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SELECTED GENES POLYMORPHISM RELATED TO BIOMARKERS AND PHYSICAL CHARACTERISTICS IN YOUNG AFRICAN CRICKET, RUGBY, SOCCER, NETBALL PLAYERS AND BULGARIAN ATHLETES

Department of Human Movement Science

FAULTY OF SCIENCE AND AGRICULTURE

SAM CHENJERAI MUGANDANI

201001581

UNIVERSITY OF ZULULAND

Supervisor: Prof. Trayana Djarova- Daniels

Co-supervisor: Prof A. K. Basson

Co- supervisor: Dr Gregory Watson

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BY

SAM CHENJERAI MUGANDANI

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ABSTRACT

This study sought to determine the frequency distribution of genotypes and alleles of the polymorphic genes ACE, ACTN3 (α-actinin-3), AMPD 1(adenosine monophosphate deaminase) among athletes. Compare the genotype and allele frequency among athletes of different sporting codes but same ethnicity. Compare the genotype and allele frequency of Zulu and Bulgarian athletes. Study anthropometric, physiological, physical performance variables as well as blood biomarkers and determine their association with gene polymorphism. Determine the association of genotype combinations of ACTN3 and AMPD1 polymorphism and anaerobic power.

Zulu cricketers (n=14) and students (n=17) as controls were genotyped for the (ACE). Systolic and diastolic blood pressure (SBP and DBP) and grip strength (kg), knee extension and flexion (Nm/kg) were measured, systolic tension time (STT) and metabolic rates (MR) were calculated. ACE genotyping for the whole group displayed a complete absence of II genotype, 67.7% DD and 32.3% ID genotypes. The frequency of D allele was 83.8% and I allele 16.2%. In cricketers DD and ID genotypes were 50% each compared to controls-83% DD and 17% ID. No differences in grip strength and quadricep/hamstring muscle strength between the groups were observed, but for the whole cohort 86% D allele frequency was associated with higher (greater than 43.3 kg) grip strength ($p<0.037$). In cricketers CRP (less than 3.0 mmol/l) was associated with 79% D allele frequency. SBP and DBP were significantly lower by 3.2 and 4.25 mmHg, whereas increased values of STT by 5.5%, and MR by 10.3% were found. A balanced display of DD and ID genotypes was observed.

Zulu female soccer and netball players (n=16, age= 20.8±3.1years) and female Bulgarian soccer players (n=23, age 22.6±2.0 years) and control groups of 23 and 42 female students respectively were genotyped for ACE polymorphism. CRP and UA were measured at rest. No statistical differences were found between netball and soccer players. Null II genotype was found in Zulu players. They displayed higher 62.5% DD genotype and 82% D allele frequency compared to 40% DD genotype and 45% D allele in Bulgarians and to respective controls and

ACE population genetic study. CRP and UA levels were within the normal range. CRP was higher in Zulu players (2.80±1.2 mg/l) compared to Bulgarians (1.37±1.03 mg/l), but lower than respective Zulu controls (4.0±1.36 mg/l). In Zulu players UA levels (217.5±60.0 µmol/l) were lower compared to 259.6±32.8 µmol/l in Bulgarians. Null II ACE genotype were found among Zulus, 62.5% DD genotype and 82% D allele frequency related to low CRP and UA, Bulgarian players showed 12% II, 21% DD and 67% ID ACE genotypes and 45% D and 55% I allele frequency.

A mixed group of Bulgarian athletes (n = 52) competing at national and international level and a matching genetic control group (n = 109) of volunteers were genotyped for ACTN3 and AMPD1. There were no significant differences in ACTN3 genotype distribution between athletes performing the Wingate test (38% RR, 46% RX, 16% XX) and controls (41.2% RR, 46% RX, 12.8% XX). AMPD1 distribution was (73% CC, 27% CT, 0% TT) and in controls (73.2% CC, 25% CT, 1.8% TT). Athletes performing Wingate test showed equal 33% frequency of RR/CC and RX/CC combination, and 12% RX/CT. Significantly higher ($P < 0.05$) peak power output (11.10 W kg^{-1}) was found in athletes with RX/CT combination compared to other combinations (range: 8.83-9.71 W kg^{-1}) and in R-power (RR + RX) and C-power (CC + CT) dominant models (9.91 W kg^{-1}). Mean power was higher ($P < 0.05$) in RX/CT combination (8.93 W kg^{-1}) compared to RR/CC (7.75 W kg^{-1}) and RR/CT (7.95 W kg^{-1}).

The effects of exercise on leukocytes and lymphocytes were determined on the Zulu athletes above with the same blood samples which were used for genotyping, and investigating levels of AU, CRP and LA. There were higher levels of leucocytes in the netball players than in female soccer players (6.8 ± 1.24 X 109/L and 6.11 ± 1.28 X 109/L respectively). The lymphocyte levels were also higher in the netball players than in female soccer players (2.60 ± 0.58 X109/L and 2.16 ± 0.49 X109/L respectively). There were also higher levels of leucocytes in the male soccer players compared to the male rugby players (6.26 ± 1.97X109/L and 5.46 ± 0.99 X109/L respectively). The lymphocyte levels were higher in the soccer players than in the rugby players (2.17 ± 0.36 X109/L and 1.85 ± 0.32 X109/L respectively), but the differences were not significant at $p< 0.05$. The changes in leucocytes could be a result (among other things) of the removal of dead cells related to exercise stress and trauma. The athletes' results for the measured blood parameters were within the health norms.

We concluded that there are ethnic differences in frequencies of genotypes and alleles as well as biomarkers. Differences were also noted among Zulus. Association mainly with power dominant genotypes and physical and physiological variables as well as biomarkers were of significant note. The changes in levels of hematological parameters during the competition period (period of the study) and the association of AU, CRP and LA suggests that they can be used to monitor athletes' response to exercise, injury, oxidative stress and inflammation in relation to their genetic endowment.

DECLARATION BY CANDIDATE

I herewith would like to declare that I was very interested in Sports Genetics and I wanted to pursue my studies in this field. The research design of the studies and the recruitment of experimental subjects (participants) was done by me under the supervision of Prof Trayana Djarova. I did all the physical performance tests. Laboratory genotyping procedures were performed by me at the Biochemistry Laboratory, University of KwaZulu Natal, Pietermaritzburg Campus under the supervision of Dr Gregory Watson except for the genotyping of Bulgarian athletes which was done in collaboration with Dr Radka Kaneva and Prof Peter Atanassov at the Genome Diagnostic Laboratory, Medical University, Sofia, Bulgaria. The statistical analysis was done by me with the help of Mr. Jack Cloete, Dr Liubomir Petrov and Prof Trayana Djarova.

I did the interpretation of results and I was involved in the writing and editing of all submitted published papers.

DECLARATION BY CANDIDATE

Signature 

Sam Chenjerai Mugandani

Date 05 November 2019

DECLARATION BY SUPERVISOR(S)

Signature: 

Signature: …….

Print Name: Prof AK Basson

Print Name: …….

Date:13 November 2019

Date: …….

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LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme gene
ACTN3	α – actinin – 3 gene
ADRB2	Adrenergic receptor beta 2 gene
AMPD 1	Adenosine monophosphate deaminase
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
AU	Uric acid
BMI	Body mass index
CAA	Cytosine Arginine Arginine
CGA	Cytosine Guanine Arginine
CRP	C-reactive protein

DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
FM	Fat mass
GWAS	Genome-wide association study
IMP	Inosine monophosphate
LBM	Lean body mass
MR	Metabolic rate
MVC	Maximal voluntary contraction
NOS3	Nitric acid synthase 3 gene
Nm/kg	Newton meters per kilogram
PCR	Polymerase chain reaction
RAAS	Rennin-angiotensin-aldosterone system
SBP	Systolic blood pressure
SNPS	Single nucleotide polymorphism
STT	Systolic tension time
TAA	Thymine Arginine Arginine
TGA	Thymine Guanine Arginine
TNF	Tumor necrosis factor
VEGFA	Vascular endothelial growth factor gene
VO_{2max}	Maximal oxygen consumption

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CHAPTER 1

PROBLEM STATEMENT AND OBJECTIVES

1.1 INTRODUCTION

Sports have become a large industry with the establishment of vibrant leagues in many countries, especially, in soccer. Events, such as the soccer world cup and the rugby world cup generate a lot of activity in diverse spheres of the economy, internationally (Molly, Chetty, 2015; World Rugby, 2017). Netball has become very significant internationally with the latest netball world cup having been staged in the United Kingdom in 2019. Netball is played by many females in South Africa with about half a million players in schools and 9700 adult players (Ferreira and Spamer, 2010). Cricket is also a very popular sport in the world (Pote, Christie, 2016; Kumarapandiyan, Keerthivarman, 2018). To meet the increasing demand for better performance of athletes and sustain this growing industry, coaches and trainers, for decades, have depended on the manipulation of factors such as physical and physiological parameters, nutrition, tactics, techniques, and psychological factors, to improve the performance of athletes. These have been referred to as the "environmental or nurture constraints" (MacArthur and North, 2004; Gibson 2009). These, however contribute only 34%, to athletes' performance with the remaining 66% being attributed to genetics (De Moor *et al.,* 2007; Pitsiladis *et al.,* 2016).

The completion of the human genome project in 2003 and advances in molecular genetic studies accelerated the search for knowledge on how genes at the molecular level, influence sport performance. This led to the growth of sports genomics which started in the early 2000s when genetic markers such as *Angiotensin 1 converting enzyme* (*ACE) gene* variation were associated with athletic performance (Bray *et al.,* 2009; Ahmetov and Fedotovskaya, 2015). A brief explanation of the human genome follows.

The human genome represents all the genetic material in a human cell (McArdle, Katch and Katch, 2001). This is arranged into 23 chromosome pairs, of which 22 are autosomal and the 23rd pair is sex determining. The chromosome is a very long, single folded thread of DNA, highly-regular, double-stranded DNA helix on which thousands of genes are packed. It is wrapped round histones and constitutes an organism's hereditary data (Gibson, 2009). The DNA molecule is a very long polymer which consists of nucleotides which are repeated throughout its length. The haploid

human genome is made up of slightly over 3 billion DNA base pairs, most of which are found on the 23 pairs of chromosomes and a few on the mitochondria. The DNA base pairs are organized into sections (genes) which encode for specific materials, which are either protein or RNA molecules. The gene can be identified at specific locations on the chromosome, some parts of it have to do with heredity while others regulate the activities of the gene. (Pearson, 2006; Sharp, 2008).

The structure of a chromosome depicts those locatable regions, where, for example, the location of the *ACE* gene is referred to as 17q23. The letter 'q' stands for the long arm of the chromosome as measured from the centromere and the letter 'p' denotes the short arm of the chromosome. Each arm has a region numbered from the centromere to the tip of the chromosome - the telomere. Each banded region within *p* and *q* is also numbered (McArdle *et al.,* 2001).

It is estimated that the human genome is made up of 20 000 to 25 000 genes (Farrell, Joyner & Caiozzo, 2012). Every gene is estimated to be made up of 3000 base pairs, with the size differing a lot among the 23 pairs of chromosomes. It is estimated that of all the human genome, only 2% constitutes genes which code for proteins. The rest is comprised of non-coding regions called 'introns'. The non-coding regions are thought to provide stability and regulation of the proteins coded for. The regions which code for proteins on the length of bases on the gene are known as 'exons' (Sharp, 2008).

An allelomorph (allele) is one or two forms of a gene that occupy the same locus on a chromosome; each of the alleles has a different DNA form/sequence. When individuals have different alleles for a gene they are referred to as being heterozygous, and those with identical alleles on each of the chromosome pair are referred to as being homozygous. Almost all (99.9%) nucleotide bases are the same in all individuals, however, scientists have identified 1.4 million locations where single-base DNA differences occur in humans. These are referred to as 'single nucleotide polymorphisms' (SNPs).

Polymorphism is a form of gene variation which occurs when two or more alleles exist in a population and is related to biodiversity and adaptation. A gene polymorphism occurs when there is a locus where two or more alleles have gene frequencies that exceed 1% in a population (McArdle *et al.,* 2001). The rate at which SNPs appear is one in every 1000 bases (Farrell *et al.,* 2012). Common, small variations (polymorphisms) occur in each of these genes, some of which will influence the 'responsiveness' of the gene (in terms of its readiness to yield associated

protein), or the functionality of the protein itself. It is the interaction of such 'functional polymorphisms' with a unique pattern of environmental stimuli which make all humans different from one another. Some of these variations may be as simple as the substitution of one base for another - in which case they are called 'single nucleotide polymorphisms' or 'SNPs'. These are generally described in 'shorthand' by the nucleotide variant an individual has (for example, -174C rather than -174G as this means a cytosine rather than guanine at a specific site). Such a variation may be 'recognized' by specific enzymes, called 'restriction' enzymes. Meanwhile, a set of common variations in a gene may occur commonly together; such a grouping is described as a 'haplotype'

The corollary of this phenomenon, in the human genome, is what forms the basis for determining allelomorph association with physical performance phenotypes and biomarkers. The hypothesis arising from this is that, different allele forms of the same gene in individuals, will lead to different physical performance and biomarker phenotypes in a cohort of athletes and the population.

Using information from the human genome project, scientists in sports science have investigated the association or linkages between genes and performance, as well as health-related fitness phenotypes. This resulted in the compilation of a data base of genes associated with performance and health related factors and 120 DNA polymorphisms related to sports genomics by December 2014 (Ahmetov and Fedotovskaya, 2015).

Most of the studies done have concentrated on Caucasian cohorts and populations, hence, more needs to be done with black athletes. Additionally, there is also a paucity of studies on the association among genetic factors, biomarkers and physical performance characteristics.

1.2 PROBLEM STATEMENT

Studies in exercise and sport genetics are very recent, having started in earnest in the early 2000s. Most of the studies were conducted among the Caucasian populations with very few focusing on athletes of African origin, specifically, among athletes of Zulu origin. The evolutionary ethnic genetic differences require that knowledge be sought among other ethnic groups such as Africans, specifically, Zulus who have a greater African genetic identity than their African Americans and Jamaican counterparts who have received more attention than ethnic groups in Africa. Most studies have focused mostly on determining the association between the various gene polymorphisms and sports performance factors or variables. There has been no deliberate attempt to compare two distinct ethnic groups, especially, with reference to

biomarkers and their association with gene polymorphisms. This means there are no studies which have been conducted to determine the association between gene polymorphism and biomarkers, such as C-reactive protein, uric acid and lactate. Similarly, there is a paucity of studies comparing African and Caucasian athletes, specifically, Africans of Zulu origin.

1.3 OBJECTIVES

This study, therefore, sought to determine the frequency distribution of genotypes and alleles of the polymorphic genes ACE, ACTN3, and AMPD 1 among athletes and their association with selected variables. The specific objectives are:

- To compare the genotype and allele frequency among athletes of different sporting codes but with same ethnicity;
- To compare the genotypes and allele frequencies of Zulu and Bulgarian athletes;
- To study the anthropometric, physiological, physical performance variables as well as blood biomarkers and determine their association with gene polymorphisms; and
- To determine the association between the genotype combinations of ACTN3 and AMPD1 polymorphisms and anerobic power.

1.4 HYPOTHESIS

The following hypotheses were identified to guide the study:

1.4.1 Research Hypothesis

There will be an association between the ACE I/D gene polymorphism in male cricket players and blood pressure, c-reactive protein, uric acid, lactate and selected physical tests.

1.4.2 Research Hypothesis

There will be significant differences in the ACTN3 gene polymorphism genotypes and alleles frequencies, anthropometric and physical characteristics among cricket, rugby and soccer players.

1.4.3 Research Hypothesis

There will be differences in the ACE D/I genotype and allele frequencies of Zulu and Bulgarian female athletes, as well as differences in CRP and UA levels. The ACE DD genotype and D allele will show association with CRP and UA levels.

1.4.4 Research Hypothesis

The ACTN3 and AMPD1 genotype power /speed combinations will show greater peak-power output than their non-power genotypes' combinations.

1.4.5 Research Hypothesis

There will be differences in the leucocyte and lymphocyte counts among netball, female soccer players, male soccer players and male rugby players.

1.5 STRUCTURE OF THE THESIS

This thesis is a compilation of published articles from the study, a format approved by the University of Zululand. It consists of eight chapters. Chapter 1 the introduction is followed by Chapter 2 the literature review; this is written in the form of a published review paper. Chapters 3 to 7 which are published articles in peer-reviewed journals constitute the findings of the research. The last section, which is Chapter 8 is made up of the outcomes, conclusions, implications for sport practitioners, suggestions for further research and limitations.

Chapter 1 is the introduction and comprises of the background to the study, the statement of the problem, the objectives and the hypotheses. Chapter 2 is the literature review which comes as a reviewed article titled – "*Athletic performance enhancing ACE, ACTN3, AMPD1genetic markers, fitness characteristics, C-reactive protein and uric acid of cricket, netball, rugby and soccer players: A Review"* - published in the "Journal of Applied Sports Science, 2019, Volume 1, pages 131 to 149". Chapter 3 is an article titled – "*Angiotensin-converting enzyme genotypes relationship with blood pressure, C-reactive protein and selected physical tests in Zulu South African cricketers"* - published in the "African Journal of Biochemistry Research", 2011, Volume 5, Issue 7, pages 197 to 205'. Chapter 4 is an article titled – "*ACTN3 (α – Actinin-3) gene polymorphism and anthropometric characteristics in Zulu rugby, soccer and cricket players"* - published in a book of conference proceedings of "The 26th International Scientific Congress Olympic Sports and Sport for All" and 6th International Scientific Congress, "Sport, Stress, Adaptation" 17- 19 May 2012, pages 414 to 416'. Chapter 5 is an article titled – "*Angiotensin-converting enzyme genotype, allele frequency, C-reactive protein, uric acid in female Zulu South African soccer, netball and Bulgarian soccer players"* - published in the "African Journal for Physical, Health Education, Recreation and Dance (AJPHERD)", 2014, Volume 20, Issue 1, pages 153 to 163'. Chapter 6 is an article titled – "*ACTN3 and AMPD1 polymorphism and genotype combinations in Bulgarian*

athletes performing the Wingate test" - published in the "Journal of Sports Science", 2015, Volume 3, pages, 1 to 10'. Chapter 7 is an article titled – "*Differences in the distribution of selected blood variables during a competition period"* - published in the "Journal of Applied Sport Sciences", 2017, Volume 2, pages 63 to 67'. Chapter 8 is made up of the following sections: Outcomes and Conclusions, Implications for Athletes, Coaches and Fitness Practitioners, Future Research and finally, Limitations.

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ATHLETIC PERFORMANCE ENHANCING ACE, ACTN3, AMPD1 GENETIC MARKERS, FITNESS CHARACTERISTICS, C-REACTIVE PROTEIN AND URIC ACID OF CRICKET, NETBALL, RUGBY AND SOCCER PLAYERS: A REVIEW

Sam Chenjerai Mugandani

Center for Biokinetics, Recreation and Sport Science,
School of Health Sciences, University of Venda, South Africa

ABSTRACT

Sports is a large industry with vibrant leagues running in many countries. Some of the most popular sports are soccer, rugby, cricket and netball. To meet the demand for better performance of athletes and sustain this growing industry, coaches and trainers have depended on the manipulation of factors such as physical and physiological parameters, nutrition, tactics, techniques and psychological factors to try and improve the performance of athletes. These have been referred to as the environmental or nurture constraints. The quest for better performance continues hence microtechnology such as accelerometers, heart rate monitors and global positioning systems are also being used to gather data to determine some of the physical and physiological demands of games. Evidence from studies with twins revealed that there are performance traits which are genetically determined. Research also shows that more than 60% of performance in sport and exercise is genetically determined. The unraveling of the human genome and advances in molecular biological studies resulted in the quest for knowledge relating to the influence of genes at the molecular level on performance in exercise and sport. The human genome project established approximately 20 000 genes in humans. To date, the gene map for performance and health-related fitness phenotypes has identified more than 200 single nucleotide polymorphisms (SNPs) associated with some performance and fitness linked traits. Among the most studied gene polymorphisms are the angiotensin converting enzyme (ACE) gene, the human α- actinin-3 (ACTN3) gene and the adenosine monophosphate deaminase (AMPD1) gene, as they relate mostly to anerobic and aerobic related activities. The use of hematological and biochemical indicators to identify injuries and exercise stress calls for exploration of association between gene polymorphisms and indicators such as C-reactive protein, uric acid and blood parameters such as red blood cells and sub-components of leukocytes.

***Key words**: Biomarkers, genes, polymorphism, sports, performance*

INTRODUCTION

Sports have become a large industry with vibrant leagues running in many countries. Although the population of participants is relatively low, female sport leagues are also beginning to be taken as seriously as male leagues. In South Africa, rugby, soccer cricket and netball are very popular sports. In 2017, it was estimated that there were 9.1 million players among the world rugby union members in 121 countries. Among these, 2.4 million were female players and 603 455 of these were from

South Africa (World Rugby, 2017). South Africa hosted the world rugby cup in 1999 and the Soccer world cup in 2010. (Molly, Chetty, 2015; World Rugby, 2017). The estimated number of people playing the game of soccer world wide as of 2006 was 265 million. (McCabe, Collins, 2018). Netball has an estimated 20 million players in 80 countries and in 2010 South Africa had half a million players in schools and 9 700 adult players (Ferreira, Spammer, 2010; Mclean, 2019).

As a result of the popularity of these sports, training demands are becoming increasingly complex and very scientific, calling for analysis and structures to meet the specific needs of each sport and player. There have been efforts to improve sport performance utilizing scientific principles. Exercise and sporting movement activities are either dynamic or static. Dynamic movements involve change in length of muscles with rhythmic joint movements and the energy metabolic processes in dynamic movements are largely aerobic (Mitchell, Haskell, Snell & Van Camp, 2005). The increasing dynamic component is rated from the estimated percent of maximal oxygen consumption ($MaxO_2$) achieved during competition, ranging from low to high, where *A.* Low is (<40% $MaxO_2$), *B.* Moderate (40% -70 $MaxO_2$) and *C.* High (>70% $MaxO_2$) (Mitchell et al., 2005). Mitchell et al., also state that there are activities on the other end of the continuum whose muscle energy metabolism is mainly anaerobic, and that they develop force with minimal or no change in muscle length or joint movement. The contraction of muscles on this anerobic side of the spectrum is also referred to as maximal voluntary contraction (MVC) and is also graded from low to high as follows; ***I.*** low (<20% MVC), *II.* Moderate (20-50% MVC) and *III.* High (>50% MVC) (Mitchell et al. 2005).

Cricket, netball, rugby and soccer can be classified by the above characterization. Soccer is classified as *IC*, which means that it has a low anerobic, low maximal voluntary muscle contraction with a high maximal oxygen consumption and high dynamic component, rugby is *IIB,* Cricket is *IA* and netball is *IIC* (Mitchell et al., 2005). This classification is largely physical and physiological whereas in any exercising scenario the muscles accomplish mechanical (physical) and metabolic (physiological) demands (Houweling et al., 2018). The above classification suggests that cricket is low on both its aerobic and anaerobic demands, netball has moderate anaerobic demand and high aerobic demand. On the other hand, rugby is moderate on both anaerobic and aerobic requirements. However, soccer like netball has a high aerobic component but low anaerobic component.

To satisfy the ever-increasing demand for better performance of athletes and sustain the growing industry of sport, coaches and trainers now depend on the manipulation of the physical and physiological parameters, as shown in the classification above. The physical, physiological, nutrition, tactics, technique and psychological factors are called environmental or nurture constraints (MacArthur, North, 2004; Gibson 2009; Pitsiladis et al., 2016). Family studies in human movement covering identical and dizygotic twins over and above what is mentioned above also show that genetics has a contributing factor to the phenotype of sport performance (Guth, Roth, 2013; Slizik et al., 2017). Evidently, performance in sport and physical activity is a factor of both environmental or nurture constraints and genetic or nature constraints (Puthucheary et al.,2011; Ahmetov & Fedotovska, 2015). The contribution of the genetic or nature factors to athletic status is estimated to be more than 60% while the environmental factors contribute a mere one third (De Moor et al., 2007). As a result, studies have progressed at the molecular lev-

el with the use of genotyping to identify the genes and their variants which contribute to the physical and physiological basis of human performance in sport and exercise. Through genetically profiling athletes, this knowledge would be critical and helpful towards talent identification, strengthening of the training of athletes as well as identifying risks such as susceptibility to injuries (Davids, Baker, 2007; Slizik et al., 2017). The use of genetic information for research in exercise and sport has been further facilitated and enhanced by the completion of the human genome project in 2003 and the advancement of sequencing technologies (Houweling et al., 2018).

The human genome project has shown that there are approximately 20 000 genes in humans. Gene nomenclature has been established for each known human gene in the form of an approved gene name and symbol (short form abbreviation). Each symbol is unique, and each gene is only given one approved gene symbol (Carninci, Hayashizaki, 2007). Using information from the human genome project, scientists in sport science have investigated the association or linkages of genes and performance as well as health related and fitness phenotypes. This resulted in the compilation of the human gene map for performance and health- related fitness phenotypes and 120 DNA polymorphisms related to sports genomics had been identified by December 2014 (Ahmetov, Fedotovskaya, 2015). Polymorphism as a form of gene variation occurs when two or more alleles exist in a population and is related to biodiversity. Alleles are one or two alternatives of the gene that occupy the same locus (place) on a chromosome. The data available for physical performance phenotypes from the genes studied are: cardio respiratory endurance, elite endurance athletes' status, muscle strength, speed and power together with some muscle performance traits including exercise intolerance of differing degrees (Bray et al., 2009; Ahmetov, Fedotovskaya, 2015). Energy systems, considered in the association studies, fit with the sport classification presented above. Sport teams such as cricket, netball, rugby and soccer variously fit as already illustrated (Mitchell et al., 2005).

Study approaches which are followed with gene markers are mostly case-control studies where mostly elite athletes (case) are compared to the general populations of non-athletic individuals (control) and cross-sectional studies where athletes and the general population are measured quantitatively (Ahmetov, Fedotovskaya, 2015; Houweling et al., 2018). Biomarkers are characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to an intervention (IOM (Institute of medicine), 2011). There are several biomarkers which are variously linked to physical performance and gene polymorphisms among which are C-reactive protein and uric acid. Allgrove et al., (2012) observe that exercise of elevated intensity compromises the immune system leaving athletes susceptible to illness. Therefore, hematological parameters such as leucocytes have been used as possible markers of a compromised immune system due to exercise (Mackinnon 1997; Gleeson, Walsh 2012). In this regard, soccer and rugby players have regular aerobic and anaerobic training regimes which expose them to oxidative stress (Yamaner, 2010).

METHOD

Data sources

Four hundred and fifty (450) searches were conducted and the following key words were used: ACE, ACTN3, AMPD1 polymorphisms; athletic performance; exercise, fitness and performance genomics; endurance and resistance training; Uric acid and C-Reactive protein

biomarkers in rugby, soccer and netball. Keyword searches identified articles from Medline (1987-), Research databases: Science direct (2009-), Human Kinetics (2002-), Human Gene Map for Performance and Health-related Fitness Phenotypes, the 2002 Update and the 2006-2007 update and Research Gate (2015-).

Inclusion criteria

The inclusion criteria for this review were a) ACE genotypes and associations with training response, aerobic endurance performance, VO_2max, long distance athletes, games (rugby, cricket, soccer, netball), high intensity activities of short duration, power output, exercise efficiency, muscle efficiency; b) ACTN3 genotype associations with anerobic performance; isometric and isokinetic muscle strength, properties of fast twitch and slow twitch muscle fibers in short distance athletes, games, Wingate test and pick power on response to strength training; c) AMPD1 genotypes and associations, anaerobic Wingate test for power, pick power output, mean power, muscle fibers distribution and overall strength; d) Associations of ACE, ACTN3 and AMPD1 genes with physical, physiological characteristics and blood parameters in various sports. The genotype frequencies of the polymorphisms included are presented in accordance with the genomic browsers of the USA, Indian and Arab populations (Bhagi et al.,2002; Al-Hinai et al., 2002; Salem, Batzer 2009).

Exclusion Criteria

Excluded were other potential performance enhancing genes associated with training responses and health-related phenotypes in endurance and power athletes which include, a) ADRB2 (adrenergic receptor beta 2 gene); b) VEGFA (vascular endothelial growth factor gene); c) BDKRB2 (bradykinin beta 2 receptor gene); d) NOS3 (nitric acid synthase 3 gene); e) PRARA (peroxisome proliferator activated receptor alfa gene) and f) PRARD (peroxisome proliferator receptor delta gene).

DISCUSSION

Performance demands cricket, rugby, netball and soccer

The discussion below further illustrates the performance demands of the four team sports which are both aerobic and anaerobic. This corresponds with the polymorphisms of the genes *ACE*, *ACTN3* and *AMPD1* which encode for either aerobic or anaerobic performance, except for *AMPD1* which only encodes for anaerobic performance.

Cricket

Modern cricketers are now exposed to greater physical and physiological demands. Heart rate can reach 190 beats/min and the predominant contribution from the anaerobic energy systems can contribute up to 60% of the total energy in multiple activities of short duration of less than 40 seconds (Noakes, Durandt, 2000). Martens (2004) identified the following demands of cricket: low to moderate aerobic capacity, moderate anaerobic capacity, moderate strength and flexibility, low to moderate endurance and moderate to high speed. Fast bowling has been linked with a mesomorphic somatotype, greater percentage of type II muscle fibers and superior phosphagenic and glycolytic metabolic pathways together with eccentric muscle strength (Stuelcken et al., 2007). A shorter stature and isokinetic knee and shoulder strength were seen to be contributory to the success of batsman (Noakes, Durant, 2000; Nunes, Coetzee, 2007).

Netball

Netball is a fast-paced contact sport (Soh et al., 2007; Chandler et al.,2014). Players must be endowed with speed to run short distances on the court. They perform repeated powerful jumps, well balanced landings, sudden chang-

es of direction and quick stops and starts which require agility (McManus, Stevenson & Finch, 2006; de Villiers, Venter, 2014). Both aerobic and anaerobic energy systems are a requirement (Soh, Husain & Soh, 2006; Soh, Husain & Soh, 2007; Terblanche, Venter, 2009). Using time motion analysis, centers (C) were found to have the highest player load while the goal keepers (GK) and the goal shooters (GS) had the least player loads (Fox et al., 2013; Bailey et al., 2017). The distances covered during matches are estimated to be 4210 meters for GS and 7984 meters for C (Chandler et al., 2014). Injuries occur mostly to ligaments of the ankle, knee, fingers, hands and wrists, (Langeveld, Coetzee & Holtzhausen, 2012; Hervert, Deakin & Sinclair, 2014).

Rugby

Rugby is a high-speed contact sport that involves aerobic and anaerobic fitness, there is a combination of both low and high intensity activities. In elite games running covers 5-8 km with speeds of 18-20 km/h. (Gabbett et al., 2007; Goh et al., 2009). Props are taller, heavier and their skin folds are thicker than other positions, their higher body mass helps them with momentum for their larger tackling role (O'Connor, 1996; Gabbett, 2006). Brower et al., 1994 showed that hookers, centers and wingers have better performance times in the 10and 40-m speed tests than props who were also slower than the back rowers and outside back positional groups, and backs showed significantly faster times than forwards. The hookers/halves and outside backs had superior VO_2max than the props positional groups with the highest figures being around 55.2 $ml.kg^{-1}.min^{-1}$ (O'Connor, 1996).

Soccer

Soccer involves physical efforts of an intermittent nature with, walking, runs, sprints with or without the ball, jumps, sudden acceleration or deceleration (Devrnja & Matkovic, 2018). Soccer players should have very high speed, power, strength and endurance (Gabbett, Wiig & Spencer, 2013; O'Reilley, Wong, 2012). During a normal game, they run a total of about 10 km and within that endurance context, there are anaerobic explosive bursts, such as sprinting, jumping and forceful contractions. Thus, within an aerobic endurance context, there are numerous anaerobic explosive bursts of activity (Stølen et al., 2005; Abbey, Rankin, 2011; Calahorro et al.,2013). The average VO_2max for elite male players is between 55 and 68 $ml.kg^{-1}.min^{-1}$, with individual values of more than $70ml.kg^{-1}.min^{-1}$(Hoff, 2005). Aerobic endurance is therefore of paramount importance (Bradley, Noakes, 2013; Sarmento et al., 2014; Varley et al., 2016). Soccer is also known to have eccentric movements such as running backwards, sudden direction changes and tackles, which lead to muscle damage (Magalhães et al.,2010; Gravina et al., 2011).

The *ACE gene*

The *ACE gene* is made up of 26 exons and 25 introns. Exons are coding sequences of DNA in the gene and introns are intragenic sequence/regions inside the gene. It stretches over 21 kilo bases on the chromosome 17q23. Its polymorphism consists of the presence of the (490bp *I* allele) or absence (190bp *D* allele) of a 287-base pair *Alu* repeat sequence resulting in three genotypes (*DD* and *II* homozygotes, and *ID* heterozygote) (Lin et al., 2001; Sipahi et al., 2006). The *I* allele refers to the presence of a 287 base *Alu* repeat segment in intron 16, the deletion or *D* is not likely to be the result of an actual deletion event. The presence of the intron 16 *Alu* on *ACE* expression is that of lowering activity levels of the ACE enzyme for individuals with the I allele.

Angiotensin converting enzyme (ACE) is

part of the rennin-angiotensin-aldosterone-system (RAAS). Renin converts angiotensinogen, to angiotensin I a peptide which is in turn converted by ACE to angiotensin II vasoactive peptide. Angiotensin II is the key agent of the RAAS. It mediates vascular resistance by binding to endothelial receptors causing vasoconstriction. ACE is a key enzyme in the generation of angiotensin, a potent vasoconstrictor as well as effector of sympathetic tone, and aldosterone stimulating peptide. Angiotensin also regulates salt and water balance via the aldosterone pathway. Its action also differs among individuals due to genetic differences (Gomez-Gallego et al., 2009). Several studies have examined the effects of *ACE* on physical performance such as aerobic capacity, muscle function, trainability, and athletic status (Scanavini et al., 2002; Lucia et al., 2005; Amir et al., 2007).

The *ACE* insertion (*ACE I*) allele is prevalent in endurance athletes hence related with endurance ability (Nazarov *et al*., 2001). Despite research being generally inconsistent, the widely held view is that the insertion (I) allele is associated with improved performance in endurance events whereas the "deletion" (D) allele is associated with better performance in power events. However, it is not known how lower circulating ACE happens to improve performance (Wang et al., 2008). Nonetheless, it can be inferred that lower ACE in circulation could mean less conversion of angiotensin I to angiotensin II and therefore reduced vasoconstriction of blood vessels during endurance activities. The I allele is theoretically associated with a decrease in circulating levels of angiotensin II (a potent vasoconstrictor) and thus a reduction in vascular resistance which might facilitate higher cardiac output during strenuous exercise. There is an unrestricted flow of oxygen and metabolic substrates necessary for the aerobic pathways for energy production in the skeletal muscles and other peripheral apparatus key to aerobic and 'cardiovascular' endurance. That would also explain the greater response to training in both the skeletal muscle and cardiovascular systems (Lucía *et al*., 2010). Athletes with the II genotype have greater aortic elasticity than the DD and ID genotypes (Tanriverdi et al., 2005). The I allele has also been associated with fatigue resistance in skeletal muscle (Montgomery et al., 1999). Greater percentage of the more aerobic type I skeletal muscle fibers have been found in athletes of the II genotype as compared to DD genotypes (Zhang et al., 2003). On the other hand, studies with Italian gymnasts showed the DD genotypes exhibiting higher relative strength than the II, (Calo,Vona, 2008). Similarly, a study with Caucasian Turkish female athletes showed better performance improvement in endurance with those of II genotype while those with the DD genotype improved more in the shorter more power inclined events (Cam et al., 2007). In a study of 50 to 70-year-old women the response to a 12-week varied training program showed that the ACE DD and ID did not show improvement from base line measurements in a sit to stand lower body strength test and aerobic test measured by a six-minute walk test respectively. They however showed improvements together with the II genotype group in an agility test and strength upper body arm curl test (Moraes et al., 2018). The results show some inconsistence with expected results, especially where DD would have shown association with both upper and lower strength and II not showing association with agility. In an ACE I/D variant case control study of Polish soccer players and controls, 106 players were divided into forwards, midfielders, defenders and goal keepers and there were 115 controls. Genotype and allele frequencies were not significantly different among the play positions of players neither were there

differences between the athletes and controls (Cięszczyk et al., 2016). In a similar study with 375 Brazilian soccer players of whom 90 where professionals and the other players formed strata of under 14, 15, 17 and 20 years of age, with 100 controls, the genotypic and allelic frequencies of the players in the different categories did not differ significantly from the controls (Coelho et al., 2016).

ACTN3 gene

In humans, there are two genes encoding skeletal α- actinin: ACTN2 and ACTN3 both for the structural Z discs (Bell et al., 2012). The human α- actinin-3 (ACTN3) gene encodes the structural protein α- actinin-3 in fast skeletal muscle fibers. It is located on the long arm of chromosome 11 (11q13-q14). A common polymorphism of the ACTN3 gene, 577X is due to a premature stop codon which results in a loss of function nonsense mutation. The replacement of nucleotide C (Cytosine) with T (Thymine) in exon 16 the normal codon (triplet) for Arginine (CGA) 577R is converted to the stop codon (TGA) 577X. This results in non-synthesis of the protein α- actinin-3 (North et al.,1999; MacArthur, North, 2007; Moran et al., 2007). This allele (577X) is not capable of encoding for α- actinin-3, however the presence of ACTN2 proteins in both type I and type II muscle fibers compensates for the absence of α- actinin-3 in individuals who are 577X homozygous (Calo, Vona, 2008). There are two alleles R and X, and three possible genotypes for the ACTN3 gene, which are the RR and XX homozygotes and the RX heterozygote.

The actinins (encoded by the R allele) are a group of ancient actin-binding proteins. They are limited to fast muscle fibers (type IIb) capable of generating force at high velocity (Mills et al., 2001; Vincent et al., 2007). They make up the predominant protein component of the Z line in the type IIb muscle sarcomere to form a structure that anchors together actinin myofibrils and stabilizes the muscle contractile apparatus (Squire, 1997). They also interact with other muscle proteins in carrying out some signaling and metabolic functions (MacArthur, North, 2007). The frequency of the 577X null allele differs among different human groups. It is approximately 10% among Africans and about 18% in Caucasian populations (MacArthur et al., 2007; Norman et al., 2009). Its persistence over evolutionary time has been hypothesized to suggest that there was need to have a muscle type which would be efficient in conserving energy and resist fatigue (Calo, Vona, 2008; Head et al., 2015).

Several studies have shown substantial evidence pointing to the association of ACTN3 with physical performance. The presence of the ACTN3 protein (577R) has been shown to favor success in activities of sprint or power performance (Macarthur, North, 2005; Calo, Vona, 2008). For example, in a study of 992 Greek adolescents, male carriers of the XX genotype recorded slower sprint times compared to their RR counterparts, though this was not true for the females (Moran et al., 2007). In another study of both men and women, examining knee extensor concentric peak power, at base line, the XX carriers showed greater strength but after a 10-week training, the RR showed greater improvement compared to both the XX and RX carriers (Delmonico et al., 2007). However, in a study of East and West African athletes, there were no significant differences between sprinters of Nigerian origin with controls and the whole group showed a very low X-allele frequency. Similar results were realized with elite Jamaican and US sprinters who classified themselves as at least 50% African American. Japanese sprinters also showed better performance by RR+RX genotypes compared to ACTN3 XX genotypes (Yang et al.,

2007; Scott et al., 2009; Mikami et al., 2014). In a study of 50 to 70-year-old women, the response to a 12-week varied training program showed that the ACTN3 XX group did not show improvement in the strength sit to stand test and the RR group did not show improvement in the 6-minute walk test (Moraes et al., 2018). This is what would be expected for XX endurance genotype and RR power genotype.

In a study of 51 untrained male Caucasians, the volume of the quadriceps was measured, knee extension, one repetition maximum and maximum power were measured through pedal sprints on an isokinetic cycle ergometer. It was established that the RR genotype was superior to the XX genotype in all the three parameters (Erskine et al., 2014). To enhance the power of association, studies which have been largely limited by small numbers, 550 best times of 346 elite Caucasian sprint athletes were collected for 100m, 200m and 400m. On average, these established that athletes with the ACTN3 577RR or the ACE DD genotype had superior best times than their ACTN3 XX and ACEII counterparts (Papadimitriou et al., 2016). However, in six studies with soccer players, a distinct relationship could not be established for sprint (anaerobic) or endurance (aerobic) performance (Santiago 2008; Pimenta et al., 2012; Pimenta et al., 2013; Eyon et al., 2014; Massidda, Scorcu & Calo, 2014; Coelho et al., 2016).

Adenosine monophosphate deaminase (AMPD1) gene

The gene AMPD1 encodes for the enzyme adenosine monophosphate 1 deaminase which catalyzes the deamination of adenosine monophosphate (AMP) to inosine monophosphate (IMP) during the formation of adenosine triphosphate (ATP) in skeletal muscle and is therefore an important muscle energy regulator during exercise (Collins, 2017; McCabe, Collins 2018). The gene is in the short arm of chromosome one 1p13-p21 and consists of 16 exons and 15 introns. A nonsense mutation C to T in nucleotide 34 (C34T) in exon 2 (rs 17602729) of AMPD1 converts the codon CAA into the premature stop-codon TAA which results in the premature cessation of adenosine monophosphate deaminase synthesis. This gives rise to three variants of the gene CC, CT and TT, where CC genotype is normal and has no deficiency in the enzyme, and the presence of the T allele signals deficiency in the enzyme (Cieszczyk et al., 2011; Feng et al., 2017; McCabe, Collins, 2018).

In a case control study of Israelis consisting of endurance and sprinters competing at elite national level and a control group of non-athletic healthy individuals, the results of the study did not show any significant differences in the distribution of the three genotypes CC, CT and TT. However, the TT genotype frequency was very low in all the groups (Meckel et al., 2012). A study of Lithuanian athletes and controls showed a greater frequency of the CC genotypes among sprint anaerobic athletes, as compared to endurance, mixed athletes and controls. The TT genotype was absent in all the athletes and with only a 2.4% frequency in the non-athletic controls (Gineviciene et al., 2014). Similar results were obtained with student athletes engaged in high speed and strength sport versus students not doing any sport. The TT genotype was totally absent among athletes with a 3.8% frequency among non-athletes, with power lifting athletes registering a 100% CC genotype (Fedotovskaya, Danilova & Akhmetov, 2013). In another study, the mutation TT did not manifest at all in the Polish rowers but was present among the non-athletes at a percentage of 1.59% (Cieszczyk et al, 2011).

C-reactive protein (CRP)

CRP is a protein of the pentraxin family and is produced by hepatic cells and its production

is regulated by several cytokines including IL-1, IL-6, and TNFα which are secreted locally in the area of harmed tissue (Ablij, Meinders, 2002; Kitsios et al., 2013; Hayashino et al., 2014). It is made up of five identical subunits (protomers) each of which is capable of binding with two calcium units. The calcium allows CRP to bind with the phosphocholine ligands found in the cell membranes and plasma lipoproteins and as a result of this binding, phagocytosis of damaged cell materials and pathogens is facilitated (Ablij & Meinders, 2002; Michigan et al., 2011). The median concentration of CRP in serum is 0.8 mg/l. and this concentration can increase drastically following microbial infection, trauma and strenuous exercise by as much as 1000 times in a space of 48 hours (Heikkila et al., 2007; Jabs et al., 2005). These concentrations may remain high if exercise induced muscle damage remains in force (Gabay, Kushner, 1999). CRP is broken down in the liver, with a small proportion being taken care of by neutrophils and macrophages. The biological half-time of the removal of the protein from blood serum is 19 hours irrespective of the physiological or infection levels, and the significant determiner of the serum levels of CRP is the rate at which it is produced by hepatocytes. This makes it a suitable marker or indicator of the inflammation or disease activity in the body (Ablij, Meinders, 2002).

Several theories have been propounded to shade light on how exercise is likely to reduce inflammation. Loss of fat has been suggested as one of them where low levels of fat are said to reduce adipocytokines such as Interleukin 6 (IL-6) which stimulate the production of CRP from hepatocytes (Mora et al., 2006; Campbell et al., 2009). Twenty five percent of the systemic IL-6 is produced by adipocytes, and it is the IL-6 which is responsible for signaling the hepatocytes to secret CRP. It follows then that higher levels of body fat are likely to be associated with higher levels of CRP (Plaisance, Grandjean, 2006; Plaisance et al., 2007; Giannini et al., 2017). Some studies have shown significant weight lose together with reduction in CRP levels following physical activity or diets lowering body fat (Plaisance, Grandjean, 2006; Saghebjoo et al., 2018). Other research suggests that the increase in protein as a result of exercise also contributes to the anti -inflammatory mechanisms of exercise, probably due to antioxidant proteins from the effects of the exercise (Donges et al*.,* 2010). Exercise generally has been shown to reduce resting levels of CRP (Mendham et al., 2011).

Aerobic based exercise programs have resulted in the reduction of CRP by various percentages. Donges et al., (2011) observed a 16.1% reduction following 10 weeks of aerobic training. Increases of up to 266% above baseline have been noted after a marathon race but returning to baseline levels by 48 hours. This has been postulated to be just a reaction to the tissue damage which is concomitant with the intense aerobic exercise. The long-term reaction is a lowered baseline level (Devrnja, Matkovic, 2018). Resistance based training programs have also been shown to reduce the levels of CRP with reductions being as much as 32% in some cases (Martins *et al.,* 2010; Donges et al., 2011). Some studies have shown that combining aerobic endurance and resistance training yields better CRP reducing results than for example the utilization of endurance training on its own (Daray et al., 2010; Ricci et al., 2018).

As earlier indicated, C-reactive protein (CRP) is a biomarker signaling subclinical inflammation and exercise induced oxidative stress in which regular physical activity of moderate intensity has been shown to reduce CRP (Mattusch et al., 2000; Fallon, Fallon & Boston, 2001). This suggests that physical activity has anti-inflammatory

properties (Powers et al., 1999).

There are however very high increases in CRP post highly competitive games in elite sports. This inflammatory response differs from sport to sport depending on the duration of the games of the sport, the metabolic load and the nature and intensity of the activities during the games. Souglis et al., 2015 compared CRP levels 13 hours post-match between soccer, basketball, handball and volleyball players. Soccer players who, on average, cover distances of 9.5 to 10.7 kilometers per game had CRP increases of 290% from base line. They were followed by basketball and handball players (120%) who, on average, cover 4 to 4.5 km per game. The lowest increase of 80% was observed among volleyball players.

Heightened levels of CRP have also been known to increase the risk of acute myocardial infarction, ischemic stroke, peripheral artery disease, type 2 diabetes and metabolic syndrome (Hu et al., 2004).

Uric Acid

Uric acid (UA) is an endogenous antioxidant. Its levels in the body at rest are <7mg/dl in men and <6 mg/dl in women and its levels have been shown to rise in proportion to exercise intensity and training levels due to oxidative stress imposed by physical activity (Brites, 1999; Yamaner, 2010). It is recognized as one of the most significant antioxidants. It makes up the final product of purine bases (adenine and guanine) metabolism (Van Hoorenbeeck et al., 2012). Its concentration levels are related to the age, gender, body area, body weight, ethnicity and geographical position of the individual. It also constitutes 70% of the salivary antioxidant activity (Hadžović-Džuvo et al., 2011). The production of UA is stimulated by exercise and exercise increases purine oxidation. Since UA is the final product of purine catabolism, UA concentration increases in the organism due to physical activity (Barros et al., 2012). Exercise may require the increase in the activity of adenylate cyclase acting as an additional source of energy by producing 1 ATP and 1 AMP from 2 ADP. While the ATP is used for energy, the AMP is degraded to IMP. The IMP is catabolized to hypoxanthine then xanthine, and ultimately to UA. In other words, UA is a final product of ATP degradation and increased adenine nucleotide turnover or degradation (Gailiūnienė et al., 2007; Tsalouhidou et al., 2007; Gatterer et al., 2013). This would explain why its levels rise in proportion to exercise intensity because it is a product of energy metabolism and hence an antioxidant associated with the metabolic processes of energy production during physical exercise (Svensson et al., 2002). The enzyme responsible for the conversion of xanthine to UA is xanthine oxidase and not xanthine dehydrogenase (González, et al., 2008). The antioxidant capabilities of UA are achieved by its ability to scavenge free radicals such as xanthine oxidase-free-radicals and in this way, the damage to cells by these species is minimized (Foksinski et al., 2007; Magalhães et al., 2010). UA is taken up from plasma into skeletal muscle where it reacts with reactive oxygen species in its antioxidant reactions. This is accompanied by increased formation of allantoin an oxidation product of UA in the skeletal muscles (Svensson et al., 2002). The rise in levels of UA due to exercise usually do not exceed one day and they also do not normally go above normal plasma concentration, a condition referred to as hyperuricemia (Tsalouhidou et al., 2007). Some studies have shown that chronic exercise has the effect of making the levels of UA in trained individuals higher both at rest and after exercise than in untrained non exercising individuals (Tsalouhidou et al., 2007).

Hematological acute and chronic response to exercise

Hematological parameters, just like the biochemical factors, are more sensitive indicators of responses to exercise than physical and body composition variables (Lombardi et al., 2011). Exercise stress has been known to increase leukocytes and the increase is more significant following intensive exercise. Hemoglobin and hematocrit on the other hand, will decrease, a condition known as athlete anemia and this is as a result of hemolysis and hemodilution (Kargotich et al., 2007; Suhr et al., 2009; Nazmi et al.,2014). Some studies with soccer players however have not shown a decrease in hemoglobin, hematocrit and red blood cells (Bekris et al., 2015; Santi Maria et al., 2013). This shows contradictions among various studies. In most cases, however, all the subtypes of leukocytes (neutrophils, monocytes, lymphocytes) increase during and after physical exertion (Kakanis et al., 2010; Morgado, et al., 2016). On the other hand, the number of eosinophils remains unchanged or decreases (Kakanis, et al., 2010).

A single bout of 2 hours of exercise at 75% of heart rate maximum caused leukocytosis in female soccer players mainly due to a significant rise of neutrophils (Avloniti et al., 2007). Similar results were obtained with male young soccer players and the increase in neutrophils was ascribed to the inflammatory nature of muscle damage (Devrnja & Matkovic, 2018). A significant decrease in percentage of hematocrit was observed immediately after a soccer game of under 21 soccer players, and only mean cell hemoglobin corpuscular increased significantly, with no change in the hemoglobin levels (Sporiš et al., 2016).

CONCLUSION

Performance in sport and exercise especially at elite level is made up of a very complex interaction of the environmental or nurture factors and the nature or genetic parameters. Research has established the association between genes and phenotype characteristics. However, it is not yet clear how the chemical and metabolic pathways function exactly to create a cause effect relationship. The exact role which genetic polymorphism plays in exercise, in individual sport and team sport still needs to be researched. This suggests that attempts by direct to consumer genetics testing by some companies to identify talent and establish what sports novice athletes may be suited for through genetic profiling is rather early and not valid. In the same vain, linking sport and exercise stress to individuals' genetic disposition also requires further research.

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Corresponding author:

Sam Chenjerai Mugandani
Center for Biokinetics, Recreation and Sport Science,
School of Health Sciences, University of Venda,
Private Bag X5050, Thohoyandou, South Africa
E-mail: sam.mugandani@univen .ac.za

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Full Length Research Paper

Angiotensin-converting enzyme genotypes relationship with blood pressure, C-reactive protein and selected physical tests in Zulu South African cricketers

Trayana Djarova[1]*, Abigail Ramakoaba[1], Albert Basson[1], Sam Mugandani[2], Jeanne Grace[2], Jacque Cloete[3] and Gregory Watson[4]

[1]Department of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa 3886, South Africa.
[2]Department of Biokinetics and Sport Science, University of Zululand, KwaDlangezwa 3886, South Africa.
[3]Department of Mathematical Sciences, University of Zululand, KwaDlangezwa 3886, South Africa.
[4]School of Biochemistry, Genetics and Microbiology, University of KwaZulu Natal, Scottsville 3209, South Africa.

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Zulu cricketers (n=14) and students (n=17) as controls were genotyped (blood spots) for angiotensin converting enzyme (ACE), gene by PCR amplification followed by agarose gel electrophoresis. Systolic and diastolic blood pressure (SBP and DBP) and grip strength (kg), knee extension and flexion (Nm/kg) were measured, systolic tension time (STT) and metabolic rates (MR) were calculated. After ANOVA, the association between these parameters and I/D gene polymorphism was probed using *Chi*^{2} maximum likelihood test and Fisher's exact test. ACE genotyping for the whole group displayed a complete absence of II genotype, 67.7% DD and 32.3% ID genotypes. The frequency of D allele was 83.8% and I allele 16.2%. In cricketers DD and ID genotypes were 50% each compared to controls-83% DD and 17% ID. No differences in grip strength and quadriceps/hamstring muscle strength between the groups were observed, but for the whole cohort 86% D allele frequency was associated with higher (greater than 43.3 kg) grip strength ($p<0.037$). In cricketers CRP (less than 3.0 mmol/l) was associated with 79% D allele frequency. SBP and DBP were significantly lower by 3.2 and 4.25 mmHg, whereas increased values of STT by 5.5%, and MR by 10.3% were found. Although, the number of participants in this study is small, it is concluded that in cricketers no over presentation of DD or ID genotypes was observed indicating a more balanced display of power and endurance required for the game.

Key words: Angiotensin converting enzyme (ACE) genotype, polymorphism, blood pressure, body mass index (BMI), lean body mass (LBM), fat mass, hand grip, quadriceps and hamstring muscle strength, Zulu cricketers.

INTRODUCTION

Researches have shown that genes play an important role in athletes' performance in various sports (Woods et al., 2002; Wolfarth et al., 2000; Williams and Folland, 2008; Bray et al., 2008; Ruiz et al., 2010). To date improvements in athletes' performance have been based mainly on the manipulation of physiological, physical, nutritional and psychological factors which have been referred to as nurture or environmental constrains (MacArthur and North, 2004; Davids and Baker, 2007). The general view is that there is an interactive influence of genetic and environmental factors on human physical performance. Therefore numerous studies have focused on the genetic make-up of the athletes and associations with fitness phenotypes, physical/physiological tests and molecular basis of adaptation to training (Rankinen et al., 2000; Calo and Vona, 2008; Lucia et al., 2010).

Angiotensin converting enzyme (ACE) polymorphism is the most investigated genetic variation linked to athletic status, physical performance, cardiovascular and muscle function, and trainability in athletes (Alvarez et al., 2000; Scanavini et al., 2002; Collins et al., 2004; Amir et al., 2007; Wang et al., 2008; Min et al., 2009; Ruiz et al.,

*Corresponding author. E-mail: tdjarova@pan.uzulu.ac.za or drdjarova@yahoo.co.uk. Tel: +2735 902 6100 or + 27826192989.

2010).

The ACE gene is located on the long arm of chromosome 17q23.3. It is made up of 26 exons and 25 introns, stretching over 21 kb. The_polymorphism analyzed here consists of the presence (insertion, I allele) or absence (deletion, D allele) of a 287 bp Alu repeat sequence, resulting in three genotypes (homozygote DD and II and heterozygote ID) (Rigat et al., 1990; Wang et al., 2008; Wagner et al., 2006; Sipahi et al., 2006). Observations have been reported that individuals carrying ACE I allele are overrepresented in elite endurance athletes (Alvarez et al., 2000; Nazarov et al., 2001; Scanavini et al., 2002) and have been associated with higher percentage of slow twitch type I muscle fibres (Zhang et al., 2003), and have shown an enhanced response to training (Wang et al. 2008). However while the research results are not widely consistent (Scot et al., 2005; Amir et al., 2007) the general findings support the hypothesis that the I allele is associated with better performance in endurance events, while the D allele is associated with success in power events (Folland et al., 2000; Pescatello et al., 2006; Lucia et al., 2010)

The ACE gene is implicated in the rennin angiotensin aldosterone system (RAS). The local RAS is active in a variety of tissues, including lung, kidney, heart, vascular smooth muscle cells and skeletal muscle (Pieruzzi et al., 1995; Jones and Woods, 2003). Angiotensin I-converting enzyme is a key enzyme in the generation of angiotensin (AT)-II from vasoinactive angiotensin (AT)-I. Angiotensin II promotes peripheral vasoconstriction. It also stimulates aldosterone and vasopressin secretion which leads to increased blood volume and increased arterial blood pressure (Sipahi et al., 2006; Calo and Vona, 2008). Individuals who are homozygous for the D allele have been shown to have higher angiotensin-converting enzyme activity in serum and tissue than in those with the I allele (Rigat et al., 1990) but it is not known by what mechanism lower circulating levels of the enzyme could improve performance. ACE protein is also responsible for the degradation of bradykinin, respiratory drive and tissue oxygenation (Ostrander et al., 2009).

Studies involving the association of ACE gene or other muscle and metabolism related genes (example ACTN3, AMPD1, ADRB2, TNF) with blood biochemical parameters and markers of inflammation and exercise-induced oxidative stress have been rare and not studied enough (Lakka et al., 2006; Payne et al., 2007; Bray et al., 2008; Wang et al., 2008; Andonov et al., 2008; Tsianos et al., 2009; Milander et al., 2009). The association of ACTN3 and TNF gene polymorphism with C-reactive protein, uric acid and lactate in cricketers was reported (Djarova et al., 2011). Considering the role of ACE protein in the regulation of some inflammatory reactions and skeletal muscle efficiency (Woods et al., 2000), it is important to explore further the possible association of ACE polymorphism with the above mentioned markers in the same group of cricketers.

Modern cricketers are now exposed to greater physical and physiological demands. Heart rate could reach 190 beats/min and the predominant contribution from the oxygen-independent glycolysis to lactate can contribute to 60% of the total energy in multiple activities of short duration of less than 40 s which are typical for the cricket game (Noakes and Durandt, 2000).

Martens (2004) has identified the following as the estimated energy and muscular/cardiovascular fitness demands of cricket: low to moderate aerobic capacity, moderate anaerobic capacity, moderate strength and flexibility, low to moderate endurance and moderate to high speed. Fast bowling has been linked with a mesomorphic somatotype, greater percentage of type II muscle fibres and a superior phosphagenic and glycolytic metabolic pathways together with eccentric muscle strength. Speed of the ball at release was seen to determine success in bowling (Stuelcken et al., 2007). Hand grip strength was also adjudicated to be an acceptable indicator for good performance in cricket (Koley and Yadar, 2009). A shorter stature and isokinetic knee and shoulder strength were seen to be contributory to the success of batsman (Noakes and Durant, 2000; Nunes and Coetzee, 2007).

The aim of the study is to explore ACE I/D polymorphism, blood pressure and association with C-reactive protein and selected physical tests in Zulu South African cricketers.

MATERIALS AND METHODS

Experimental subjects

The participants of this study were 31 Zulu South African males (14 cricketers age 22.85±0.65 from the University of Zululand cricket team and 17 students age 22.64±0.66 as controls). All experimental subjects were volunteers and a written consent was obtained prior to the study. Experimental protocols were approved by the Ethic Committee of the Research Board of University of Zululand. The participants of the control group reported leisure physical activities once or twice weekly. Cricket players participated in regular 2 h training sessions 5-6 times weekly and played club matches in the Uthumgulu District, KZN over the weekend and inter-universities matches during the season.

Measurements of body mass index (BMI), fat percentage (Fat %), lean body mass (LBM) and fat mass (FM) were taken according to the procedures of the American College of Sports Medicine (Thompson et al., 2000). The evaluation testing procedure as suggested by Ashton and Myers, (2004) was used for the measurement of grip strength. The IsoKnee α was applied to determine the relative strength of the quadriceps (knee extension) and hamstring (knee flexion) muscles as suggested by Coetsee (1995). The speed of rotation was set at 60° for the measurement of the peak muscular strength. A warm-up routine of two to three sets of 6 (six) repetitions interspaced by 30 s rest followed by 3 (three) maximum. The subject was allowed to recover for a few minutes. Data was recorded with the subject performing maximal knee extension and knee flexion for the duration of 10 s.

The estimates of daily energy requirements and metabolic rate (MR) were done using Cunningham equation (Thompson and Manore, 1996). The equation estimates metabolic rate at rest which

Table 1. ACE genotype and allele frequency (%) in cricket players and controls.

Group	Genotype frequency in % and number in brackets)					Allele frequency in %	
	DD		ID		II	D	I
Cricket players (n=14)	50.0	(7)	50.0	(7)	Null	75.0 (10.5)	25.0 (8.5) [a]
Controls (n=17)	82.4	(14)	16.7	(3)	Null	91.2 (15.5)	8.8 (1.5) [a]
Total (31)	67.7	(21)	32.3	(10)	Null	83.8 (26)	16.2 (5.0)[a]

[a]p= 0.004 Fisher's test - two tailed based on %.

is multiplied by an activity factor (within range 1.2 to 1.9) to establish mean daily energy requirements. This estimation has been shown to be the best energy requirement prediction equation for metabolic rate in athletic population (Watson et al., 2005).

All participants were advised not to change their dietary habits and to refrain from physical exercise 24 h before blood sampling. Blood samples were collected at rest from the antecubital vein into vacutainers and analysed in the accredited Lancet laboratory at Bay Hospital, Richards Bay according to the South African standards of good laboratory practice. The Dimension Xpanda (Siemens, Germany) equipment was used for the determination of C-reactive protein (CRP, range 0-8 mg/L), uric acid (UA, range 0.26-0.45 mmol/L and lactate (LA, range 0.63-2.4 mmol/L).

Genotyping

Blood spots were collected on FTA® Classic cards according to the manufacturer's instructions (Whatman International, UK). Samples were prepared by punching 1.2 discs from the cards and washing with FTA® purification reagent and TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) according to the manufacturer's instructions. PCR was then performed directly from the dried disc. The detection of the insertion (I) and deletion (D) alleles of the *ACE* gene was performed by a modified method of Alvarez and Coto (1998). The primer sequences were ACE F (forward): 5´-CTGGAGACCACTCCCATCCTTTCT -3´ and ACE R (reverse): 5´-GATGTGGCCATCACATTCGTCAGAT -3´. PCR reactions were performed using the SensiMix™ dT kit according to the manufacturer's instructions (Quantace, UK). The final reaction mixtures contained 1× SensiMix (with a final Mg^{2+} concentration of 3 mM) and 200 nM of each of each primer. 20 µl of the PCR mix was added to a single dried disc in a thin-walled 200 µl PCR tube. All amplifications were performed in a Rotor-Gene 6000 (Corbett Research, Australia) using the following conditions: activation step 95°C for 10 min. followed by 40 cycles of 95°C for 10 s, 60°C for 20 s 72°C for 20 s. 1 µl 6× loading buffer was added to 5 µl of each PCR reaction which was then loaded and analysed in a 2% (w/v) agarose 1× TBE gel. All genotypes were determined in duplicate.

Statistical analysis

The Student's *t*-test was used to analyze the statistical difference in the blood biomarkers and physical characteristics between the cricket players and the control group. The results are presented as mean ± SEM. Statistical significance was accepted at $p<0.05$. Statistical analysis for the genotype associations was done using GenStat Discovery Edition 3. The distribution of some variables was skewed; hence these variables were transformed for the Analysis of Variance (Unbalanced design). For the association tests, CRP levels were categorized as less than 3 mg/L (low) and less than 3 mg/l (high) according to Pearson et al. (2003). Other variables were categorized according to their median (M). After ANOVA the association was examined using Chi^2 maximum likelihood test and Fisher's exact test.

RESULTS

ACE genotyping and allele frequencies are shown in Table 1. ACE genotyping showed a complete absence of II genotype (Figure 1). For the whole group 67.7% DD and 32.3% ID genotypes were observed (Figure 2) In cricketers, DD and ID genotypes were 50% each compared to controls – 83% DD and 17% ID (Figure 2). The total frequency of 83.8% D allele for the cohort was significantly higher ($p=0.004$) compared to 16.2% I allele. It was also found that in cricketers 25% ACE I allele frequency was higher ($p=0.004$) than 8.8% in controls, and 75% D allele frequency was lower ($p=0.004$) compared to 91.2%, respectively (Figure 3 and Table 1).

The blood pressure results are shown in Table 2. In cricket players SBP was lower by 3.2 mmHg ($p<0.05$) and DBP by 4.25 mmHg ($p<0.001$), where the values of STT were increased by 5.5% ($p<0.05$) compared to controls. No differences in heart rate and pulse pressure and no associations between blood pressure and allele frequencies were noted.

Cricketers have shown higher ($p<0.05$) basic metabolic rate and increased values ($p<0.001$) of metabolic rate by 10.3% and energy requirements by 14% (Table 3).

C-reactive protein (Table 4) in controls is much higher ($p<0.001$) than in cricketers, but still within the reference range of 0-8 mg/L accepted by Lancet Laboratory, South Africa. The results in cricketers have shown that 79% D allele frequency was associated ($p<0.001$) with lower CRP levels (<3.0 mg/L). Uric acid (<0.30 mmol/L) was associated ($p=0.001$) with 43% D allele frequency.

BMI, LBM and FM were higher ($p<0.001$) in cricket players (Table 5). High D allele frequency (91-94%) were associated with BMI and FM in cricketers ($p=0.001$) and in controls ($p=0.029$), where LBM has shown an association with 71% D allele ($p<0.041$) and with 94% D allele ($p<0.029$) respectively.

No differences in grip strength and the strength of the quadriceps (knee extension) and hamstring (knee flexion) muscles between the groups were observed (Table 6).

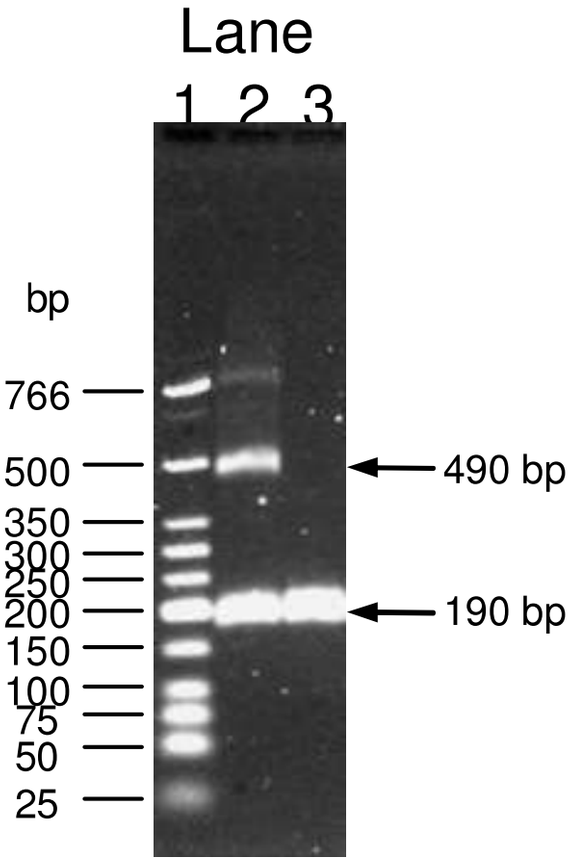

Figure 1. Analysis of ACE ID and DD genotypes. Amplified fragments were resolved in 2% (w/v) agarose, 1 ×TBE gels. Lane 1 - Low molecular weight DNA ladder (New England Biolabs); Lane 2 - ID genotype; Lane 3 - DD genotype. II genotype is absent.

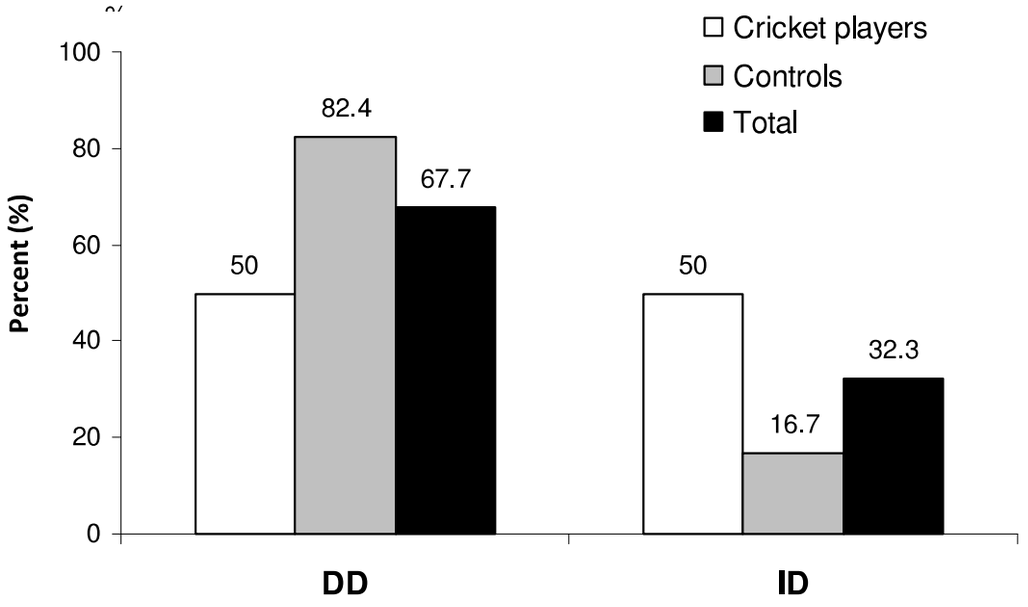

Figure 2. ACE DD and ID genotype frequencies (%) in cricketers, controls and the whole group.

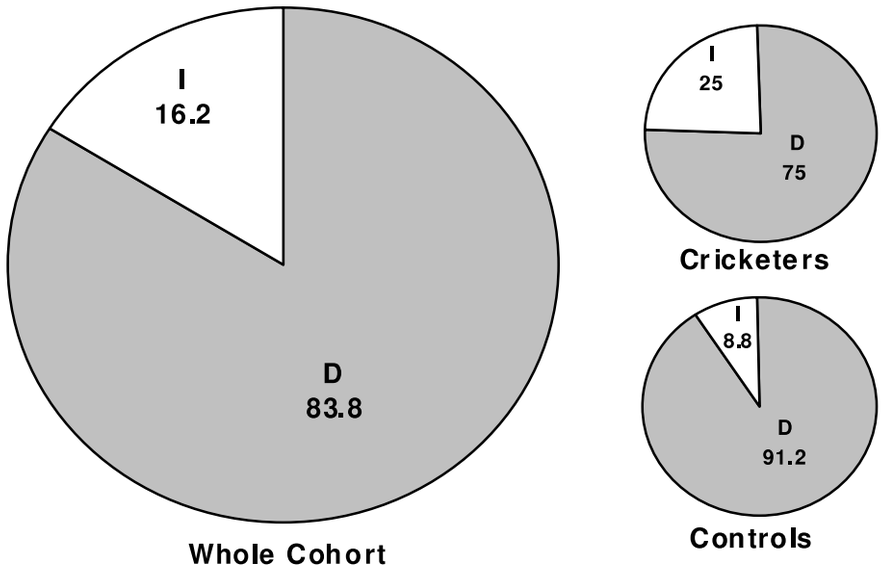

Figure 3. D and I allele frequencies (%) for the whole cohort, cricketers and controls.

Table 2. Systolic and diastolic blood pressure (mmHg) pulse pressure, heart rate (beats/min) and systolic tension time in cricket players and control group (mean ± SEM).

Parameters	Cricket players	Control group
Systolic blood pressure mmHg	120.81 ± 2.03	123.75 ± 1.07*
Diastolic blood pressure mmHg	76.88 ± 1.4	81.13 ± 1.31**
Pulse pressure mmHg	43.00 ± 1.29	42.00 ± 2.01
Heart rate beats/min	60.75 ± 0.85	59.80 ± 1.11
Systolic tension time (SBP x HR)	7654.27 ± 147.59	7253.60 ± 124.48*

*p < 0.05; *p > 0.0001.

Table 3. Basic metabolic rate, metabolic rate and energy requirements (kilojoules) in cricket players and control group (mean ± SEM).

Parameters	Cricket players	Control group
Basic metabolic rate	1502.40 ± 25.91	1480.31 ± 13.34*
Metabolic rate	2246.99 ± 102.24	2035.40 ± 18.34**
Energy requirements (kilojoules per food intake)	9737.41 ± 76.79	8520.18 ± 76.79**

*p < 0.05; *p > 0.0001.

Table 4. C-reactive protein (CRP), uric acid (UA) and lactate (LA) blood levels at rest in cricket players and control group (mean ± SEM) and ACE association tests.

Biomarkers	Cricket players	Control group
C-reactive protein (mg/L)	1.81 ± 0.37 [a,]	5.81 ± 0.51** NS
Uric acid (mmol/L)	0.31 ± 0.008 [b]	0.29 ± 0.007* NS
Lactate (mmol/L)	1.55 ± 0.08 NS	1.95 ±. 0.11** NS

Student's *t*-test: *p<0.05 control group vs. cricket players; **p<0.001 control group vs. cricket players.
Association tests: [a]p=0.001 CRP <3 mg/L in cricketers – ACE D allele frequency (79%), I allele (21%); [b]p=0.001 UA <0.30 mmol/L in cricketers – ACE D allele frequency (43%), I allele (57%); NS – no significant association tests.

Table 5. Physical characteristics of cricket players and control group (mean ± SEM) and ACE association tests.

Physical characteristics	Cricket players	Control group
Weight (kg)	68.68 ± 2.54	61.00 ± 1.61 **
Stature (cm)	175.08 ± 1.25	170.58 ± 0.33 **
Body mass index-BMI (kg/m^2)	22.40 ± 0.81 [a,]	20.79 ± 0.36 **, [C]
Lean body mass-LBM (kg)	61.81 ± 2.01 [b]	55.41 ± 1.49 **,[C]
Fat mass-FM (kg)	6.87 ± 0.54 [a,]	5.59 ± 0.22 **,[C]
Fat %	9.84 ± 0.39 NS	9.13 ± 0.32 * NS

Student's *t*-test: *p< 0.05 control group vs. cricket players; **p<0.001 control group vs. cricket players.
Association tests for BMI, LBM and FM: [a]p=0.001 BMI below 22.4 kg/m^2 , and FM below 6.9 kg – ACE D allele frequency (91%), I allele frequency (9%) in cricketers; [b]p=0.041 LBM below 61.8 kg – ACE D allele frequency (71%), I allele frequency (29%) in cricketers; [c]p=0.029 BMI below 22.4 kg/m^2 , LBM below 55.4 kg and FM below 5.6 kg – ACE D allele frequency (94%), I allele frequency (6%) in controls; NS – no significant association tests.

Table 6. Grip strength (kg), quadriceps strength (knee extension - Nm/kg) and hamstring strength (knee flexion - Nm/kg) of cricket players and control group (mean ± SEM) and ACE association tests.

Physical characteristics	Cricket players	Control group
Grip strength – L (kg)	44.00 ± 2.12 NS	42.57 ± 1.97 NS
Grip strength – R (kg)	45.79 ± 2.25 NS	45.11 ± 1.84 NS
Knee extension – L (N/kg)	3.73 ± 0.11 [a,]	3.71 ± 0.13 [e,]
Knee extension – R (N/kg)	3.63 ± 0.08 [b]	3.59 ± 0.12 [e]
Knee flexion –L (N/kg)	2.04 ± 0.08 [c]	2.08 ± 0.88 [f]
Knee flexion –R (N/kg)	2.00 ± 0.02 [d]	2.00 ± 0.08 [f]

L = left; R = right. Association tests: NS – no significant association tests for grip strength (L) and (R) per group of cricketers and controls. p=0.037 for the whole cohort grip strength (L) and (R) above 44.3 kg – ACE D allele frequency (86%) and I allele frequency (14%). [a]p=0.010 Knee extension (L) .above 3.7 Nm/kg ACE D allele frequency (86%) and I allele frequency (14%) in cricketers. [b]p=0.014 Knee extension (R) above 3.6 Nm/kg – ACE D allele frequency (79%); I allele frequency (21%) in cricketers. [c]p=0.014 Knee flexion (L) above 2.0 Nm/kg – ACE D allele frequency (78%) and I allele frequency (22%) in cricketers. [d]p=0.014 Knee flexion ® above 2.1 (Nm/kg) –ACE D allele frequency (83%) and I allele frequency (175) in cricketers. [e]p=0.001 Knee extension (L) above 3.7 Nm/kg and (R) above 3.6 N/kg – ACE D allele frequency (83%) and I allele frequency (17%) in controls.[f]p= 0.001 Knee flexion (L) and (R) above 2.0 Nm/kg – D allele frequency 87% and I allele frequency (13%) in controls

Knee extension L (>3.73 Nm/kg) and R (>3.63 Nm/kg) was associated with D allele frequency of 86% (p=0.010) and 79% (p=0.014). Knee flexion L (>2.04 Nm/kg) and R (>2.0 Nm/kg) was associated (p=0.014) with D allele frequency of 78% and 83%. For the whole cohort (Table 7) 86% D allele frequency was associated (p=0.037) with grip strength L (>43.3 kg) and R (R>45. 5 kg).

DISCUSSION

In our study a complete absence of ACE II genotype was established for the first time in Zulu South Africans. The genotype distribution for the whole cohort was skewed (67.7% DD and 32.3% ID). Low frequency of II genotype was reported in African Americans, Kenyans, Jamaicans (Scott et al., 2005), Nigerians (Woods, 2009) and Xhosa South Africans (Payne et al., 2007). The ACE distribution in Caucasian Europeans (Woods, 2009) was found to be in ratio 1:2:1 (e.g. 26% DD, 50%ID, and 24% II in British males).

Collins et al. (2004) tested a mixed group of South African-born athletes participating in Ironman triathlons and observed genotype frequencies of 24.3% DD, 54% ID and 21.6% II compared to 32.5% DD, 50.6% ID and 16.95% II in controls, pointing out that significantly higher 51.5 % ACE I allele frequency was found in the fastest South African finishers. The frequency of I allele was higher in Lithuanian elite athletes than in controls (Gineviciene et al., 2010). On the other hand, a large study of East African distance runners did not find any association between ACE genotypes and elite endurance athletic status (Scott et al., 2010). In the present study no over presentation of DD and ID genotypes was displayed in Zulu cricketers.

Our findings of high D allele frequency in Zulu South Africans are in line with the trend reported in Afro-Caribbean people (Berley et al., 1996), Nigerians (Woods, 2009) and elite Taekwondo athletes of Turkish and Azerbaijan origin (Gunay et al., 2010). The excess of D allele was represented more in athletes participating in power-oriented and short-distance/high intensity events (Myerson et al., 1999; Woods et al., 2000; Nazarov et al., 2001; Cerit et al., 2006; Charbonneau et al., 2008).

Previously, we used the same cohort to investigate the

Table 7. ACE D and I allele frequency (%) association with left (L) and right (R) grip strength in the whole cohort.

Physical test		Whole cohort		
		D (%)	I (%)	P
Grip Strength (L)	< M	92	8	-
	> M	81	19	-
Total	> M	86	14	0.037
Grip Strength (R)	< M	92	8	-
	> M	81	19	-
Total	> M	86	4	0.037

association of ACTN3 gene polymorphism and CRP, uric acid and lactate. Therefore in this study after performing ACE genotyping we conducted the association tests using the same results of the above mentioned biomarkers.

We found that the high frequency of power linked ACE D allele is also associated with lower CRP and uric acid levels in cricketers. It is important to mention that this is in concordance with our previous findings of strong association in the same cohort between these biomarkers and another power-related R allele of the ACTN3 gene (Djarova et al., 2011). C-reactive protein might be involved via cytokines in triggering metabolic signaling pathways in the exercising muscles that could be under genetic control by both genes.

It is important to emphasize that associations of the same trend as the above mentioned between high ACE D allele and high ACTN3 R allele frequencies, and BMI, LBM and FM were also found. ACE I/D polymorphism associations with BMI and body fat have been reported (Thompson et al., 2007) suggesting that it may affect adherence to exercise training.

In sports events like cricket requiring short power/sprint bursts, considering the fact that both ACE D and ACTN3 alleles have shown similarity in the association tests is a finding of interest that needs further studies. The other similarity found was the complete absence of ACE II genotype and ACTN3 XX genotype.

When comparing cricketers to control subjects we established no differences in heart rate, lower BP and higher SST, metabolic rate and energy requirements. The I/D polymorphism may play a role in enhanced performance but this is not mediated by differences in the heart rate/VO_2 relationship to training (Woods, 2009). Lower blood pressure and higher systolic tension time at rest indicate better cardiac efficiency in cricketers. Despite the interaction between ACE genotypes and serum ACE activity and the fact that D allele has been related to higher circulating/tissue ACE levels and enhanced performance, no associations between I and D alleles and BP have been reported (Bloem et al., 1996; Ostrander et al., 2009). Significantly higher estimated energy requirements were noted in cricketers. This corresponds to the findings of Noakes and Durandt (2000) that the energy demands of different cricket activities varied from 760 kJ/h in fielding to 1064 kJ/h in bowling and 1368 kJ/h in batting.

Strength, flexibility and speed parameters are among many factors contributing to the success in cricket (Nunes and Coetsee, 2007). The high D allele frequency association with grip strength that was established in the whole cohort in our study is in accordance with the findings that the D allele is related to the power/sprint output (Ruiz et al., 2010). Associations between the higher values of quadriceps/hamstring strength in cricketers and ACE D allele frequency has been observed for the first time. In batting and especially in fast bowling the trunk must flex, extend and rotate within a short period. The knee circumvents through flexion, rotation and extension. The bowling arm circumvents through extension, abduction, external rotation, thrusting flexion and internal rotation (Stuelcken et al., 2007). The average running sprint between the wickets was found to be 18.7 km/h which reflects high intensity work bouts (Christie and King, 2008).

The interpretation of association studies has always been controversial especially when the limitation is the small sample. Genetic studies need large population samples, but it is difficult to reconcile this premise with the scarce number of world-class champions or a given ethnicity and sport event (Ruiz et al., 2010). The analysis of a single sport discipline and association with I/D ACE gene polymorphism has been done in groups of athletes from 25 elite climbers, 37 swimmers and 27 up to 291 runners (Woods, 2009).

The unique demands of cricket may require specific physical characteristics and genetic traits play a substantial role. The perspective is to consider individual genetic endowment and develop training programmes that allow it to be optimized (Ostrander et al., 2009; Djarova et al., 2011). This study also might provide insight in talent identification and nurturing of young South African cricketers of various ethnicities.

Although, the number of participants in this study is small, it is concluded that ACE I/D genotyping has shown a complete absence of II genotype. Zulu cricket players

display a balanced DD and ID genotypes distribution in conjugation with significantly higher D allele frequencies associated with physical tests and beneficial differences in blood pressure and systolic tension time compared to controls. This could be considered as a competitive advantage in the cricket training and performance.

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ACTN3 (α-ACTININ-3) GENE POLYMORPHISM AND ANTHROPOMETRIC CHARACTERISTICS IN ZULU RUGBY, SOCCER AND CRICKET PLAYERS

Mugandani, Sam[1], Ramakoaba, Abigail[2], Watson, Gregory[3], Cloete, Jacque[4], Grace, Jean[1], Basson, Albert[2] and Djarova, Trayana[2]

[1]Department of Biokinetics and Sport Science, University of Zululand

[2]Departments of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa

[3]School of Biochemistry, Microbiology and Genetics, University of KwaZulu Natal

[4]Mathematical Sciences, University of Zululand, KwaDlangezwa

Email: smuganda@pan.uzulu.ac.za

***Key words**: Polymorphism, α- actinin-3 gene, lean body mass (LBM), fat mass (FM), Zulu.*

Introduction

Genetic information can be used for profiling athletes to understand their suitability for specific team positions and roles, as well as gaining insight into player development for various sports or physical activities [1].

Polymorphism is a form of gene variation. It occurs when two or more alleles exist in a population. It is related to biodiversity and adaptation; hence diversity in sport performance. An allele represents one or two forms of a gene that occupies the same locus on a particular chromosome [8, 5].

The α- actinin-3 (ACTN3) gene contains a polymorphism, R577X located on the long arm of chromosome 11 (11q13-q14) There are 2 alleles X and R, the X allele is due to a nonsense mutation. The R allele codes for the structural Z line protein α- actinin-3 in fast skeletal muscle fibers. Homozygosis for the X-allele results in complete deficiency for α-actinin-3 in humans. There are three genotypes- RR and XX homozygote and RX heterozygote [10]. The 577XX has been found more in endurance athletes, while the 577RR favors success in power performance. [11,7]. The Physical-Physiological needs of cricket, rugby and soccer in rugby players' excess body fat has been shown to negatively influence performance [9]. High playing ability has also been associated with high body mass and low fat [4]. Fat percentages are generally lower in soccer players compared to sedentary individuals [6] Fat is also known to impact negatively on jumping, sprinting, endurance and agility [6]. A leaner body is advantageous [16]. Studies have shown variance in height of soccer players with midfielders and forwards being shorter than defenders [16]. In cricket fast bowling has been linked with a mesomorphic somatotype, greater percentage of type II muscle fibers [15]. A short stature and isokinetic knee and shoulder strength were seen to be contributory to the success of batsman [12]. The aim of the study was to investigate the ACTN3 R577X gene polymorphism and physical characteristics in Zulu rugby, soccer and cricket players.

Methodology

Experimental subjects

There were 76 participants all of South African Zulu origin, (n=48) were rugby, soccer and cricket players and (n=28) were control students. All experimental subjects were volunteers and a written consent was obtained prior to the study. Body mass index (BMI), lean body mass (LBM), fat mass (FM) and Fat percentage (Fat %) were measured following the procedures of the American College of Sports Medicine [17].

ACTN3 Genotyping

Blood spots were collected on FTA® Classic cards. Samples were prepared by punching 1.2 discs from the cards and washing with FTA® purification reagent and TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) PCR was then performed directly from the dried discs. Modified method of Mills [10] was used to detect R577X polymorphism with the primers ACTN3 F:5CTGTTGCCTGTGGTAAGTGGG-3' and ACTN3 R:5'-TGGTCACAGTATGCAGGAGGG-3'. PCR was performed using the SensiMix™ dT kit according to the manufacturer's procedures (Quantace, UK). Amplifications were performed in a Rotor-Gene 6000 (Corbett Research, Australia). Amplicons were resolved in 2.5% (w/v) agarose 1x TBE gels.

Data Analysis: Data was analysed using the SPSS package. The Chi-square test with likelihood ratio was used for the analysis of genotype frequency. Allele frequency analysis was done using Fisher's exact test.

Results

ACTN3 Genotype Frequencies

When Rugby players were compared to the controls, they showed higher RX (29.4%), XX (5.9%) and lower RR (64.7%). Control genotypes were RX (7.2%), XX (0%) and RR (92.8%) ($p = 0.022$). Comparison of soccer players to controls showed no statistical difference ($p = 0.382$). The same was true for cricket players when compared to controls ($p = 0.407$). It was also the case between cricket and Rugby players ($p = 0.146$). There also was no statistical difference between Cricketers and Soccer players ($p = 0.196$). There were significant differences between Rugby and Soccer sports persons. Soccer showed a higher and unexpected RR (100%), RX (0%) and XX (0%) ($p = 0.004$). There however were no statistical differences between the combined sport groups and the controls. The whole cohort's ($n = 76$) demonstrated genotypes were 86.8% RR, 11.9% RX and 1.3% XX.

Allele frequency

There were significant differences between Rugby players and the controls ($p = 0.0002$), where Rugby had a lower frequency of R (79.4%) and higher X (20.6%), compared to controls' R (96.4) and X (3.6). Significant differences were also noted between Cricket players and controls ($p = 0.0002$), Cricketers had a lower R (74%) frequency and higher X (20.6%) frequency. There were no significant differences between the soccer sportsmen and the controls. Comparisons were made for the three sportspersons' groups. Rugby and Soccer reflected significant differences ($p = 0.0001$), with Soccer showing a higher frequency for R (100%) and an absence of the X (0%) allele. Rugby had a lower R (79.4%) and higher X (20.6%). There were no significant differences between Cricket and Rugby ($p = 1.000$).There were significant differences between Cricket and

Soccer (p = 0.00001), in which soccer showed higher frequencies of R (100%) and null X (0%) against cricket frequencies of R (79.4%) and X (20.6%).When the combined sports groups were compared to the controls there were no significant differences which were found (p = 0.09619).

Physical characteristics

When the rugby players were compared to the soccer, cricket and controls they showed significantly higher weight values ($p<0.001$). They also showed a higher body mass index (BMI) than the rest of the groups. When these two factors are, high they have been shown to be potential contributors to rugby performance [3]. With reference to stature cricket players showed higher stature compared to the rugby and soccer players and the controls ($p<0.001$). Stature is a contributory factor in bowling, more so when it is accompanied by long arm reach. The Cricketers' lean body mass was significantly higher than that of the rugby and soccer players by 3.8 and 4.6 % respectively ($p<0.001$). Higher lean body mass is necessary for greater strength and speed in all the three sports. Rugby players compared to soccer and cricket players showed higher fat mass by 42.38 to 45.47% respectively ($p<0.001$) A significant difference from the other three groups. Reduced levels of fat mass are essential for lighter body carriage during sprints [4]. All physical characteristics were found lower ($p<0.001$) in the control group.

Discussion

ACTN3 R/X polymorphism is related to muscle efficiency and the ability to produce fast contractions [19]. R allele is associated with power output and X allele with endurance performance. ACTN3 genotyping of Zulu rugby, soccer and cricket players was done for the first time. The absence or very low frequency of XX genotype observed in this study confirms previous findings [18, 14, 2]. Lower R allele frequency established in rugby players was unexpected finding that might be linked to their higher fat mass and fat %.

Conclusions

Complete absence of XX and RX genotype was established in soccer players and XX genotype was not found in cricketers. Soccer players displayed overrepresentation of RR genotype and the highest R allele frequency. Cricketers had the highest LBM. More favorable distribution of RR and RX genotypes which happen to be related to endurance performance was found in rugby players. The probing of genetic make-up in young Zulu players may provide evidence about genetic endowment and useful information needed for training, performance and future talent selection.

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Angiotensin-converting enzyme genotypes, allele frequency, C-reactive protein, uric acid in female Zulu South African soccer, netball and Bulgarian soccer players

S.C. MUGANDANI[1], T. DJAROVA[2], L. ANDREEVA[3], L. PETROV[3], P. ATANASOV[3] AND G. WATSON[4]

[1]*Department of Biokinetics and Sports Science, University of Zululand, South Africa. E-mail: scmugandani@gmail.com*
[2]*Department of Biochemistry and Microbiology, University of Zululand, South Africa*
[3]*Department of Physiology and Biochemistry, National SportsAcademy, Bulgaria*
[4]*Department of Rehabilitation and Health Sciences, Leeds Metropolitan University, City Campus, Leeds, United Kingdom, LS1 3HE.*

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Abstract

Athletes of various ethnicities and gender have been genotyped for Angiotensin-converting enzyme (ACE) I/D (insertion/deletion) genetic variants. The I allele is related to endurance and D allele to power performance. The study purpose was to investigate the ACE genotypes and allele frequency and possible association with C-reactive protein (CRP) and uric acid (UA) in female soccer and netball players. Players from University of Zululand, RSA (n=16, age= 20.8±3.1years) and from National Sports Academy, Bulgaria (n=23, age 22.6±2.0 years) and control groups of 23 and 42 female students respectively were genotyped for ACE polymorphism. CRP and UA were measured at rest. *Chi*^{2} – test, Fisher's exact test and Student *t* - test were used for statistical analysis. No statistical differences were found between netball and soccer players. Null II genotype was found in Zulu players. They displayed higher 62.5% DD genotype and 82% D allele frequency compared to 40% DD genotype and 45% D allele in Bulgarians and to respective controls and ACE population genetic study. CRP and UA levels were within the normal range. CRP was higher in Zulu players (2.80±1.2 mg/l) compared to Bulgarians (1.37±1.03 mg/l), but lower than respective Zulu controls (4.0±1.36 mg/l). In Zulu players UA levels (217.5±60.0 µmol/l) were lower compared to 259.6±32.8 µmol/l in Bulgarians. Findings of null II ACE genotype, 62.5% DD genotype and 82% D allele frequency related to low CRP and UA, favour strong sprint/power performance in Zulu athletes. Bulgarian players showed 12% II and 67% ID ACE genotypes and 45% D and 55% I allele frequency.

Keywords: Angiotensin-converting enzyme gene polymorphism, alleles, C-reactive protein, uric acid.

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Introduction

The optimization of sport performance lies in being able to understand the training needs of individual athletes, rather than using a one size fits all approach to sport training. Already there are teams who are claiming to have an edge over their opponents by using genetic profiling to personalize the training programmes of their players (Dennis, 2005). Physical performance is a complex phenomenon that is influenced by several environmental and genetic factors (MacAthur & North 2005). Individual variations in human performance could be related to various genetic traits that can potentiate the outcome of athletic challenges (Ruiz et al., 2009*).* Some of the parameters which are critical are oxidative stress and inflammation which result from heavy training and tight competition schedules. To date variants of more than 214 genes have been linked to human performance and fitness (Bray et al., 2009), with an indication that many of these genes play a significant contributory role in various sport performances (Woods, 2009; Wolfarth, 2001; Bray et al., 2009; Ruiz et al., 2010). The ACE *gene* is among these genes and athletes of various ethnic origin and gender have been genotyped for analysis of the link of (ACE) I/D (insertion /deletion) genetic variants to sports performance. ACE polymorphism is to date the most investigated gene variation (Alvarez et al., 2000; Scanavini, Bernardi & Castoldi, 2002; Amir, Amir & Yamin, 2007; Wang, Fedoruk & Rupert, 2008; Ruiz et al., 2010).

Polymorphism is a form of gene variation that occurs when two or more alleles exist in a population and is related to biodiversity and adaptation (McArdle et al., 2001). The ACE *gene* is located on chromosome 17q23.3, it encodes for the synthesis of angiotensin converting enzyme, which is related to cardiovascular and skeletal muscle function, muscle growth, training response, muscle efficiency, strength gains, and hypertrophy (Gomez-Gallego et al., 2009). The ACE polymorphism consists of an insertion of an Alu repeat sequence (I allele) or deletion (Dallele) leading to 3 genotypes, the homozygotes II and DD genotypes and the heterozygote ID genotype.(Wang et al., 2008; Wagner, Thaller, Dahse & Sust, 2006). Studies though not conclusive have shown that individuals carrying the ACE I allele are associated with better performance in endurance events, while those with the D allele have been associated with power oriented, short distance and high intensity events (Wang et al., 2008) As athletes undergo rigorous training and engage in tournaments thereafter they are subjected to systemic inflammation and oxidative stress, which are linked among others to CRP and UA (Banfi et al., 2006).

CRP is a protein sythesised in the liver. Its function is to activate the compliment system which is part of the innate immune system. This leads to components of the immune system ingesting dead or dying cells, such as those affected by eccentric muscle contractions or oxidative stress (Ablij & Meinders, 2002). CRP

is therefore a sensitive biomarker of the inflammatory status of the individual and exercise–induced oxidative stress (Djarova et al., 2009a; Gravina, Ruiz, Lekue, Irazusta & Gil, 2011).

UA on the other end is an end product of purine metabolism (González, Marquina & Rondón, 2008). It is a powerful endogenous antioxidant conferring protection against exercise-induced oxidative stress. The contribution of UA in the total plasma antioxidant capacity is approximately 35 – 65% (Andersson, Karlsen, Blomhoff, Raastad & Kadi, 2010; Hadzovic–Dzuvo, Pjanić, Mekić & Kapur, 2011; Hammouda et al., 2012).It is also an indirect marker of oxidative stress (Gatterer, Schenk, Wille, Murnig & Burtscher, 2013).

Research has shown that aerobic based, resistance based and combination protocols all cause oxidative stress and inflammation (Michigan, Johnson & Master, 2011). An analysis of the physiological demands of both netball and soccer has clearly shown that both games have a combination of aerobic and anaerobic demands. Hence (Holdys,Krysciak, Stanislawski & Gronek,2011), in a study of ACE polymorphism in athletes of various sport disciplines, classified soccer as an endurance-speed-strength (E-Sp-St) sport, suggesting that the sport requires both anaerobic and aerobic energy metabolism.

Soccer players should have very high speed, power, strength and endurance (Gabbett, Wiig & Spencer, 2013; O'Reilley & Wong, 2012). During a normal game they run a total of about 10km within that endurance context there are anaerobic explosive bursts of activity, such as sprinting over short distances, jumping and forceful contractions to maintain balance. Thus within an aerobic endurance context there are numerous anaerobic explosive bursts of activity (Stølen, Chamari, Castagna & Wisløff, 2005; Abbey & Rankin, 2011; Calahorro, Torres-Luque, Lara-Sánchez & Zagalaz-Sánchez, 2013). Soccer is also known to have eccentric movement components which lead to muscle damage, such as running backwards, sudden direction changes and tackles (Magalhães, Rebelo, Oliveira, Silva & Marques, 2010; Gravina, Ruiz, Leku, Irazusta & Gil, 2011).

Netball is afast paced contact sport. Players have to be endowed with speed to run short distance on the court. They perform repeated jumps, sudden change of direction and quick stops and starts which require agility. Both aerobic and anaerobic energy systems are a requirement (Soh, Husain & Soh, 2006; Soh, Husain & Soh, 2007; Terblanche & Venter, 2009).

The aim of this study was to investigate the ACE genotype distribution and allele frequency and possible association with C-reactive protein and uric acid in female soccer and netball players of African and Caucasian origin.

Design and Methods

Experimental Subjects

The participants were African Zulu female soccer and netball players from the University of Zululand, Republic of South Africa (n=16), age, 20.8±3.1 years), and Bulgarian Caucasian female soccer players from National Sports Academy, in Sofia Bulgaria (n=23, age 22.6±2.0 years). There were control groups of 23 and 42 female students and population cohorts (population genetic study) of 104 and 114 subjects respectively. In all cases participants gave their written consent prior to the study. The study was approved by the Research Boards of University of Zululand and National Sports Academy and was conducted in accordance with the Helsinki Declaration for Ethical Treatment of Human Subjects.

Blood samples for determination of CRP and UA were collected at rest from the antecubital vein into vacutainers and analysed in the accredited Lancet laboratory at the Bay hospital in Richards Bay, South Africa using Dimension Xpanda (Siemens Germany) and in Ciba laboratory, Sofia, Bulgaria, using Hitachi 911, Japan and Sedin 3500 Abbot, according to the South African and European Union standards of good laboratory practice. All participants were advised not to change their dietary habits and to refrain from physical exercise 24 hours before blood sampling and were questioned about current and previous transient infections.

G*enotyping*

Genotyping was done on blood spots collected on FTA® Classic cards according to the manufacturer's instructions (Whatman International, UK) or with blood samples collected in vacutainers. The detection of the insertion (I) and deletion (D) alleles of the *ACE* gene was performed by a modified method of Alvarez et al. (1998). The primer sequences were ACE F (forward):
5′- CTGGAGACCACTCCCATCCTTTCT -3′ and ACE R (reverse):
5′- GATGTGGCCATCACATTCGTCAGAT -3′. Polymerase chain reactions (PCR) reactions and amplifications were performed by using the SensiMix™ dT kit according to the manufacturer's instructions (Quantace, UK) and Rotor-Gene 6000 (Corbett Research, Australia) in South Africa and Quanta Biotech QB96 Server Gradients Thermocycler in Bulgaria, followed by restriction digestion and by agarose gel electrophoresis.

Statistical analysis

The Student *t*-test was used to analyse the statistical difference in the blood biomarkers between the Zulu and Bulgarian athletes, and between the athletes and controls. The results are presented as mean ± SD. Statistical significance was

accepted at $p<0.05$. Statistcal analysis for the genotype associations was done using GenSta Discovery Edition 3. For the association tests, CRP levels were categorised as less than 3 mg/l (low) and more than 3 mg/l (high) and for UA less than 0.26 mmol/l (low) and more than 0.45 mmol/l (high). After ANOVA the association was examined using Chi^2 maximum likelihood test and Fisher's exact test.

Results

ACE genotype and allele frequencies are shown in Table 1 for Zulu Africans and Table 2 for Bulgarians. There were no statistically significant differences between the Zulu soccer and netball players.

Table 1: ACE gene polymorphism in female soccer and netball Zulu African players

Group	**n**	**Genotype distribution in % and numbers in brackets**						**Allele frequency (%)**	
		DD		**ID**		**II**		**D allele**	**I allele**
Soccer	9	66.7	(6)	33.3	(3)	0	(0)	83.3	16.7
Netball	7	57.2	(4)	42.8	(3)	0	(0)	78.6	21.4
Players (total)	16	62.5	(10)	37.5	6	0	(0)	81.2	18.8
Controls	23	21.7	(5)	60.9	(14)	17.4	(4)	52.2	47.8
ACE gene population cohort	111	45.9	(51)	43.3	(48)	10.8	(12)	67.6	32.4

Table: 2. ACE gene polymorphism in female Bulgarian soccer players, control group and population gene study group

Group	**n**	**Genotype distribution in % and number in brackets**						**Allele frequency (%)**	
		DD		**ID**		**II**		**D allele**	**I allele**
Soccer	20	40	(8)	50	(10)	10	(2)	45	55
Controls	42	21	(9)	67	(28)	12	(5)	56	44
ACE gene population study	114	29	(32)	60	(69)	11	(13)	59	41

In Bulgaria netball is not a popular sport and therefore no netball players participated in the study. Null II genotype was found in all female Zulu players. The Zulu players had higher DD genotype 62.5% ($p = 0.036$), and also displayed

an 83.3% D allele frequency (p=0.003), compared to 40% DD genotype and 45% D allele frequency in Bulgarian players, and also to respective controls.

Table: 3. C- reactive protein (mg/L) and uric acid (mmol/L) in female Zulu African soccer and netball players and Bulgarian soccer players (means ±SD)

Biomarkers	**Zulu South African players**				**Bulgarian players**	
	Soccer	**Netball**	**Total**	**Controls**	**Soccer**	**Controls**
C-reactive protein mg/L	2.70 ±0.97	2.91 ±1.31	2.80* ±1.21	4.00 ±1.36	1.37** ±1.03	2.58 ±0.98
Uric acid mmol/L	0.24 ±0.08	0.18 ±0.050	0.22 ±0.065	0.23 ±0.060	0.26** ±0.033	0.29 ±0.073

*p < 0.001 Zulu South African players vs controls; **p < 000.1 Zulu South African player vs Bulgarian players.

The Bulgarian players showed a fairly balanced distribution of the DD (40%) and ID (50%) genotypes with D (45%) and I (55%) allele frequency respectively. No statistically significant differences in ACE genotype distribution and allele frequency were observed between Zulu African and Bulgarians population cohorts.

The CRP levels at rest for all the groups were within the normal range (0.0 – 8.0 mg/l). It was higher in Zulu players compared to Bulgarian players (p< 0.001), but lower than the respective Zulu controls. Both controls had higher CRP levels than their respective players.

The UA baseline levels were too in the normal range for all the groups (0.26 – 0.45 mmol/l). Zulu UA levels were lower (0.22 mmol/l) compared to those of Bulgarian players (0.26 mmol/l) (p<0.001). Both controls had higher levels of UA than their respective players.

Discussion

The findings of null II ACE genotype among Zulu players are similar to those reported in an earlier study of Zulu Cricket players from the same population by Djarova et al. (2011). Other studies elsewhere of people of African descent also showed low frequency of the II genotype: in Nigerians (Woods, 2009) and Xhosa South Africans (Payne et al., 2007). While on the other hand in a study of Polish Caucasian athletes (Holdys et al. 2011), women athletes in the disciplines classified as endurance-speed-strength (among which was soccer), had a genotype distribution of DD 27%, ID 45% and ID 27%. This frequency shows a dominance of the ID genotype like in the Bulgarian Caucasian players. In yet another study concerning genotype distribution among top-level Caucasians

soccer players the ID genotype was found to be more prevalent (Juffer et al., 2009). In this study the 62.5% DD genotype and 82% D allele frequency favours a strong sprint /power performance in previously unexamined homogenous cohort of Zulu female athletes. Both soccer and netball are games which require athletes to have sprinting speed and power for jumping (Terblanche and Venter, 2009; Stølen et al., 2005). The lack of II genotype which predisposes aerobic and endurance capacity may call for deliberately targeted endurance training for the Zulu players to compensate for the possible deficiency; hence training can vary widely based on an individual's genetic make-up (Bouchard, Malina, & Peruse, 1997). The Bulgarian soccer players, (with their display of a balanced 45% D and 55% I allele frequency) are likely to have an association with endurance, sprint and power performance.

Both UA and CRP levels were within the normal range. The lower resting levels of UA in individuals who exercise on a regular basis as is the case with the athletes in this study, versus that of controls have been seen in other studies (Magalhaes, Rebelo, Oliveira, Silva & Marques, 2009). Also cardiorespiratory fitness, a component of both netball and soccer, has been associated with 6-35% lower CRP levels (Plaisance & Grandjean, 2006; Martins et al., 2010). Other studies have also demonstrated that, with exercise such as training and competition female soccer players' antioxidant capacity increases (Gravina, Ruiz, Lekue, Irazusta & Gil, 2011), and subsequently reflects lower levels of the biomarkers. The differences in the biomarker levels, particularly uric acid between the Zulus and the Bulgarians maybe due to ethnicity as differences attributable to ethnicity have been demonstrated in other studies (Hadžović-Džuvo, Pjanić, Mekić & Kapur, 2011).

There was association between the DD genotype and D allele frequency with low CRP and UA. Similar association was found with another power- related R allele of the ACTN3 gene (Djarova et al., 2011).This could suggest that athletes with the DD genotype might have better protective status from oxidative stress in the muscle cells. Studies have shown that the ACE DD genotypes are genetically susceptible to left ventricular development as a result of training, which may require individual considerations during training for those athletes (Pellicia and Thompson, 2006). Individuals with ACE genotype that do not show association with CRP and UA may also be more susceptible to oxidative stress and inflammation. Through genotyping and tests of the biomarkers individualized training programmes can be designed, such as personalized rate of recovery between training bouts and sessions to minimize adverse effects during training and competition.

It should be noted however that the complex nature of athletic performance cannot be explained by a "single-gene-as-magic-bullet" philosophy (Davids & Baker, 2007).The many single gene variants work cooperatively together with

other environmental constraints of a diverse and varied nature to produce the athletic behaviors which we ultimately observe in the many and different sporting disciplines.

Furthermore the interpretation of association studies has always been controversial especially when the limitation is the small sample. Genetic studies need large population samples, but it is difficult to reconcile this premise with the scarce number of world-class champions or a given ethnicity and sport event (Ruiz et al., 2010). The analysis of a single sport discipline and association with I/D ACE gene polymorphism has been done in groups of athletes from 25 elite climbers, 37 swimmers and from 27 up to 291 runners (Woods, 2009).

Despite the small size of the sample in this study, we conclude that ACE I/D genotyping among Zulu female netball and soccer players has a complete absence of the II genotype with high D allele frequency related to speed/power performance. The Caucasian participants displayed a balanced DD and ID genotype and allele distribution. Both C-reactive protein and uric acid could be positively associated with the DD genotype and D allele which is likely to confer a protective advantage against oxidative stress and inflammation.

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ACTN3 and *AMPD1* Polymorphism and Genotype Combinations in Bulgarian Athletes Performing Wingate Test

Peter Atanasov[1], Trayana Djarova[2], Michael Kalinski[3], Lubomir Petrov[4], Radka Kaneva[5], Sam Mugandani[6], Gregori Watson[7] and Monem Jemni[8]

1. Department of Theory and Methods of Physical Education, Faculty of Pedagogy, Paissii Hilendarski Plovdiv University, Plovdiv 4000, Bulgaria

2. Department of Biochemistry and Microbiology, University of Zululand, Private Bag X1001, KwaDlagenzwa 3886, South Africa

3. Department of Applied Health Sciences, Murray State University, Murray, Kentucky 42071-3347, USA

4. Department of Physiology and Biochemistry, National Sports Academy, Sofia 1700, Bulgaria

5. Molecular Medicine Centre, Medical Academy, Sofia 1431, Bulgaria

6. Department of Biokinetics and Sport Science, University of Zululand, KwaDlangezwa 3886, South Africa

7. Department of Rehabilitation and Health Sciences, Leeds Metropolitan University, Leeds LS1 3HE, United Kingdom

8. Department of Sport Science, College of Arts and Sciences, Qatar University, Al Tarfa, Doha 2713, Qatar

Abstract: The aim of the study was to investigate *ACTN3* (α-actinin-3) and *AMPD1* (adenosine monophosphate deaminase) polymorphism and genotype combinations in Bulgarian athletes competing in various sports and the relation to peak power output. A mixed group of athletes (n = 52) competing at national and international level and a matching genetic control group (n = 109) of volunteers were recruited. Participants were genotyped for *ACTN3* and *AMPD1* by polymerase chain reaction. There were no significant differences in *ACTN3* genotype distribution between athletes performing Wingate test (38% RR, 46% RX, 16% XX) and controls (41.2% RR, 46% RX, 12.8% XX). *AMPD1* distribution was (73% CC, 27% CT, 0% TT) and in controls (73.2% CC, 25% CT, 1.8% TT). Athletes performing Wingate test showed equal 33% frequency of RR/CC and RX/CC combination, and 12% RX/CT. Significantly higher (P < 0.05) peak power output (11.10 W kg^{-1}) was found in athletes with RX/CT combination compared to other combinations (range: 8.83-9.71 W kg^{-1}) and in R-power (RR + RX) and C-power (CC + CT) dominant models (9.91 W kg^{-1}). Mean power was higher (P < 0.05) in RX/CT combination (8.93 W kg^{-1}) compared to RR/CC (7.75 W kg^{-1}) and RR/CT (7.95 W kg^{-1}). In conclusion, the low frequency of T *AMPD1* allele in Bulgarian athletes might indicate that this mutant allele is related to the physical performance. The prevalence of R *ACTN3* and C *AMPD1* alleles suggests that they could contribute to anaerobic performance. Higher peak power in Wingate test is associated with RX/CT genotype combination and R- and C-power dominant models.

Key words: Genetics, metabolism, exercise, performance, peak power.

1. Introduction

Athletic performance in various sports is influenced by genetic traits and phenotype characteristics and is associated with the presence of certain gene polymorphisms [1-10].

The polymorphism of ACTN3 (α-actinin-3) and AMPD1 (adenosine monophosphate deaminase) genes have been extensively studied in athletes [11-17]. In both genes, the polymorphism is due to single base mutations [18]. The ACTN3 gene encodes the α-actinin protein in human skeletal muscle. Its expression is limited to type II fast muscle fibres [19]. The mutation of the gene is due to C-T nucleotide

Corresponding author: Trayana Djarova, Ph.D., professor, research fields: biochemistry and sports genetics. E-mail: drdjarova@yahoo.co.uk; djarovat@unizulu.ac.za.

transition in exon 16, leading to the replacement of arginine (577R) with a stop codon (577X) leading to premature termination of α-actinin-3 synthesis [1]. The resulting polymorphism of ACTN3 gene is presented in three genetic variants (RR, XX and RX). It has been reported that RR genotype and R allele are associated with power/sprint performance and strength in various sports and different ethnic groups [3, 7-9, 17, 20-23]. Several studies have pointed out that elite power/sprint athletes (jumping, throwing, 100m runners and soccer players rarely exhibit XX genotype [5, 14, 24, 25]. The function of X allele is not fully understood. The XX genotype and X allele may alter the metabolism of muscle cells in aerobic direction resulting in poorer sprint/power performance [20, 21]. However, a handful of athletes with the RX genotype have shown excellent results in power/sprint events [22]. Recently, the research has been focused on the influence of ACTN3 R-power allele dominant (RR + RX) model alone [22, 24] or in combination with other genes [16, 26].

The AMPD1 polymorphism is a nonsense (C34T) mutation in exon 2 resulting in AMPD enzyme deficiency [27, 28]. The AMPD1 gene is presented in three genotypes (CC, TC and TT). The AMPD enzyme catalyses the deamination of AMP (adenosine monophosphate) to IMP (inosine monophosphate), thereby reducing the accumulation of ADP (adenosine diphosphate) and shifting the balance of the myokinase reaction towards ATP generation [6]. The AMPD1 gene is an important regulator of cellular energy metabolism during high-intensity physical exercise [13, 21]. Individuals with the unfavourable mutant TT genotype might show diminished exercise capacity and cardio-respiratory response to exercise [7]. The CC genotype and C allele are considered to be related to performance in power-oriented athletes, and T allele might be a negative factor for athletic performance [29]. However, it was reported that high intensity anaerobic performance was not influenced by AMPD1 genotypes [13].

In various sport disciplines, despite the specific energy demands of a particular sport, an objective assessment of the individual's physical fitness (aerobic and anaerobic capacities) is therefore a necessity for planning purposes [7, 25, 30-32]. Training regimens and conditioning have to be specifically designed and focused on the achievement of maximal responses in the most contributing physical and physiological characteristics in contemporary sports [10, 33].

The potential for achieving maximal performance under anaerobic conditions in power/sprint events is determined by the genetic endowment, properties of muscle fibres, the neuromuscular facilitations and energy metabolism [9, 34, 35]. The Wingate test is one of the gold standards and one of the most popular laboratory protocols for assessment of anaerobic capacity [25, 31, 33, 34, 36]. Research data about its relationship with genetic traits is scarce [13, 24, 37].

The rational for the study is to probe this insufficiently investigated topic taking into consideration that both ACTN3 and AMPD1 genes play significant contributory role in various anaerobic power-linked sport performances.

The purpose of this study was set in two folds: (1) to compare ACTN3 and AMPD1 genotypes and genotype combinations of Bulgarian athletes with a control group of unrelated healthy individuals; (2) to identify the following:

- Any possible association of the ACTN3 and AMPD1 genetic variants;
- Any potentially beneficial genotype combinations;
- Any R-(RR+RX) and C-(CC+CT) power allele dominant models related to the anaerobic capacity of the athletes performing Wingate test.

2. Methods

2.1 Subjects

Two groups of participants (athletes and controls) were recruited for the study. All subjects received details on the study during an information meeting

and signed an informed consent form. Experimental procedures were conducted in accordance with the Helsinki Declaration for Ethical treatment of Human Subjects. The study was approved by the Ethic Committee of the Research Board of the National Sports Academy, Sofia and by the Ethic Committee of the National Genetic Laboratory, Medical Academy, Sofia. All participants completed a health screening questionnaire via an interview with a medical doctor. Physical examination of all participants was conducted; blood pressure, heart rate and ECG were recorded at rest. The exclusion criteria included irregularities in ECG and blood pressure, history of chronic diseases, current infection, use of antibiotics and herbal, antioxidant or steroid containing supplements.

The group of athletes consisted of 52 Bulgarian male athletes (age: 21.3 ± 1.5 years, stature: 179.3 ± 1.9 cm and weight: 76.6 ± 1.8 kg). They were elite and sub-elite athletes [1] that belonged to the Bulgarian national teams and were qualifiers and participants at international level competitions. The recruited subjects represented a mixed group of athletes competing in various sports such as power events (long and triple jump, javelin throw), power/sprint orientated events (running: 100, 200, 400 m, swimming: 200, 400 m, sprint cycling, boxing, wrestling) and power/sprint-endurance games (soccer, volleyball, handball). The events were stratified on the basis of the relative anaerobic/aerobic energy system contribution, the duration and intensity of the competitive exercise performance in each sport [38].

The control group comprised of 109 unrelated healthy volunteers (male students with very low level of leisure physical activities), matched for (age: 20.6 ± 1.9 years, stature: 175.5 ± 2.8 cm and weight: 72.5 ± 2.3 kg). The students were asked to complete a questionnaire about their physical activity habits.

2.2 Experimental Approach

2.2.1 Genotyping

All participants in the current study were Caucasian Europeans. Venous blood samples (10 mL) were obtained from all individuals at rest in EDTA (ethylenediaminetetraacetic acid) anti-coagulant vacutainers for genome DNA isolation from white blood cells using a reagent kit Macherey, Nagel, Germany. Genotyping for detecting the ACTN3 R577X variants (rs 1815739) was performed [39] by using forward 5'-CTGTTGCCTGTGGTAAGTGGG-3' and reverse 5'-TGGTCACAGTATGCAGGAGGG-3' primers (Tib Molecular Biology, Germany) and RFLP (restriction fragment length polymorphism) technique [39]. The PCR amplification (Quanta Biotech QB96 Server Gradient Thermocycler) was performed by 35 cycles of denaturation at 95 °C, annealing at 65 °C and extension at 72 °C. The amplified fragment subsequently underwent digestion by Ddel enzyme (NEB, UK). The digested products were electrophoresed in 3% (*w*/*v*) agarose gels and visualised by ethidium bromide staining.

The genotyping of the AMPD1 variants (rs 17602729) was performed using a detection method for C34T mutation [28]. The following primers were used: forward 5'-CTCTGACAAATGGCAGCAAA-3' and reverse 5'-TGTCTACCCCAAAGCAGTGA 3' (Tib Molecular Biology, Germany). PCR was carried out for 30 cycles at 94 °C denaturing temperature, 64 °C annealing temperature and 72 °C extension temperature. Ethidium bromide staining of 3% (*w*/*v*) agarose gels was done after the digestion with HpyCH4 IV enzyme (NEB, UK).

After completing the stage of genotyping, the results were analysed. ACTN3 and AMPD1 genotype distribution in both groups was found to be in Hardy-Weinberg equilibrium. No statistically significant differences were observed between the groups. Therefore, to further explore the potentially beneficial genetic traits that might be related to attaining higher anaerobic performance, we tested only the group of athletes by using Wingate test.

2.2.2 Wingate Test Protocol

The Wingate protocol consisted of 30 seconds maximal effort on a mechanically braked, pan-loaded Monark 828E cycle ergometer (Monark, Varberg, Sweden). Resistance was set up at 0.075 kg/kg body mass [34] and was preloaded onto weight pan for immediate application at the beginning of the test. The subject's feet were firmly strapped to the pedals, and the seat height was adjusted for optimal comfort and pedaling efficiency. All participants were previously familiarised with the test. The protocol began with 5 minutes warm up period with light cycling resistance and 5 seconds of sprint cycling at the end of every consecutive minute. After 2 minutes of recovery period (very low resistance cycling), 15 seconds of acceleration at 70 rpm with 1/3 of the work resistance was used. Afterwards the full load was applied and the electronic revolution counter activated. Power output for each second was recorded during the 30 seconds period. A computerised system was used to determine the peak power output (P_p) in watts per kg body weight (P_p W kg$^{-1)}$. On the basis of the total work accomplished in 30 s the anaerobic capacity or mean power (P_m) for period of 30 s was calculated in watts per kg body weight (P_m W kg^{-1}).

2.2.3 Statistical Analysis

Differences in genotype distributions, allele frequencies, and associations of genotypes and genotype combinations with the Wingate parameters (peak power output and mean power) were evaluated by GenStat Discovery Edition 3 and by ANOVA with post hoc Bonferroni test. The Wingate test variables were categorised according to their median. After ANOVA, the associations were examined using Chi2 maximum likelihood test and Fisher's exact test. The Wingate test parameters were expressed in mean value and standard deviation (s). Statistical significance was accepted at $P < 0.05$. The statistical power of the study was calculated post hoc using a statistic power calculator [40] for alpha (α) error level criterion set at 0.05 or 5% confidence level (5% chance the null hypothesis to be rejected incorrectly).

3. Results

The distributions of ACTN3 and AMD1 genotypes and allele frequencies in athletes and in the control group are shown in Table 1. No significant differences in *ACTN3* genotype distribution and allele frequencies were found between the athletes and controls. In both groups, the prevalence of R allele (61.5%-64%) was notable (Table 1). No differences in the R-allele power (RR + RX vs. XX) dominant model were found between the athletes (n = 44, 84.6%) and the controls (n = 95, 87.2%).

In *AMPD1* genotype distribution, a null TT genotype was found in athletes, whereas in the control group, the frequency of TT genotype was 1.8% (Table 1).

Table 1 Distribution of genotypes and allele frequencies (%) of ACTN3 and AMPD1 genes in athletes and in the control group.

Gene	Genotype						Allele frequency	
ACTN3	RR		RX		XX		R	X
Groups	*n*	%	*n*	%	*n*	%	%	%
Athletes (n = 52)	20	38	24	46 %	8	16	61.5	38.5
Controls (n = 109)	45	41.2	50	46 %	14	12.8	64	36
AMPD1 groups	CC		CT		TT		C	T
	n	%	*n*	%	*n*	%	%	%
Athletes (n = 52)	38	73	14	27 %	0	0	86.5	13.5
Controls (n = 109)	80	73..2	27	25 %	2	1.8	86	14

Table 2 Distribution of genotype combinations (%) of AMPD1 and ACTN3 genes in athletes and in the control group.

Groups	RR/CC		RR/CT		XX/CC		XX/CT		RX/CC		RX/CT		RR/TT		RX/TT	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Athletes (*n* = 52)	17	33	3	6	4	8	4	8	17	33	7	12.0	0	0	0	0
Control (*n* =109)	33	30	9	8.3	9	8.3	3	2.8	39	36	14	12.8	1	0.9	1	0.9

No differences in CC and CT genotypes were found between the groups. The prevalence of CC genotype (73%-73.2%), high frequency of C allele (86%-86.5%) and low frequency of T allele (13.5%-14%) was observed in both groups. With regards to the C-allele power dominant model (CC + CT vs. TT), all athletes displayed 100% (*n* = 52; CC + CT genotype) compared to 98.2% (*n* = 107) in the controls.

The *ACTN3* and *AMPD1* genotype combinations in the athletes and the control group are presented in Table 2. Both groups displayed similar combinations of RR/CC (30%-33%), RX/CC (30%-36%), RX/CT (12%-12.8%), XX/CC (8%-8.3%) and RR/CT (6%-8.3%) genotypes. No significant differences between the two groups were found. However, in athletes, the null RR/TT and RX/TT genotypes should be noted.

The results of Wingate test parameters of athletes are shown in Figs. 1-4. Higher ($P < 0.05$) peak power output (Fig. 1) was found in athletes with RX (P_p = 10.03 ± 0.60 W kg^{-1}) and CT (P_p = 10.30 ± 0.54 W kg^{-1}) genotypes. The association tests showed that values above the median are associated with 75% of RX genotype ($P = 0.006$) and 71% of CT genotype ($P = 0.011$), respectively.

Athletes with RX/CT genotype combination (Fig. 2) displayed a significantly higher ($P < 0.05$) peak power output of 11.10 ± 0.86 W kg^{-1} when compared to RR/CC, RX/CC, RR/CT and XX/CT combinations. The lowest value of 8.83 ± 0.99 W kg^{-1} was found in XX/CC combination. Athletes with RX/CT genotype combination showed the highest ($P < 0.001$) peak power output compared to the average value of P_p = 9.41 ± 0.82 W kg^{-1} derived from all other genotype combinations pooled together. P_p values above the median are associated with 71% of RX/CT combination ($P = 0.030$). In addition, the P_p of RX/CT combination was significantly higher ($P < 0.05$) compared to R-allele power dominant model (P_p = 9.92 ± 0.52 W kg^{-1}) and C-allele power dominant model (P_p = 9.90 ± 0.50 W kg^{-1}). No significant differences were observed in the mean power (Fig. 3) among ACTN3 and AMPD1 genotypes. When investigating the six genotype combinations (Fig. 4), the results showed that athletes with RX/CT combination have significantly higher ($P < 0.05$) P_m = 8.93 ± 0.61 W kg^{-1} when compared to RR/CC and RR/CT genotype combinations. P_m values above the median are associated with 75% of RX/CT genotype combination ($P = 0.032$).

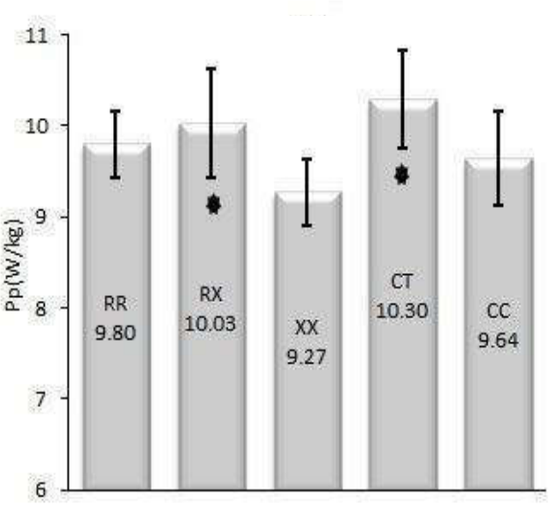

Fig. 1 Peak power output (P_p W kg^{-1}) of *ACTN3* and *AMPD1* genotypes in athletes.

*$P < 0.05$—P_p values of RX and CT genotypes versus XX and CC genotypes.

Study power alpha (α)—RX genotype (95%) and CT genotype (99.6%) versus XX genotype and CT genotype (98.2%) versus CC genotype.

Association tests:

$P = 0.006$—P_p values above the median associated with 75% of RX genotype.

$P = 0.011$—P_p values above the median associated with 71% of CT genotype.

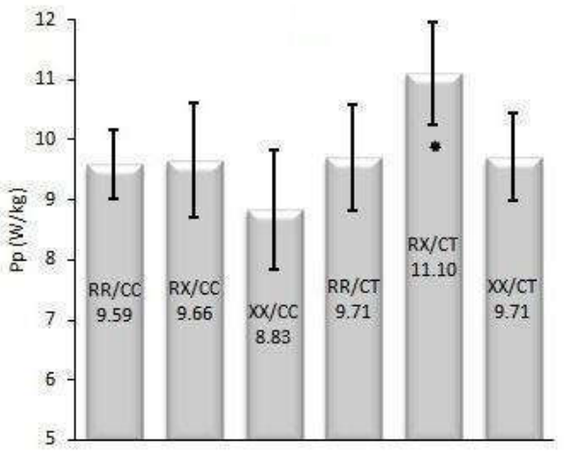

Fig. 2 Peak power output (P_p W kg^{-1}) of *ACTN3* and *AMPD1* genotype combinations in athletes.

*P < 0.05—RX/CT genotype combination versus RR/CC, RX/CC and XX/CC genotype combinations

Study power alpha (α)—RX/CT combination versus RR/CC (α = 98.7%), RX/CC (α = 95%), XX/CC (α = 96.9%), RR/CT (α = 63.5%) XX/CT (α = 81.2%).

Association test: P = 0.030—Pp values above the median associated with 71% of RX/CT genotype combination.

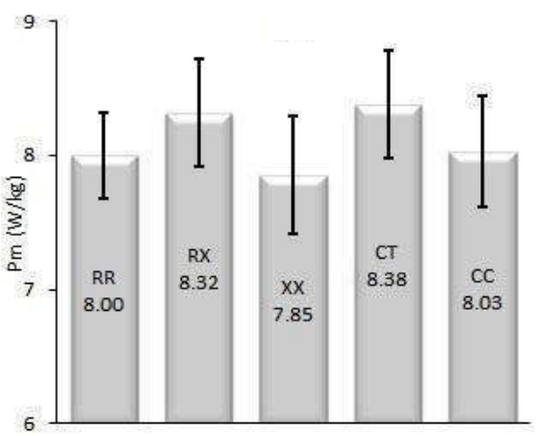

Fig. 3 Mean power (P_m W kg^{-1}) of ACTN3 and AMPD1 genotypes in athletes. No significant differences.

4. Discussion

In this study, the highest peak power obtained during Wingate test were found in carriers of RX/CT genotype combination compared to all other combinations and to the carriers of R and C power allele dominant model. A null TT genotype and low T allele frequency were observed in athletes.

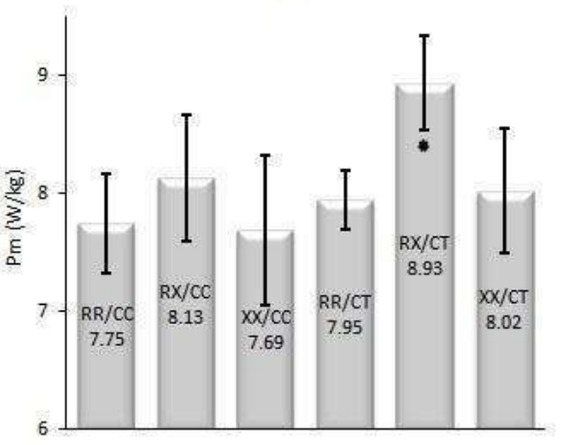

Fig. 4 Mean power (P_m W kg^{-1}) of *ACTN3* and *AMPD1* combinations in athletes.

*P < 0.05—P_m values of RX/CT genotype combination versus RR/CC and RR/CT combinations.

Study power alpha (α %)—RX/CT combination versus RR/CC (α = 98.5%), RX/CC (α = 75.2%), RR/CT (α = 95%), XX/CT (α = 64.4%) and XX/CT (α = 50.4%) genotype combinations.

Association test: P = 0.032—P_m values above the median associated with 75% of RX/CT combination.

The Wingate test peak power results are in accordance with our previous investigations of male Polish elite athletes of the national basketball, volleyball, handball, rugby and soccer teams [31] and with the data reported in male athletes (track, cycling, soccer, boxing, wrestling, water polo, lacrosse and American football) from Division 1, US Air Force Academy [36].

We expected athletes with RR/CC combination to have the highest anaerobic performance, taking into consideration that R and C alleles are both associated with power/strength and sprint events. In the present study, the unexpected finding was that only 12% of the athletes who carried RX/CT genotype combination have shown higher anaerobic performance. Therefore, the small sample size of this subgroup was of major concern when analysing the results. However, despite the small percentage of the carriers of this genetic trait, our findings were supported by the calculation of the statistical power of the study, the association tests and

the comparison with the R and C power allele dominant models. The association of CT and TT genotypes with anaerobic performance is not clear. It was reported that the Wingate test results are unaffected by AMPD1 genotypes [13]. In contrast, a faster power decrease and a lower mean power was demonstrated in individuals with TT and CT genotypes during the 30 s cycling test [27]. However, despite the 10% decline in the mean power, a better circulatory adaptation to exercise was found in TT and CT individuals with diminished muscular AMPD enzyme activity. It was suggested that it is probably due to an AMPD1 genotype-dependent increase in adenosine, which is known to be an important vasodilator [6]. Our findings suggest that C34T mutation might play an important role in energy metabolism by effecting both AMPD's and AMPK's enzyme activities [13, 21], thereby altering ATP and AMP cellular levels and the anaerobic performance.

Our data about ACTN3 genotype distribution in athletes and controls are in agreement with the findings established by numerous other studies of Caucasian Europeans [5, 11, 14, 17]. Our results confirmed that peak power was not different among ACTN3 genotypes, and support the finding that peak power was significantly higher in the R-allele dominant model as it was found in Japanese athletes [24].

The evidence for beneficial effects of RR genotype and R allele has been provided by numerous studies of mixed sprint/power cohorts of athletes in various sports (sprints, swimming, skating, gymnastics, track and field, throwing, weightlifting, soccer, basketball, volleyball, cricket) [1, 7, 8, 11, 17, 21, 30]. In contrast, the role of XX genotype and X allele on athletic performance was not quite clear. Recent publications have shown that α-actinin-3 deficiency results in a fundamental shift in metabolism from the anaerobic pathway towards the oxidative muscle metabolism and enhanced endurance performance [2, 9].

The expression of the skeletal muscle α-actinin-3 protein is almost exclusively restricted to fast twitch (type II) muscle fibres, where it constitutes one of the major components of the Z-disk. The α-actinin-3 stabilises the muscle contractile apparatus, which may confer a higher capacity for force absorption/transmission compared to type I fibres [22, 37]. The protein interacts with potassium channel proteins, glycolytic enzyme fructose—1,6-biphosphatase, glycogen phosphorylase and the calsarcins which bind to and regulate the expression of calcineurin. This protein is a signalling factor which plays a role in the specification of muscle fibre type and other signalling cascades on the cell membrane of the working muscle [19].

In our study, the simultaneous display of R and C alleles related to power/sprint and the mutant X and T alleles in RX/CT genotype may indicate that all contribute to enhanced anaerobic performance

The limitation of this preliminary study is the small sample size of the group of athletes. Bulgaria is a small country and limited numbers of elite and top athletes in each of the sport disciplines are available. Other studies have also recruited small number of athletes when investigating the influence of genotypes on Wingate test [33] and explosive leg muscle power [7]. The other limitation is that the inexperienced volunteers from the control group could not be exposed to Wingate due to the risk factors related to the metabolic and performance demands of the test.

Experimental studies recruiting large cohorts have to be carried out to validate our current exploratory results. More polygenic profiles should be probed to consider the optimization of training regimen and focussed training interventions to achieve better trainability [10, 18]. Our study addresses the need of exploring the influence of selected genotype combinations and power allele dominant models on the anaerobic capacity.

The findings of this study are applicable in the contemporary sports practice by implementing interpretations of individual genetic profiling and

Wingate test results, leading to personalized training programme. Athletes who are carriers of R- and C-power dominant model are likely to achieve improvement of strength, speed and power training. Carriers of X and T allele should be more realistic about their speed/power potential. Athletes with TT genotype might have limited ventilatory adaptation to higher intensity exercises. Genetic testing on sport participation [3] and talent selection could be appropriate to aspiring young athletes towards training and competing in the most suited sport discipline.

5. Conclusion

The purposes of this study were: (1) to compare ACTN3 and AMPD1 genotypes and genotype combinations of Bulgarian athletes with a control group of unrelated healthy individuals; (2) to identify any possible association of the ACTN3 and AMPD1 genetic variants; any potentially beneficial genotype combinations; any R-(RR + RX) and C-(CC + CT) power allele dominant models related to the anaerobic capacity of the athletes performing Wingate test.

The low frequency of T allele in Bulgarian athletes might indicate that this mutant allele is related to the physical performance. The prevalence of R ACTN3 allele and C AMPD1 allele suggests that they could contribute to anaerobic performance. Higher peak power output in Wingate test is associated with RX/CT genotype combination and R- and C-power dominant models.

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DIFFERENCE IN THE DISTRIBUTION OF SELECTED BLOOD VARIABLES AMONG ATHLETES DURING A COMPETITION PERIOD

Sam Mugandani[1], Trayana Djarova-Daniels[2]
[1]University of Venda, Thohoyandou, South Africa
[2]Department of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa, South Africa

ABSTRACT

Exercise is known to cause considerable changes in leucocyte counts and functions. The aim of the present study was to investigate the effect of exercise on leukocyte counts in athletes of different sporting codes during the competition time of the season.

Forty-two university athletes voluntarily participated in the study, rugby players, male soccer players, female soccer players and female netball players. Blood samples were collected and analysis for whole blood count was done in Lancet laboratory, Richards Bay, RSA. Data were analyzed using unpaired t-test for treatment independent samples.

It is noteworthy to point out the higher levels of leucocytes in the netball players than in female soccer players (6.8 ± 1.24 X 10^9/L and 6.11 ± 1.28 X 10^9/L respectively). The lymphocyte levels were also higher in the netball players than in female soccer players (2.60 ± 0.58 X10^9/L and 2.16 ± 0.49 X10^9/L respectively). There were also higher levels of leucocytes in the male soccer players compared to the male rugby players (6.26 ± 1.97X10^9/L and 5.46 ± 0.99 X10^9/L respectively). The lymphocyte levels were higher in the soccer players than in the rugby players (2.17 ± 0.36 X10^9/L and 1.85 ± 0.32 X10^9/L respectively), but the differences were not significant at $p< 0.05$.

The changes in leucocytes could be a result (among other things) of the removal of dead cells related to exercise stress and trauma. It was expected, considering the levels of physical contact, that the leucocyte counts and the lymphocytes in particular were going to be higher among female netball players and male soccer players. The athletes' results for the measured blood parameters were within the health norms. These findings could be related to the less intensive training protocols and lower levels of physical contact and stress in players from the students teams compared to the professional players.

***Key words**: athletes, exercise stress, leucocytes, lymphocytes.*

INTRODUCTION

Training of athletes is intended to ensure that athletes reach their peak condition and produce performance which ensures that they succeed during competition. There is a complex relationship between training and competition. There are, therefore, processes which can be used to monitor how athletes respond to training. There are a number of procedures which are used by coaches and sports scientists to monitor how athletes respond to both training during preparation for competition and during the competition period itself. A number of biochemical markers are being used to monitor fitness and fatigue of athletes (Coutts & Cormack, 2014). Muscle damage has been detected through blood markers and enzymes such as creatine kinase (CK) (Clarkson et al., 2005; Yamin et al., 2007). Myoglobin, troponin, urea, uric acid and ammonia have also been used as biomarkers of muscle damage (Kirwan et al., 1990). The hormone cortisol from saliva has been used as a marker and has been shown to be elevated soon after competition (Elloumi et al., 2003; Haneish et al., 2007). Elloumi et al., 2003 have also re-

ported the use of the hormone testosterone as an exercise marker. White blood cells and platelets increase following exercise, hence are biomarkers of oxidative stress (Djarova et al., 2010), and may be useful for a clinician to better assess and evaluate the benefits of training and/ or supplementation programs (Banfi et al., 2006). It is established that exercise of elevated intensity compromises the immune system leaving athletes susceptible to illnesses (Allgrove et al., 2012). Leucocytes and cytokines have been used as possible markers of compromised immune system due to exercise (Mackinnon 1997; Gleeson & Walsh 2012).

Exercise is known to cause considerable changes in leucocyte counts and functions. The aim of the present study was to investigate the effect of exercise on leukocyte and lymphocyte counts in athletes of different sporting codes during the competition time of the season.

METHODOLOGY

Forty-two (n=42) active university athletes voluntarily participated in the study; the participants' ages were 21.76±3.24. They were all students recruited from the University of Zululand as follows: rugby players (n=9), male soccer players (n=17), female soccer players (n=9) and female netball players (n=7). They had regular training sessions of two hours or more with a frequency of five to six times a week. They also took part in the inter university games. The net ball and male soccer players were also involved in club competitions of the UThungulu District in the Kwa-Zulu Natal province. As part of their obligation in the clubs and university games, they played competitive matches during the weekends. All participants participated in the study on a voluntary basis. The objectives of the study were explained to them and the possible risks of participating in the study were clearly elucidated, after which written consent was obtained from each one of the participants. A medical professional sought information on any diseases they might have had before, their present health status and any medication which they could have been taking. The following constituted the exclusion criteria; current infection, history of chronic disease use of antibiotics, herbal, antioxidant and steroids containing supplements. The study protocols were conducted in accordance with the Helsinki Declaration for the Ethical Treatment of Human Subjects and were evaluated and approved by the ethics committee of the Faculty of Science and Agriculture at the University of Zululand.

Blood samples were collected and blood analysis for whole blood count was done in Lancet laboratory, Richards Bay, RSA. Data were analysed using unpaired t-test for treatment of independent samples.

RESULTS

It is noteworthy to point out the higher levels of leucocytes in the netball players than in female soccer players (6.8 ± 1.24 X 10^9/L and 6.11 ± 1.28 X 10^9/L respectively). The lymphocyte levels were also higher in the netball players than in female soccer players (2.60 ± 0.58 X10^9/L and 2.16 ± 0.49 X10^9/L respectively). There were also higher levels of leucocytes in the male soccer players compared to male rugby players (6.26 ± 1.97X10^9/L and 5.46 ± 0.99 X10^9/L respectively). The lymphocyte levels were higher in the soccer players than in the rugby players (2.17 ± 0.36 X10^9/L and 1.85 ± 0.32 X10^9/L respectively), but the differences were however not significant at $p< 0.05$. When looking at the results of the four groups collectively, the netball players had the highest levels of both leucocyte and lymphocyte counts. They were followed by the male soccer players, and then the female soccer players, the male rugby players had the lowest counts for both the leucocytes and lymphocytes.

Table 1. *Athletes' lymphocyte and leucocyte blood counts*

Athletes	**Lymphocytes** X 10^9/L	**Leucocytes** X 10^9/L
Male soccer	2.17±0.36	6.26±1.92
Male Rugby	1.85±0.32	5.46±0.99
Female Netball	2.60±0.58	6.80±1.24
Female soccer	2.16±0.49	6.11±1.28

DISCUSSION

Soccer is the most popular and widely played sport among blacks in South Africa especially among men (Hammond 2011; Fredrick and Llewellyn 2016). It is also much more popular among blacks than Rugby; as a result, the University of Zululand which has a predominantly black student population has a soccer league within the university itself where students' teams from within the same university compete against each other. The best students from the same teams form a university select team which competes in the local district league and the inter university competitions. Compared to their rugby counter parts the male soccer players are exposed to more competitions. There is only one rugby team in the university and therefore the rugby players do not have a local university league within the university and the district, because of that they only wait for the national inter universities rugby tournament. In the interim they play occasional friendly matches. The rugby players are therefore not exposed to the usual extensive exercise, training and competition stress as is the norm with regular rugby league players. In comparison to their soccer counterparts in the university they are subjected to less stress which may cause rise in the leucocytes and lymphocytes. The changes in leucocytes could be a result (among other things) of the removal of dead cells related to exercise stress and trauma especially due to contact impact during play. It was expected, considering the higher levels of physical contact in rugby generally than in soccer, that the leucocyte counts and the lymphocytes counts were going to be higher among the male rugby players compared to their male soccer counterparts. It would appear however that the rugby players' counts of the two blood parameters were lower than that of the male soccer players due to the very low levels of competition compared to their male soccer counter parts who had more competitions, as discussed above.

With regards to females' netball is a relatively more popular sport among women compared to female soccer (Fabrizio, 2005). Just like the university male soccer players the netball athletes had more tournaments in the local district than their female soccer counterparts. It would appear therefore that the netball players experienced great workloads during the competition season than their female soccer counter parts resulting in them registering high levels of both leucocytes and lymphocytes. The relatively higher levels of leucocytes and lymphocytes in male soccer and female netball players reflect what has been obtained in other studies which show an increase of constituents of leucocytes such as the insulin-like growth factor 1 (IGF-1) which have a regulatory role in the immune response for muscle repair (Fragala et al., 2014). High competition physical exercise stress was seen to induce oxidative stress and activation of leucocytes

in adolescents in fairly the same way as seen in individuals in our study who were also adolescents (Santos-Silvaa et al., 2001). Lymphocytes and their subsets have also been known to increase with exercise (Hong et al., 2004).

CONCLUSIONS

The athletes' results for the measured blood parameters were within the health norms. These findings could be related to the less intensive training protocols and lower levels of physical contact and stress in players from the students' teams compared to the professional players. The method used with the two blood parameters can always be used with other methods of monitoring athletes' response to training to increase the reliability of the athletes' assessment process.

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Correspondence:

sam Chenjerai Mugandani
University of Venda - Centre for Biokinetics
School of Health Sciences University of Venda Private
Bag X5050 Thohoyandou Limpopo 0950
South Africa
E-mail: scmugandani@gmail.com

CHAPTER 8

OUTCOMES AND CONCLUSIONS

This chapter addresses the outcomes and conclusions of this research by revisiting the hypotheses set in Chapter One. The outcomes are also articulated by showing the implications of the findings for coaches, exercise practitioners and athletes. This is then followed by a discussion of possible further research, as well as the limitations of the current research.

8.1 HYPOTHESES

8.1.1 Research Hypothesis

There will be an association between the ACE I/D gene polymorphism in male cricket players and blood pressure, c-reactive protein, uric acid, lactate and selected physical tests.

There were no associations between blood pressure and allele frequencies. The results of the cricketers showed that 79% D allele frequency was associated ($p<0.001$) with lower CRP levels (<3.0 mg/L). Uric acid (<0.30 mmol/L) was associated ($p=0.001$) with 43% D allele frequency. High D allele frequency (91-94%) were associated with BMI and FM in cricketers ($p=0.001$) and in controls ($p=0.029$), LBM showed an association with 71% D allele ($p<0.041$) and with 94% D allele ($p<0.029$), respectively. Knee extension L (>3.73 Nm/kg) and R (>3.63 Nm/kg) was associated with D allele frequency of 86% ($p=0.010$) and 79% ($p=0.014$). Knee flexion L (>2.04 Nm/kg) and R (>2.0 Nm/kg) was associated ($p=0.014$) with D allele frequency of 78% and 83%. For the whole cohort (Table 7) 86% D allele frequency was associated ($p=0.037$) with grip strength L (>43.3 kg) and R (R>45. 5 kg).

8.1.2 Research Hypothesis

There will be significant differences in the ACTN3 gene polymorphism genotypes and alleles frequencies, anthropometric and physical characteristics among cricket, rugby and soccer players.

There were no statistically significant differences between the genotype frequencies of rugby and cricket players. There were also no statistically significant differences between the genotype frequencies of cricket and soccer players. Significant differences were, however, found between the genotype frequencies of rugby and soccer players. Notably, the soccer players had a 100% RR

frequency distribution. Compared individually, the sport groups' genotype frequencies were not significantly different from the controls. When combined, the sport groups' genotype frequencies were not significantly different from those of the control group.

With reference to the allele frequencies, rugby and soccer showed significant differences. There were no significant differences between cricket and rugby, however, there were significant differences between cricket and soccer. Soccer had a higher frequency of R compared to both cricket and rugby. The combined frequencies of the alleles of these sport groups were not different from those of the control group.

The rugby players showed higher body mass values than the cricket, soccer and controls ($p<0.001$). The cricket players had higher stature compared to the rugby and soccer players ($p<0.001$). The cricket players also had higher lean body mass than the rugby and soccer players, while all physical characteristics were lower in the control group, as compared to all the sport groups ($p<0.001$).

8.1.3 Research Hypothesis

There will be differences in the ACE D/I genotype and allele frequencies of Zulu and Bulgarian female athletes, as well as differences in CRP and UA levels. The ACE DD genotype and D allele will show association with CRP and UA levels

There were no statistically significant differences in the ACE genotype and allele frequencies between the netball and soccer players. There were, however, statistically significant differences between Zulu and Bulgarian athletes in the genotypes ($p = 0.036$) and allele frequencies ($p= 0.003$). CRP levels were higher in the Zulu players than in the Bulgarian players ($p< 0.001$). Uric acid levels were lower in Zulu players as compared to those of the Bulgarian players. Both controls had higher levels of the biomarkers CRP and UA compared to their respective non-control players.

8.1.4 Research Hypothesis

The ACTN3 and AMPD1 genotype power /speed combinations will show greater peak power output than their non-power genotypes combinations

The genotype and allele frequencies for both the ACTN3 and AMPD1 genes were not different between the athletes and the controls. There was, however, null TT genotype among the athletes, as well as, a low T allele frequency among athletes. While we expected the RR/CC genotype

combination to show the highest anaerobic performance it was, however, the RX/CT heterogenous combination which showed the highest anaerobic performance. This was followed by the RR/CT and the XX/CT combinations whose results were the same.

8.1.5 Research Hypothesis

There will be differences in the leucocyte and lymphocyte counts among netball, female soccer players, male soccer players and male rugby players.

There were higher levels of leucocytes in the netball players than in female soccer players (6.8 ± 1.24 X 10^9/L and 6.11 ± 1.28 X 10^9/L, respectively). The lymphocyte levels were also higher in the netball players than in the female soccer players (2.60 ± 0.58 X 10^9/L and 2.16 ± 0.49 X10^9/L, respectively). There were also higher levels of leucocytes in the male soccer players as compared to male rugby players (6.26 ± 1.97 X 10^9/L and 5.46 ± 0.99 X 10^9/L, respectively). The lymphocyte levels were higher in the soccer players than in the rugby players (2.17 ± 0.36 X 10^9/L and 1.85 ± 0.32 X 10^9/L, respectively), but the differences were, however, not significant at $p< 0.05$. When looking at the results of the four groups collectively, the netball players had the highest levels of both leucocyte and lymphocyte counts. They were followed by the male soccer players, and then the female soccer players; the male rugby players had the lowest counts for both the leucocytes and lymphocytes.

Conclusion

The observations from the genotype and allele frequencies for the genes ACE, ACTN3 and AMPD1 are that the ACE gene polymorphism among athletes of Zulu origin are characterized by a dominance of the homozygous DD genotype, followed by the heterozygous ID genotype with the exception of the cricket players where DD and ID were in a ratio of 50:50. There was a null frequency of the II genotype for all the athletes and only 4% for the controls; the null I was recorded here for the first time, to the best of our knowledge. The same is true of the allele frequencies where the D allele is dominant in all the athlete groups as well as the control groups. The I allele frequency was below 35%, when the athletes and players were put together. The Bulgarian Caucasians had a higher percentage frequency of ACE ID (50%) compared to the DD (40%) frequency. The I allele for the Bulgarian athletes was higher than the D allele frequency as well as being higher than that of the I allele of the Zulu athletes. This ethnicity difference between the Caucasians and the African Zulus where there is a polymorphism in the Zulus

which is more inclined towards power dominance (D allele) and a greater presence of an ACE I allele in the Bulgarian Caucasians which favors an efficient metabolic production of energy through aerobic pathways; this is evolutionary, hence, is documented in the literature. The II genotype and a prevalence of the I allele are characteristic of populations found in the harsher temperate climatic regions of the earth dominated by Caucasian populations, as opposed to tropical climatic regions where the African Zulus live. The more energy efficient metabolic pathways helped with the survival of Caucasian populations in those harsh conditions.

A similar trend of the differences of the ACE gene polymorphism between Zulus and Bulgarians where the power genotype was expressed at a high level than in the Bulgarians, was also observed in the ACTN3 gene polymorphism. The trend, therefore, is that if the two genes are combined to constitute a polygenic scenario, the Caucasians will continue to reflect a more energy-efficient phenotype characterized by a lower presence of the ACTN3 RR power genotype compared to the very high RR genotype of the Zulu cohort. The Zulu soccer athletes had an unexpected 100% RR genotype, further strengthening the fact of a dominant power/speed genotype occurrence. The null ACTN3 XX which was found in the soccer and cricket players was encountered for the first time.

The genotype combination observed with the Bulgarian athletes performing the Wingate strongly suggest that phenotypes are inherited from a combination of genes coding for similar functions. In this case, it would be the actinin 3 protein in the Z line of type 2 fast-switch muscle fibers being coded by the ACTN3 gene R allele and the anerobic enzymes coded by the AMPD1 gene C allele. These two, therefore, contribute towards high peak power output. In other words, this is evidence that the observed phenotypes are the results of polygenic expressions. The observation is that the power genotypes RR for ACTN3 and CC for AMPD1 show higher peak-power output than the ACTN3 XX. The metabolically unfavorable AMPD1 TT genotype was null in the athletes' group. The favorable power-genotype combinations of RR/CC, RR/CT, RX/CC and RX/CT showed better peak-power outputs where all were above 9.5 Pp W kg^{-1} as compared to the low of 8.83 Pp W kg^{-1} of the XX/CC combination. The performances in the Wingate tests support the association of the AMPD1 CC polymorphism and the ACTN3 RR polymorphism into a combined power phenotype is reflected in the literature, in Chapter 2. The established association of the physiological and physical characteristics, such as BMI, LBM, FM to the ACE and ACTN3 gene polymorphisms is critical for an understanding of the multifactorial nature of the contribution of genetic polymorphism and those factors to performance in sports. The association of the D allele with the higher systolic tension time (STT) and the lower blood pressure in athletes also

suggests better cardiac efficiency of the athletes, hence, better performance. Further the association of the D allele with handgrip strength, quadriceps and hamstring muscle strength, confirms the D allele as a power/strength allele in Zulu athletes. The association of the D allele with low levels of uric acid and C-reactive protein is, to the best of our knowledge, being established for the first time. There is evidence here that there are possibly ethnic differences in the levels of these biomarkers as are reflected in the difference in the levels between Zulu and Bulgarian players. The lower levels of C-reactive protein and uric acid and the association among athletes with the ACE D allele genotype are, probably, signs that this association confers on these athletes, protection against oxidative stress and muscle damage related to eccentric muscle contraction during exercise and game play. There were differences in the levels of C-reactive protein and uric acid between athletes of different sporting codes, however, the uric acid and C-reactive levels were within the normal range. These differences, among the different sporting codes were also noted in the levels of leucocytes and lymphocytes. The levels of the leucocytes and lymphocytes while they were different among the different groups of athletes, were both within their normal ranges. C-reactive protein levels, uric acid levels, leucocyte counts, and lymphocyte counts are all variables which can be used to monitor the injury status of athletes, as well as their inflammatory and oxidative status.

8.2 IMPLICATIONS FOR ATHLETES, COACHES AND FITNESS PRACTITIONERS

Traditionally, talent identification of potential athletes has been done through observation analysis with prospective candidates being engaged in matches or competition, complemented by anthropometrical assessments, as well as physical tests. With developing knowledge in sports genomics and genetic polymorphism, coaches and teams can complement this process by genotyping prospective athletes. Based on genetic makeup, athletes can, for example, be selected for a specific sport which may be, either power / anerobic dominant or endurance / aerobic dominant. In such cases polymorphic genotypes such as AMPDI CC, ACTN3 RR, and ACE DD would be sought for power /speed sports or positions in team sports which demand speed, like strikers in soccer. Beginning athletes will, therefore, not waste a lot of time in a sport for which they may not be genetically endowed. Given that 60% of performance in sport and exercise is genetically determined, genotyping could become a vital and more objective tool in talent identification. It will, however, only become viable and reliable when polygenic genotype and allele polymorphic combinations have been improved through more research on the subject. Biomarkers, such

as C-reactive protein, uric acid, lactate, leucocytes and lymphocytes can be used in relation with the genotypes and alleles they associate with. This will help to identify those athletes who are susceptible to specific injuries as a result of their genetic makeup; for example, the association of ACE DD and ACTN3 RR with low levels of CRP and AU may confer protective status on individuals with that genetic disposition. Such knowledge about athletes will enable coaches to design individualized or personalized training programs. Athletes with genotypes which are not protective against exercise-induced muscle damage (ACTN3 XX genotype or X allele) such as eccentric muscle damage, therefore, can be asked to take longer recovery periods if using interval training or long periods of recovery in between training days; their trainers can also use cold-water immersions to enhance their recovery. Similarly, those athletes with genotypes and alleles which show increased muscle damage following eccentric exercise can be helped to reduce such damage through protocols, such as the Nordic hamstring exercises which will specifically protect hamstring muscles from eccentric damage. Individuals with a RR genotype may not have good trainability status for aerobic metabolism, which may be required of them especially in sports like soccer which depend on high levels of both anerobic and aerobic energy. Personalized training would be required to boost this energy system which will not be well supported by their power /speed genotypic and allele polymorphic endowments. In that situation, those individuals carrying power-related genotypes and alleles and need to improve their VO_2max, can receive individualized training prioritizing high intensity interval training (HIIT).

8.4 FUTURE RESEARCH

Research has established quite conclusively, that performance variations among individuals in athletic and exercise performance are greatly influenced by genetic endowment; it is now also clear that athletic performance can be explained at the genetic molecular level. By 2014, cumulative studies had established about 53 autosomal genes as being associated with elite athletic performance; as many as 120 gene polymorphisms have shown association with sport and exercise performance. Epigenetic research will also help with an understanding of how cells read genes and affect the expression of genes. That knowledge will increase our understanding of how SNPs of genes operate and how they differ from each other. Further research needs to be done to establish how many more genes are linked to sport and exercise performances out of the approximately 25 000 known human genes as established at the end of the human genome project in 2003. There is, therefore, the need for GWAS research, which will enable researchers to understand genetic markers using large numbers of athletes and individuals. Research in

proteomics needs to be done beyond associations which will be able to establish a cause/effect relationships of performance variables and metabolic processes that are controlled by the proteins which are coded for by the various polymorphisms and mutations associated with specific athletic performances. There is a need to sequence genetic profiles which will give more information on the suitability of individuals for specify roles in team sports. More replication studies also need to be conducted with research designs with more power.

8.5 LIMITATIONS

The small sample sizes of the participants were the main limitation of the research and hence a reduction in the power and generalizability of the study. There is, hence, a possibility that some of the frequencies for genotypes and alleles were influenced by the small size of the sample, although a lot of credible studies have been done with small sample sizes as with our study. One reason for this challenge is that, it is quite difficult to get large samples of athletes as there are usually few people engaging in sport at collegiate and elite levels. Attempts to establish homogeneity in the study by involving Zulus only as the participating athletes from South Africa contributed to the constraints of the small sample size. Similarly, the Caucasian participants were also a small sample.

APPENDIX A

PARTICIPANT INFORMED CONSENT FORM

INTRODUCTION

You are invited to volunteer for a research study. This information form is to help you to decide if you would like to participate. Before you agree to take part in this study, you should fully understand what is involved. If you have any questions, which are not fully explained in this form, do not hesitate to ask.

You should not agree to take part unless you fully understand the procedure involved.

By signing this document, you indicate that you understand the information and that you give your consent to the medical procedures and performance physical tests to be performed and to participate in the research study. Please read this document carefully.

NATURE AND PURPOSE OF THE STUDY

- To assess genetic and biochemical markers associated with physical performance in cricket, netball, rugby and soccer players.
- To establish the polymorphism in ACE, ACTN3 and AMPD1 genes.
- To assess the levels of red and white blood cells C-reactive protein, uric acid and lactate in control and experimental participants.
- To assess the physical and physiological characteristics (height, weight, BMI, FFBM, isometric grip strength, isokinetic strength of quadriceps and hamstring muscles, pulse and blood pressure) of athletes and controls.

WHAT IS THE DURATION OF THE STUDY AND WHAT PROCEDURES ARE INVOLVED?

During three periods of 4 weeks each, volunteers are going to be examined. Your blood pressure, heart rate, weight, handgrip strength and quadriceps and hamstring strength will be measured. Blood tests will be taken and sent to the laboratory for measurement of the biochemical markers and assessment of the gene polymorphism.

At this time of the study, the researchers will review your past and present medical history and you will have a routine medical examination. You will have to answer to few questions and to fill a questionnaire.

HAS THE STUDY RECEIVED ETHICS COMMITTEE APPROVAL?

The study protocol was submitted to the Ethics and Biosafety Committee of University of Zululand.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Your participation in this study is entirely voluntary and you can refuse to participate or stop at any time without stating any reasons. Your withdrawal will not affect your access to other medical care.

POTENTIAL BENEFITS

During this study you will receive a very high standard of medical advice and assessment of your physical performance by the researchers who are very experienced in this field.

Lastly, the information obtained from your participation, may benefit other athletes.

CONFIDENTIALITY

Any information uncovered regarding your test results or state of health as a result of your participation in this study, will be held in strict confidence.

INFORMED CONSENT TO PARTICIPATE

I have read the above information.

The content and meaning of this information have been explained to me.

I have read and understood the written information in this form.

I may, at any stage without prejudice, withdraw my consent and participation in the study.

I have had an opportunity to ask questions about this study and I am giving permission to take part in the study.

Participant's name Participant's Signature Date

I Sam Chenjerai Mugandani, herewith confirm that the above participant has been informed fully about the meaning of this study.

Researcher's Name Researcher's Signature

APPENDIX B

DATA CAPTURE SHEET

Participant Name:		
Date of Birth:		
Sport Code:		
Position played:		

Pulse beats/min:		
Diastolic blood pressure (mmHg):		
Systolic blood pressure (mmHg):		
Pulse pressure (mmHg):		
Systolic tension time (SBP x HR)		

Stature:		
Mass:		
BMI:		

Triceps:		
Midaxillary:		
Chest:		
Subscapular:		
Suprailliac:		
Abdominal:		
Thigh:		
Lean body mass-LBM:		
Fat mass-FM:		
Fat %:		

Grip strength - L (kg):		
Grip strength - R (kg):		
Knee extension – L (N/kg):		
Knee extension – R (N/kg):		
Knee flexion – L (N/kg):		
Knee flexion – R (N/kg):		

Peak power output (Pp W Kg^{1}):		
Mean power output (Pm W Kg^{1}):		

APPENDIX C

RISK ASSESSMENT QUESTIONNAIRE

Name of participant:	**Date:**

Question	**Yes** (Please give more detail)	**No**
1.Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?		
2.Do you feel pain in your chest when you do physical activity?		
3.In the past month, have you had chest pain when you were not doing physical activity?		
4.Do you lose your balance because of dizziness or do you ever lose consciousness?		
5.Do you have a bone or joint problem that could be made worse by a change in your physical activity?		
6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?		
7**.** Do you know of any other reason you should not do physical activity?		

APPENDIX D

Ethics Committee
Faculty of Science and Agriculture
University of Zululand
C/O Mr L Vivier
Department of Zoology
University of Zululand
Private Bag 1001
KwaDlangezwa
3886
Tel: 035 – 902 6741
Email: lvivier@pan.uzulu.ac.za

8 April 2011

To whom it may concern

ETHICS EVALUATION OF RESEARCH PROJECT PROPOSAL

This letter serves to confirm that **S M Mugandani** (Student No **201001581**), registered for a PhD Degree in the Department of Biokinetics and Sport Sciences, Faculty of Science and Agriculture, at the University of Zululand, in accordance with appropriate rules submitted a research project proposal to the Ethics Committee of the Faculty of Science and Agriculture. The research project will investigate: **Selected gene polymorphism related to biomarkers and physical characteristics in young African rugby, soccer and netball players**. Based on the research protocol stipulated, the Ethics Committee of the Faculty of Science and Agriculture could find no reason from an ethical standpoint to reject the proposed research.

Yours sincerely

Mr L Vivier
Chairperson
Ethics Committee
Faculty of Science and Agriculture
University of Zululand