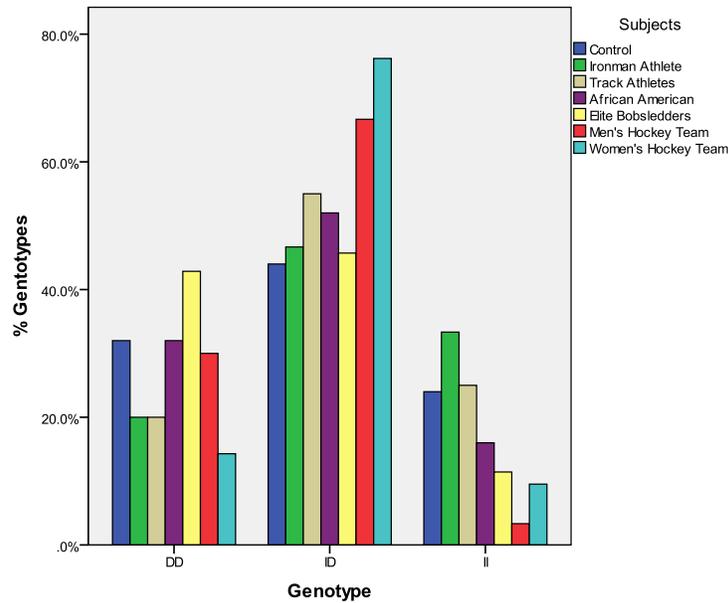


Figure 3. Distribution of genotype results within the experimental groups and the control. The elite bobsledders had the highest percentage of the D/D genotype; while the Ironman athletes had the greatest percentage of the I/I genotype.

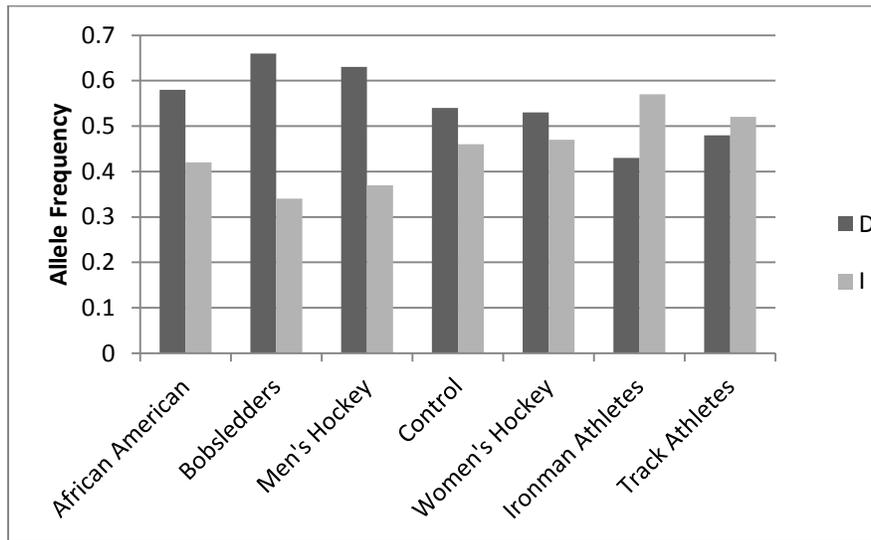


Figure 4. Allele frequencies within the experimental and control groups. The bobsledders had the highest frequency for the D allele, while the Ironman triathletes had the highest I allele frequency. Table I shows the experimental results compared to the control along with results from other publications. There were significant differences ( $p < 0.05$ ) observed in the distribution of ACE genotype polymorphisms between the control and both the men's and women's hockey teams (Figure 3, Table 1), while there was no significant differences between the control and the elite bobsledders and African American groups ( $p$  value 0.151 and 0.357, respectively). It was observed that within the experimental groups, the ACE DD genotype showed no significant differences when compared to the control (data not shown). Whereas, the ID genotype was significantly ( $p < 0.05$ ) higher in the women's hockey team when compared against the control. The frequency of the D allele was significantly ( $p < 0.05$ ) higher in the elite bobsled group, it was also higher in the men's hockey team but not significant (Figure 4, Table 1).

It should be noted that within the control group (n=100), there were 23 African Americans, but there were no bobsledders and/or hockey players. Additionally, there were no African Americans within the bobsledder test group or the hockey teams.

<b>Table 1. ACE Genotype and Allele Distributions</b>								
<b>Groups</b>	<b>DD (%)</b>	<b>ID (%)</b>	<b>II (%)</b>	<b>P value</b>	<b>D</b>	<b>I</b>	<b>Reference</b>	<b>n</b>
<b>Elite Endurance Swimmers</b>	6.2	75	18.8	0.021	0.44	0.56	Tsianos et al., 2004	16
<b>Elite Short Distance Swimmers</b>	51	36	13	0.113	0.69	0.31	Costa et al., 2009	39
<b>Control</b>	39	45	16		0.62	0.32	Costa et al., 2009	100
<b>Hypertension in Business World</b>	46	32	22	0.0013	0.49	0.51	Badaruddoza et al., 2009	50
<b>Control</b>	20	54	26		0.53	0.47	Badaruddoza et al., 2009	50
<b>Fast Ironman Triathletes</b>	27.7	50.0	22.2	0.005	0.48	0.52	Collins et al., 2004	72
<b>Slow Ironman Triathletes</b>	24.65	46.57	28.76	0.019	0.52	0.48	Collins et al., 2004	73
<b>Control</b>	32.5	50.6	16.9		0.58	0.42	Collins et al., 2004	166
<b>African American</b>	32.0	52.0	16.0	0.357	0.58	0.42	<b>Present Study</b>	50
<b>Elite Bobsledders</b>	42.86	45.71	11.43	0.1518	0.66	0.34	<b>Present Study</b>	35
<b>Men's Hockey Team</b>	30.0	66.67	3.33	0.0118	0.63	0.37	<b>Present Study</b>	30
<b>Women's Hockey Team</b>	14.29	76.19	9.52	0.0121	0.53	0.47	<b>Present Study</b>	21
<b>Ironman Athletes</b>	20.0	46.7	33.3	0.569	0.43	0.57	<b>Present Study</b>	15
<b>Track Athletes</b>	20	55	25	0.536	0.48	0.52	<b>Present Study</b>	20
<b>Control</b>	32	44	24		0.54	0.46	<b>Present Study</b>	100

**DISCUSSION**

Of the five studied groups: African Americans, men's and women's college hockey teams, elite (international) bobsledders, Ironmen triathletes and members of the college's track team, the findings showed that the only significant differences were between the women's hockey team and control for the ID genotype, not in any other groups (Figure 3, Table 1). A possibility for the large frequency in ID genotype for this group can be due to the fact that the athletes on the women's hockey team are only division three, they are not division one or like the elite bobsledders, where they are the best of the best at that sport. If a study was conducted using either division one, or even, professional hockey athletes, a different outcome could more likely be observed. Even though the bobsledders had the highest DD genotype percentage compared to the other groups in the study, it wasn't significantly different when compared to the ID genotype (Figure 3, Table 1), not the expected results that were predicted. However, when each allele was examined through gel electrophoresis, there was a significant difference between the elite bobsledders and the control (not shown for alleles) (Figure 4, Table 1). This was expected, since the elite bobsledders had competed in international events including the 2010 & 2014 Winter Olympics. Bobsledders are expected to have the power and quick sprinting ability required to push a sled at the start of their competition. Even though ice hockey requires fast responses, bursts of speed and power, the expected DD genotypes were not seen; maybe the hypothesis would have been supported if professional ice hockey players were tested instead. The expected results from the Ironman triathletes and the track team were also not supported. Results of both groups showed no significant difference (p value 0.569 and 0.536 respectively), as shown in Table 1. However when looking at the homozygous genotype I/I (Figure 3), and I allele frequency (Figure 4), in all groups one can see a linear increase in the presence of this genotype and allele as the distance required by an athlete/individual increases. However, to further investigate this trend, a larger sample size would be needed for the ironman triathletes. In regards to the African American group, coronary artery disease has been found to be associated with the DD genotype. In 2009, the leading cause for death in African Americans was cardiovascular disease, 31.7% in men and 34.2% in females (provided by the American Heart Association). This study was conducted to see if African Americans had a higher frequency of the DD genotype compared to the control. For future research on this topic a study should be done where both the experimental and control group fill out a survey asking if any of them had a family history of parents that were already diagnosed with coronary heart disease. Instead, a general population of African American's was used for this study.

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